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Contributions to the Journey on Oncofertility

Providing evidence-based information for shared decisions concerning fertility preservation and supporting a more accurate assessment of infertility risk associated with cancer treatments

Tese de Doutoramento em Ciências Farmacêuticas, ramo de Farmacologia e Farmacoterapia, orientada pela Professora Doutora Ana Cristina Costa Ribeiro Rama e co-orientada pela Professora Doutora Ana Teresa Almeida-Santos e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

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Front page: Original image kindly provided by Dr. Miguel Pina, Executive Coordinator of the Centre Regional Section of the Portuguese League Against Cancer.

[*Capa: Imagem original gentilmente cedida pelo Dr. Miguel Pina, Coordenador Executivo do Núcleo Regional do Centro da Liga Portuguesa Contra o Cancro.*]

This research was conducted at the **Centre for Fertility Preservation**, in the Reproductive Medicine Unit of the Coimbra Hospital and University Centre, CHUC, EPE.

Motivation

At the time this thesis was planned, during the year of 2012, the concept of oncofertility was still relatively unknown in Portugal. Although information on the potential gonadotoxicity of cancer treatments already existed and the concept of oncofertility as a medical field was recognized since 2006, the subject of infertility risks related with cancer treatments was rarely mentioned to male or female cancer patients of reproductive age by their oncologists, at the time of diagnosis. At the stage of planning this PhD research, at the end of 2012, my supervisor, Professor Ana Cristina Rama suggested that we approached Professor Ana Teresa Almeida Santos, as she saw the opportunity to make a relevant contribution, through our pharmacist's expertise, for a very innovative, pertinent and patient-centred service that was being developed at the *Human Reproduction Service* of the *Coimbra Hospital and University Centre* (CHUC, EPE).

The *Centre for Fertility Preservation* (CFP) had been created in 2010 and was fully operational but only about twenty female cancer patients had been consulted in the period from 2010 to 2012. The CFP already provided access to all the techniques for the preservation of female fertility, (including the experimental technique of ovarian tissue preservation) but appointments for consultations had been made largely by patients self-initiative or as a result of occasional encounters, and very few upon referral by oncologists. Also, too many of those patients were coming to the CFP after they had already initiated, or even completed, the potential gonadotoxic cancer treatments. At that time, the risks of infertility associated with cancer treatments, on one hand, and the availability of fertility preservation (FP) options, on the other, were hardly known or recognized issues. The published international literature was full of reports on the information deficits of professionals and cancer patients regarding oncofertility issues. Moreover, a survey conducted in a sample of cancer care clinicians from the CHUC, EPE had revealed a generalized lack of information, especially regarding the possibility of FP in female patients. However, evidence was already growing that the issues of fertility and parenthood after cancer were amongst the most relevant concerns of young cancer patients and their partners, and that FP techniques could help them to successfully overcome the gonadotoxic effects of cancer treatments. In this context it was urgent to plan and implement initiatives to raise local and national awareness on those risks, to help cancer care professionals and patients initiating discussions on these subjects and to disseminate information about the possibility of cryopreserving embryos, gametes or ovarian tissue before

the potentially gonadotoxic treatments. So, when the opportunity came up to build a PhD thesis around such an emergent and relevant issue, the challenge was immediately accepted and the team started to delineate a draft of what could be done to give a positive contribute.
The subject was totally fitted for a PhD thesis on Pharmacology and Pharmacotherapy and to my background as a medicine's information specialist, as it perfectly integrated the pharmaceutical issue of preventing, identifying and managing cancer medicines' adverse effects with the clinical, investigational and patient health information perspectives.

At that time point, some civil society institutions, namely the *Portuguese League Against Cancer* (LPCC) and the *Portuguese Society of Reproductive Medicine* (SPMR), at that time headed by Professor Carlos Freire de Oliveira and Professor Ana Teresa Almeida Santos, respectively, were attentive to this problem and had the determination and motivation to change the paradigm. One of the first signs for change was the establishment, in the same year of 2012, of a patient's centred research grant from the LPCC, in partnership with the pharmaceutical company Celgene (Research Grant LPCC/Celgene 2012), with the clear purpose of increasing the knowledge and health literacy of Portuguese cancer patients. This grant was seen as an opportunity for gaining financial support for the development of a broad information program concerning infertility risks and FP in cancer patients, and also as a chance of benefiting from the expertise and communication influence of the LPCC, the leading non-profit cancer patients' organization in Portugal. Clearly, the successful application for this grant was one of the main driving forces for the initiation, continuation and successful conclusion of this research work.

Considering the above mentioned context, one of the investigation areas that was identified as a priority was the need to understand the information needs and gaps in knowledge of both Portuguese health professionals and cancer patients and subsequently, to develop an information program to promote awareness on the subject of infertility risks and FP options, according to those identified needs. Our main goal was to provide to every reproductive-age patient facing a cancer diagnosis, information to support their participation in the decision-making process, and also to inform healthcare professionals, particularly those working in cancer care, on this theme.
The second area of research that was identified in the course of this work was related with the estimation of the risk of infertility in cancer patients, recognized as the first and fundamental step in the process of decision-making concerning FP. After a thorough analysis of the research available at that time, there was an obvious need to gather additional data in order to: 1)

clearly identify the factors that influence the risk; 2) quantify specific risks associated with specific treatments and 3) identify the best markers and/or predictors of infertility risk. We decided to focus our research on the population of reproductive-age female cancer patients and, more specifically, in the group of breast cancer patients, for a number of reasons. FP decisions in women present a higher complexity, as the available FP techniques are invasive, present risks and some may even require a delay in cancer treatment initiation. In opposition to what occurs in male patients, decisions concerning FP in women are multifaceted and preference-sensitive, *i.e.* different options may be differently valued by distinct patients, according to their personal values and preferences (O'Connor BMJ 1999). Additionally, estimating a female's fertility after cancer treatment is significantly dependent on personal factors like the woman's age and initial fertility. We focused on breast cancer as it is the most common cancer in reproductive-age women and the most common diagnosis among women referred to oncofertility programs. Additionally, the standard treatment for BC (surgery followed by adjuvant chemotherapy) provides a window of opportunity of several weeks for FP. We also realized that data on the impact of the various BC treatments (chemotherapy, molecular targeted therapies and hormonal therapy) on fertility was still insufficient, both in quantity and quality, despite the increasing survival of breast cancer in women of reproductive-age. New treatment agents like taxanes, trastuzumab or ovarian suppression drugs are being increasingly included in BC treatment regimens, even though traditional drugs to fight breast cancer like cyclophosphamide or anthracyclines remain in use. Additionally, most studies reporting effects of antineoplastic agents for breast cancer in female fertility had been conducted using retrospective methodologies and poor surrogate markers of fertility, like the presence or absence of amenorrhea. According to these circumstances, two parallel studies were planned to achieve the core aims of supporting a more accurate assessment of infertility risks and to support a quality decision-making process in the context of FP in the female BC setting.

In the research work presented in this thesis, we have tried to overcome several of the unsolved issues around infertility risks associated with cancer treatments. The results brought us one step closer to give, to every cancer patient, the opportunity to be effectively and timely informed about his/her specific risks, to get involved in the decision-making process and to make a better use of the available FP resources. Through these achievements we have conquered a bit more for the quality of life for those who will be, fortunately more and more, cancer survivors.

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Os meus sinceros agradecimentos

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À Professora Doutora Ana Cristina Rama, por ter trilhado (mais) este caminho comigo, partilhando sempre a sua visão e sabedoria, e pelo apoio, carinho e entusiasmo sempre demonstrados, nesta e em muitas outras jornadas ao longo de 17 anos de convivência.

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In tribute to those who are now cancer patients or survivors,

and to all who will become.

“You can’t go back and make a new start, but you can start right now and make a brand new ending.”

James R. Sherman

PUBLICATIONS

The results presented in this dissertation are available in the following publications:

1. JOURNAL ARTICLES

Antineoplastic agents and (in)fertility: Informing patients to improve decisions.

Silva C, Almeida-Santos AT, Melo C, Rama ACR.

J Adolesc Young Adult Oncol. 2018 Jan 3. [Epub ahead of print]

DOI: 10.1089/jayao.2017.0094.

Decision on Fertility Preservation in Cancer Patients: Development of Information Materials for Healthcare Professionals.

Silva C, Almeida-Santos AT, Melo C, Rama ACR.

J Adolesc Young Adult Oncol. 2017 Jun;6(2):353-357.

DOI: 10.1089/jayao.2016.0064.

Factors associated with ovarian function recovery after chemotherapy for breast cancer: a systematic review and meta-analysis.

Silva C, Caramelo O, Almeida-Santos T, Ribeiro Rama AC.

Hum Reprod. 2016 Dec;31(12):2737-2749.

DOI: 10.1093/humrep/dew224.

Recommendations for the preservation of the reproductive potential in cancer patients.

[*Recomendações para a preservação do potencial reprodutivo no doente oncológico*.]

Ana Teresa Almeida Santos, Gabriela Sousa, Adriana Teixeira, Pepe Cardoso, Cláudia Melo, Alexandra Teixeira, Joaquim Andrade, Cristina Silva, Emanuel Gouveia, Iris Bravo, Isabel Augusto, Joana Magalhães, Nuno Louro, Bruno Pereira, Rita Ramalho, Vanda Patrício, Sueli Pinelo.

***Portuguese Journal of Oncology* - official publication of the *Portuguese Oncology Society*. 2016; 2(1): 5-24.**

Accessible in

https://www.sponcologia.pt/fotos/editor2/articulo_recomendaciones.pdf

[*Revista Portuguesa de Oncologia – Órgão Oficial da Sociedade Portuguesa de Oncologia. 2016; 2(1): 5-24*].

Acessível em

https://www.sponcologia.pt/fotos/editor2/articulo_recomendaciones.pdf

2. INFORMATION RESOURCES

Patient Information Handouts

Fertility in Women with Cancer: Know the risks

[*Fertilidade na Mulher com Cancro: Conheça os Riscos*]

Fertility in Men with Cancer: Know the risks

[*Fertilidade no Homem com Cancro: Conheça os Riscos*]

Patient Decision Aids

Fertility in Women with Cancer: Know the Fertility Preservation Options

[*Fertilidade na Mulher com Cancro: Conheça as Opções de Preservação de Fertilidade*]

Fertility in Men with Cancer: Know the Fertility Preservation Options

[*Fertilidade no Homem com Cancro: Conheça as Opções de Preservação de Fertilidade*]

Information booklet for healthcare professionals

Fertility Preservation in Cancer Patients: General Information for Healthcare Professionals

[*Preservação da Fertilidade em Doentes Oncológicos: Informação Geral para Profissionais de Saúde*]

Information booklet for oncologists

Fertility Preservation in Cancer Patients

[*Preservação da Fertilidade em Doentes Oncológicos*]

Note: Some of the results included in Chapter I and Chapter II and the results in Chapter III are formatted according to the style of the journal where the papers were published, with minor modifications.

Abstract

Infertility is a potential adverse effect of cancer treatments and oncofertility is an emergent multidisciplinary field that addresses cancer patients' concerns regarding their future reproductive ability. As the number of cancer survivors increase, shared decisions concerning fertility preservation (FP) must take place at the time of diagnosis. This decision has to be informed and meet patients' preferences. However, national and international reports on FP needs and practices reveal that many patients remain unaware of the risks and not all are referred to FP counselling.

Breast cancer (BC) is the most common cancer in women under 40 years and future fertility is an important issue for quality of life in survivorship. Multi-agent chemotherapy (CT) regimens in association with targeted therapy (TT) and/or hormonal therapy (HT) are used to treat BC but much is still to be known about the mechanisms and gonadotoxic effects of specific regimens and treatment combinations. The identification of (in)fertility in female cancer patients has been traditionally based on the presence or absence of amenorrhea but, currently, the use of more specific surrogate markers of OR such as the Anti-Mullerian Hormone (AMH) is recommended.

The aims of this work are: 1) to provide significant contributions to a shared decision-making process concerning FP and 2) to support a more accurate assessment of infertility risk associated with cancer treatments, with a special focus in young female patients with BC.

A comprehensive information program directed to both cancer patients and health professionals, and involving all the relevant stakeholders in the context of cancer care, was established. These information resources were developed through a systematic approach and are currently available to Portuguese health professionals and cancer patients in many Portuguese institutions of primary, cancer and reproductive healthcare. They are currently supporting an informed and shared decision-making process in the context of FP and, by including information on risks associated with specific cancer treatments and on the factors that may influence that risk, they are also contributing for a more accurate infertility risk assessment.

In parallel, the results of this information program have contributed to significant advances in the oncofertility field that have been happening in our country in the last years, from which

the establishment of national clinical guidance concerning FP, endorsed by several Portuguese medical societies, must be highlighted.

In order to support a more accurate assessment of infertility risks in young patients with BC, two investigation approaches were used. The first was to carry out an innovative systematic review and meta-analysis of published studies with the aim of confirming the existence of one or more factors that would help to predict, in each specific BC patient, the chance of recovering post-treatment ovarian function. The main results of this review support that younger patients are more likely to recover menses and addition of taxanes to standard CT regimens is negatively associated with recovery. The second approach was a prospective observational study in young female BC patients, developed to assess the impact of modern treatment associations combining CT with TT and/or HT agents, by measuring reliable fertility surrogate markers, and focusing on relevant reproductive health outcomes. This research found that many young women with BC will not recover to their age-expected levels of OR and that some will be at risk for premature ovarian failure. AMH was confirmed to be the most relevant OR marker in this setting. Lower age, higher AMH and exposure to trastuzumab were associated with higher post-treatment OR and better reproductive health outcomes. In addition, the lack of reliable markers of OR in patients exposed to some form of HT was highlighted by the results of this study. Notably, the results from the systematic review and from the clinical study are in accordance and support each other.

In conclusion, all the various results of this work have given important contributions both for shared decisions concerning FP and for an easier and more accurate assessment of the risk of infertility in each cancer patient, especially in the case of young pre-menopausal patients with BC. The overall results of this thesis are very significant contributions to a multitude of aspects related with oncofertility, both at national and international levels. Due to a high and immediate applicability in clinical practice, they will support and facilitate a more conscious journey on oncofertility.

Resumo

A infertilidade é um potencial efeito adverso dos tratamentos oncológicos e a oncofertilidade é uma especialidade multidisciplinar emergente que aborda as preocupações dos doentes oncológicos no que diz respeito à sua futura capacidade reprodutiva. À medida que o número de sobreviventes de doença oncológica aumenta, é importante permitir que decisões partilhadas sobre preservação da fertilidade aconteçam no momento do diagnóstico. Estas decisões devem ser informadas e atender às preferências dos doentes. No entanto, estudos nacionais e internacionais sobre as necessidades e práticas de PF revelam que muitos doentes permanecem inconscientes dos riscos e que poucos são encaminhados para os serviços de preservação da fertilidade disponíveis.

O cancro da mama é o tipo de cancro mais comum em mulheres até aos 40 anos e a fertilidade futura é uma questão importante para a sua qualidade de vida na sobrevivência. Atualmente, são utilizados regimes de quimioterapia multiagente, em associação com terapêuticas dirigidas e/ou terapêutica hormonal mas ainda há muito a ser conhecido sobre os mecanismos e efeitos gonadotóxicos de regimes e combinações específicas de tratamento. A identificação da (in)fertilidade em mulheres com cancro tem sido tradicionalmente baseada na presença/ausência de amenorreia, mas, atualmente, o uso de marcadores mais específicos e fiáveis de reserva ovárica, como a hormona Anti-Mulleriana, é recomendado.

Os objetivos desta investigação são: 1) contribuir de forma significativa para um processo de tomada de decisão informada e partilhada sobre a preservação da fertilidade em doentes oncológicos; 2) apoiar uma avaliação mais precisa do risco de infertilidade associado aos tratamentos oncológicos, com um foco especial na população de mulheres jovens com cancro da mama.

Foi implementado um programa de informação abrangente, dirigido a doentes oncológicos e profissionais de saúde e envolvendo todos os intervenientes relevantes no contexto da doença oncológica. Os recursos de informação foram desenvolvidos através de uma abordagem sistemática e estão disponíveis, para profissionais e doentes, em instituições portuguesas de cuidados de saúde primários, oncológicos e reprodutivos. Atualmente, contribuem para facilitar a tomada de decisões no contexto da preservação da fertilidade e para uma avaliação mais precisa do risco de infertilidade em cada doente oncológico. Em paralelo, estes resultados contribuíram para os avanços significativos da oncofertilidade em Portugal, dos quais se destaca a publicação de recomendações clínicas nacionais sobre a proteção do

potencial reprodutivo no doente oncológico, em colaboração com várias sociedades médicas. Com o objetivo de apoiar uma avaliação mais precisa do risco de infertilidade em mulheres jovens com cancro da mama, foram utilizados dois métodos de investigação. O primeiro foi a realização de uma revisão sistemática, com meta-análise, com o objetivo inovador de confirmar a existência de fatores, relacionados com o doente e/ou com o tratamento, que pudessem predizer a probabilidade de recuperação da função ovárica após exposição à quimioterapia. Os principais resultados deste estudo mostraram que as mulheres mais jovens têm maior probabilidade de recuperar a menstruação e que a adição de taxanos influencia negativamente essa recuperação. O segundo método foi um estudo observacional prospetivo em mulheres jovens com cancro da mama, que pretendeu avaliar o impacto de esquemas modernos de tratamento, através da avaliação de marcadores intermédios de fertilidade fiáveis e com foco em *outcomes* relevantes de saúde reprodutiva. Os resultados mostraram que muitas mulheres jovens com cancro da mama não recuperam níveis de reserva ovárica que seriam esperados para a idade e que algumas estão em risco de insuficiência ovárica prematura. Confirmou-se a relevância da hormona Anti-Mulleriana como marcador de reserva ovárica no contexto do cancro da mama. Verificou-se ainda que uma menor idade, maior nível de hormona Anti-Mulleriana e exposição ao trastuzumab são fatores associados a maior reserva ovárica pós-tratamento e a melhores resultados de saúde reprodutiva. Os resultados deste estudo salientaram ainda a ausência de marcadores fiáveis de reserva ovárica em doentes expostas a terapêutica hormonal. Notoriamente, os resultados das duas abordagens são concordantes e reforçam-se mutuamente.

Em conclusão, foram produzidas contribuições muito significativas para uma variedade de aspetos relacionados com a oncofertilidade, tanto a nível nacional como internacional. Os resultados desta tese irão apoiar decisões partilhadas e informadas sobre preservação da fertilidade e uma avaliação mais precisa do risco de infertilidade em cada doente oncológico, especialmente no contexto específico de mulheres jovens com cancro da mama. Tendo em conta a elevada e imediata aplicabilidade destes contributos à prática clínica, a jornada de oncofertilidade será agora, e no futuro, mais apoiada e consciente.

GENERAL INDEX

FIGURES AND TABLES INDEX

FIGURES INDEX

TABLES INDEX

Introduction

Results

Chapter I

ANNEXES INDEX

Acronyms and abbreviations

AC	Adriamycin (Doxorubicin) and Cyclophosphamide
AFC	Antral Follicle Count
AMH	Anti-Mullerian Hormone
ASCO	American Society of Clinical Oncology
BC	Breast Cancer
BMI	Body Mass Index
BRCA	BReast CAncer susceptibility gene
C	Cyclophosphamide
Cap	Capecitabine
CFP	Centre for Fertility Preservation
CHUC	Coimbra Hospital and University Centre
CT	Chemotherapy
D	Doxorubicin
DD	Dose-dense
E	Epirubicin
ESHRE	European Society of Human Reproduction and Embriology
5-FU	Fluorouracil
EC	Epirubicin and Cyclophosphamide
Epi	Epirubicin
Exem	Exemestane
FAC	Fluorouracil, Adriamycin (Doxorubicin)and Cyclophosphamide
FEC	Fluorouracil, Epirubicin and Cyclophosphamide
FP	Fertility Preservation
FSH	Follicle-Stimulating Hormone
Gos	Goserelin
HC	Hormonal Contraception
Her_2	Human Epidermal growth factor receptor 2
HR	Hormone-receptor
HT	Hormonal Therapy
GnRHa	Gonadotropin-Releasing Hormone agonist
ICSI	Intracytoplasmic Sperm Injection
IUI	Intrauterine Insemination
IVF	In Vitro Fertilization
LoQ	Limit of Quantification
LPCC	Portuguese League Against Cancer
OR	Ovarian Reserve
P	Paclitaxel
POI	Premature Ovarian Insufficiency
PPS	Portuguese Pharmaceutical Society
PZM	Pertuzumab
SPMR	Portuguese Society of Reproductive Medicine
SPO	Portuguese Society of Oncology

T	Docetaxel
TC	Docetaxel and Cyclophosphamide
TE	Docetaxel and Epirubicin
TT	Targeted Therapy
Tam	Tamoxifen
TESA	Testicular Sperm Aspiration
TESE	Testicular Sperm Extraction
TZM	Trastuzumab

INTRODUCTION

INTRODUCTION

1. (In)fertility in cancer patients

1.1. Reasoning

The number of cancer survivors of reproductive age has been rising in the last decades mainly due to considerable advances in cancer diagnosis and treatment. For children with cancer, the survival statistics have been the most impressive – about 85% of pre-pubertal cancer patients (age 0–14 years) are likely to survive their disease (Smith, Altekruse et al. 2014). Another relevant example is that of breast cancer, where survival rates currently range from 72 to 85% in young female patients (Anders, Johnson et al. 2009, UK Cancer Research 2013). These significant improvements in cancer patients survival led both patients and practitioners to think well beyond cancer cure and focus on survival issues and future quality of life (Woodruff 2015). Therefore, addressing the late effects associated with cancer treatments including reproductive and endocrine issues, has recently taken on a new urgency: in addition to facing the consequences of the disease, these patients will have to address the consequences of cancer treatments in their fertility (Schover 2005).

1.2. General concepts on male and female fertility

Human fertility is defined as the ability to produce offspring i.e. to conceive a baby (Northwestern University 2011). The internationally accepted definition for infertility describes it as “a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner.” (Zegers-Hochschild, Adamson et al. 2017).

The function of the male reproductive system is to produce sperm and transfer them to the female reproductive tract. Spermatogenesis, *i.e.* the process by which male gametes (sperm cells or spermatozoa) are formed in the seminiferous tubules of the testis, is a vital component of the reproductive function. This process occurs in the seminiferous tubules that form the bulk of each testis and begins at puberty, after which time sperm are produced constantly throughout a man’s life. Spermatogenesis involves three distinct phases: mitotic division of the spermatogonia (proliferation), meiotic division of the spermatocytes to produce spermatids (meiosis), and differentiation of round spermatids to form elongated spermatids (spermiogenesis) (Figure i.1). Germ cells remain in contact with Sertoli cells throughout spermatogenesis. After spermatogenesis in the testis, spermatozoa are still immotile and must

go through further maturation processes in the epididymis and female reproductive tract before they are able to fertilize an egg (Northwestern University 2011).

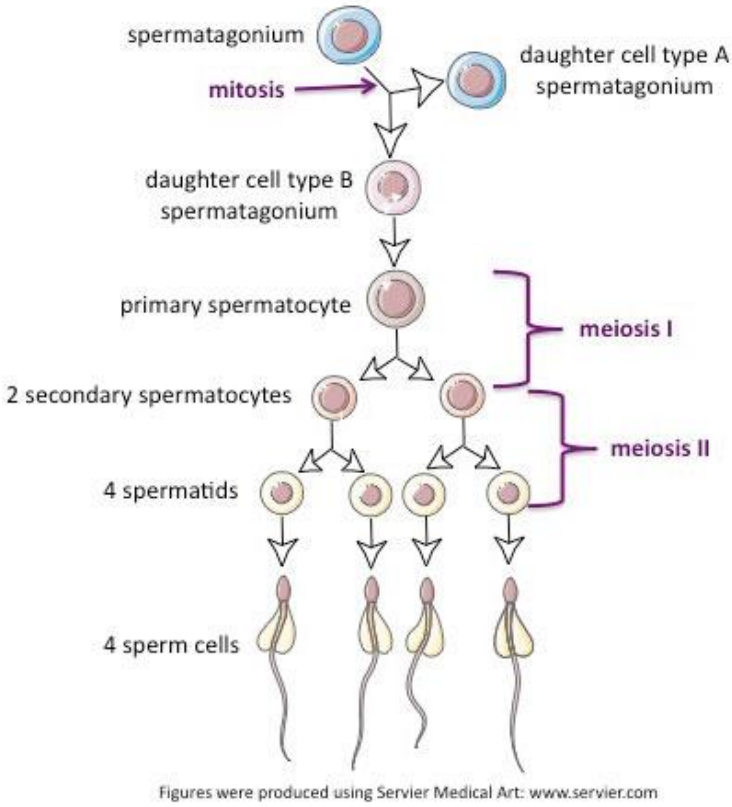

Figure i.1 Process of spermatogenesis. Accessible in http://www.repropedia.org/spermatogenesis.

The female reproductive system functions to produce gametes and reproductive hormones, just like the male reproductive system; however, it also has the additional task of supporting the developing foetus and delivering it to the outside world. In women, the reproductive potential is mainly limited by the number of available gametes, called oocytes (Knopman, Papadopoulos et al. 2010). Oocytes are produced in the ovary, where the functional unit is the ovarian follicle, composed of an oocyte that is surrounded by somatic cells, including granulosa cells and theca cells (Northwestern University 2011). Each woman develops a finite pool of follicles during foetal development, corresponding to 6-7 million by the 4th month. From these follicles, only 300 to 400 thousand remain in the ovaries at the age of menarche. After puberty, a cohort of follicles is recruited each month but only one will become dominant; the others undergo atresia, accounting for a progressive decline in the number of follicles (Broekmans, Soules et al. 2009).

1.3. Fertility assessment in male cancer patients

After cancer treatment, endocrine dysfunction (e.g. reduction of testosterone levels) only occurs in rare occasions, with pre-pubertal males showing greater sensitivity to high doses of radiation (Ginsberg 2012). The manifestation of toxicity of highest concern is the prolonged reduction in sperm count to the point of azoospermia. Damage to other aspects of sperm function, such as loss of motility or morphological abnormalities is less pronounced (Meistrich 2009). Spermatogenesis begins at puberty and continues throughout life and semen analysis remains the gold standard for assessing male fertility status: it can be performed at any age after puberty and it is an easy, cost-effective, and non-invasive method of determining fertility potential. Important aspects of a semen analysis for review include semen volume, sperm concentration, sperm motility and morphology (The Oncofertility Consortium 2015).

1.4. Fertility assessment in female cancer patients

Ongoing pregnancy and occurrence of live birth are stated as the ideal primary outcomes in female fertility trials (Barnhart 2014, Braakhekke, Kamphuis et al. 2014). However, using pregnancy as a measure of fertility has obvious limitations: the need to wait until a childhood cancer survivor has grown into an adult and has attempted to get pregnant, or the fact that not all adult survivors attempt to get pregnant. Therefore, fertility surrogate measures are needed to assess the effect of cancer and cancer treatments on fertility (Gosiengfiao and Gomez-Lobo 2015). The absence of menses, known as amenorrhea, has traditionally been used as the primary surrogate measure of infertility in cancer patients. However, this clinical indicator is currently known to be a poor and late marker of damaged ovarian function (Ruddy and Partridge 2012). A history of irregular menses or amenorrhea suggests a lack of normal ovarian hormone production and anovulation. However, a woman who is amenorrheic or presents irregular menses (oligomenorrhea) may not be ovulating but may maintain a normal ovarian reserve (OR), i.e. the pool of primordial non-growing follicles present in the ovaries. By opposition, women may have regular menstrual cycles for several years after chemotherapy, but may have a lower likelihood of pregnancy occurrence during this period and an increased risk for premature menopause due to a significant reduction in OR (Letourneau, Ebbel et al. 2012, Bedoschi, Navarro et al. 2016).

1.4.1. Ovarian reserve markers

Although oocyte number and quality decline with age, fertility can be variable in distinct women of similar age. At a given age, the OR is the most important female reproductive potential indicator which, however, is difficult to measure. There is no *in vivo* technique for

counting primordial follicles and the estimation of the OR at various chronological ages has been made by analysing *post-mortem* or post-oophorectomy tissues (Kelsey, Anderson et al. 2012). To overcome this problem, a number of tests involving biochemical measures and ovarian imaging, collectively known as OR tests, have been proposed to help in predicting OR and/or reproductive potential (Practice Committee of the American Society for Reproductive Medicine 2015). These markers can be organized in two different types:

- Endocrine markers, such as serum levels of the Anti-Mullerian Hormone (AMH) and Follicle-Stimulating Hormone (FSH), measurable by available hormonal assays;
- Ultrasound markers that measure physical factors, such as ovarian volume (OV) and antral follicle counts (AFC), by transvaginal sonography.

Accelerated atresia coincides with a decrease in the quantity and quality of oocytes (Knopman, Papadopoulos et al. 2010). Besides age, other factors like smoking, stress, parity and body mass index also contribute to follicular atresia (Bedoschi, Navarro et al. 2016). The loss of follicles occurs continuously during reproductive years, until the end of the OR (which normally occurs at around 50 years of age). At the time of menopause less than 1000 follicles are found in the ovaries (Broekmans, Soules et al. 2009).

In parallel, the quality of oocytes also diminishes and this decrease in quality occurs more significantly after the age of 30. Underlying mechanisms may involve differences between germ cells at the time they are formed (foetal life), accumulated damage of oocytes in the course of a woman's life and age-related changes in the quality of the granulosa cells surrounding oocytes (Broekmans, Soules et al. 2009).

Despite the profound changes in the number and quality of follicles during the third and fourth decade of life, it is only when menstrual cycles become irregular that women usually first notice the signs of the ongoing reduction in follicle numbers. Oligomenorrhea (prolonged intervals between menstrual cycles) and amenorrhea (cessation of menses) are late markers of ovarian ageing and infertility. Several ultrasound and endocrine markers have emerged that may express more accurately the decline in the number of follicles i.e. the decreased ovarian reserve.

1.4.1.1. Ultrasound markers

Ultrasound measures of OR include antral follicle count (AFC) and ovarian volume (OV). More recently, the assessment of ovarian blood flow by measuring the resistance index (RI) and the

pulsatility index (PI) has been proposed as an additional relevant marker of ovarian function after chemotherapy (Ben-Aharon, Meizner et al. 2012).

- Antral Follicle Count

The AFC describes the total number of follicles measuring 2–10 millimetres in diameter that are observed during an early follicular phase transvaginal scan (Practice Committee of the American Society for Reproductive Medicine 2015). An antral follicle (or Graafian follicle) has reached the most mature ovarian follicle stage of folliculogenesis. It is characterized by its large diameter and the presence of a liquid-filled space, known as antrum (Northwestern University 2011). The number of antral follicles correlates with the size of the remaining follicular pool (Hansen, Hodnett et al. 2011) and a low AFC has high specificity for predicting low OR (Bedoschi, Navarro et al. 2016). Moreover, its measurement shows good inter-cycle and inter-observer reliability in experienced centres. Ideally, antral follicles should be counted between days 2 and 4 of a spontaneous menstrual cycle to avoid the effect of intra-cycle variation (Broekmans, de Ziegler et al. 2010).

- Ovarian volume

Ovarian volume is calculated using three ovarian diameters as follows: d1 x d2 x d3 x π/6. The result is reported in cm^3. Typically, the mean volume of the two ovaries is reported and an ovarian volume of less than 3 cm^3 per ovary predicts poor response to ovarian stimulation with high specificity (Practice Committee of the American Society for Reproductive Medicine 2015). However, this measure has limited clinical utility as an OR marker as low ovarian volume has it has been reported to show low sensitivity for predicting low OR (Bedoschi, Navarro et al. 2016).

1.4.1.2. Endocrine markers

- Follicle-Stimulating Hormone (FSH)

The ovarian function depends on gonadotropin production by the pituitary gland. Follicle-Stimulating Hormone (FSH) stimulates the growth of granulosa cells of growing follicles as well as the production of oestradiol by the follicles (Bedoschi, Navarro et al. 2016). However, this hormone has, clearly, a much lower correlation with primordial follicle counts and follicular recruitment rates than other indirect measures of OR and shows a limited ability to diagnose ovarian dysfunction (Nelson 2013). Nevertheless, in women with OR compromised by chemotherapy (CT), follicular depletion correlates to an increase in FSH levels (Bedoschi, Navarro et al. 2016). Assays for FSH measurement have, however, significant inter- and intra-

cycle variability which limits their reliability (Practice Committee of the American Society for Reproductive Medicine 2015). Levels of FSH that exceed 10 mIU/mL on menstrual cycle days 2 or 3 are highly specific but poorly sensitive for predicting low OR (Bedoschi, Navarro et al. 2016). Although women who have suffered significant damage to the ovaries may present normal FSH levels, the test is still clinically useful as one can be fairly certain that women having an abnormally elevated value will have a decreased OR (Practice Committee of the American Society for Reproductive Medicine 2015).

Studies have shown that FSH levels were significantly higher in women presenting amenorrhea after oncological treatment. However, FSH levels were within the normal range in women with diminished OR but regular menstrual cycles (Jung, Shin et al. 2010, Anderson and Wallace 2013). When FSH levels are within the normal range, basal oestradiol levels may provide additional useful information for the evaluation of OR (Practice Committee of the American Society for Reproductive Medicine 2015).

- Anti-Mullerian Hormone (AMH)

The Anti-Mullerian Hormone (AMH) is produced by granulosa cells from pre-antral and small antral follicles and is involved in the regulation of primordial follicle recruitment by inhibiting the initial follicular recruitment from the primordial to the antral pool (Peigne and Decanter 2014). AMH levels strongly correlate with the ovarian follicular pool and the AFC (Hansen, Hodnett et al. 2011, Kelsey, Anderson et al. 2012). AMH is considered, currently, the most reliable and accurate marker of OR (Tal and Seifer 2017). Furthermore, unlike other reproductive hormones, AMH is detectable in girls of all ages and rises steadily through childhood, thus being of value in the assessment of ovarian function in pre-pubertal girls (Dewailly, Andersen et al. 2014). Serum concentrations of AMH are gonadotropin-independent and therefore remain relatively constant within and between menstrual cycles (Bedoschi, Navarro et al. 2016, Tal and Seifer 2017). This fact represents a clear advantage towards other available markers when considering the frequent time constraints in the cancer setting. It is important to note that women who smoke and those exposed to long term hormonal contraception may present significantly reduced AMH levels (Dolleman, Verschuren et al. 2013, Dewailly, Andersen et al. 2014). There are still some technical limitations in the assessment of AMH levels, as different assays and different procedures in sample handling can influence AMH determinations (Nelson 2013). Low AMH threshold values have good sensitivity and specificity for low OR although published data does not show a correlation with pregnancy (Dillon, Sammel et al. 2013, Hamy, Porcher et al. 2016, Steiner, Pritchard et al. 2017).

- Inhibin B

Inhibin-B is a glycoproteic hormone mainly secreted during the follicular phase by granulosa cells of pre-antral and antral follicles. It regulates the pituitary FSH secretion by negative feedback mechanisms: as inhibin-B levels decrease with advancing reproductive age and decreased OR, FSH levels increase (Bedoschi, Navarro et al. 2016). Although the levels of this hormone are generally lower in women with diminished OR, inhibin B shows less correlation with the pool of primordial follicles and serum levels vary widely during and between menstrual cycles. Therefore, it is not considered a reliable OR marker (Practice Committee of the American Society for Reproductive 2012, Bedoschi, Navarro et al. 2016).

2. Effects of cancer and cancer treatments on human fertility

2.1. Epidemiology

A vast amount of epidemiological data demonstrates decreased fertility in cancer survivors. Reports from the *Childhood Cancer Survivor Study* (CCSS) are amongst the largest studies (6,224 male and 5,149 female survivors) and the most recent results confirm that survivors have a decreased likelihood of having a pregnancy versus siblings (male survivors: hazard ratio [HR] 0.63, 95% CI 0.58-0.68; p<0·0001; female survivors: 0.87, 0.81-0.94; p<0·0001) and of having a livebirth (male survivors: [HR] 0.63, 0.58-0.69; p<0.0001; female survivors: [HR] 0.82, 0.76-0.89; p<0.0001) (Chow, Stratton et al. 2016). Other report from the CCSS identified acute ovarian failure in 6.3% of female survivors and premature nonsurgical menopause in 8% of participants versus 0.8% of siblings (rate ratio = 13.21; 95% CI, 3.26 to 53.51; P < .001). Offspring of women who received pelvic radiation doses of more than 5 Gy were more likely to be small for gestational age but, overall, there were no differences in the proportion of offspring with malformations, cytogenetic syndromes or single-gene defects (Green, Kawashima et al. 2009). In another report that studied infertility rates and reproductive interventions, CCSS female survivors had an increased risk of clinical infertility (relative risk [RR] 1·48 [95% CI 1·23-1·78]; p<0·0001) and an increased time to pregnancy compared with their siblings (p=0·032) (Barton, Najita et al. 2013). These results are corroborated by a recently published meta-analysis of 45 studies that focused on the specific pregnancy outcomes in women after BC treatment. The pregnancy rate for survivors was on average 40% lower than the general population pregnancy rate (Gerstl, Sullivan et al. 2018). The mentioned results from the CCSS were confirmed by another large cohort study from Norway that found a lower pregnancy rate in cancer survivors, except for those of malignant melanoma or thyroid cancer. The pregnancy rate was higher in male than in female survivors (hazard ratio [HR]

=0.74 (95% confidence interval (CI) 0.71-0.78) and [HR] =0.61 (95% CI 0.58-0.64), respectively) (Stensheim, Cvancarova et al. 2011). Specifically for male survivors, additional data from a Norwegian cohort study confirms reduced paternity ([HR] =0.72) and a greater likelihood of using assisted reproduction in survivors compared to cancer-free controls (relative Risk [RR] =3.32) (Gunnes, Lie et al. 2016). Moreover, this latter study corroborates previous results of non-increased adverse outcomes in the survivors' offspring.

2.2. Effects of cancer disease

Several studies have reported that semen quality is poor in male patients with cancer, indicating that some cancer patients have potentially decreased fertility even before starting any cancer treatment (Agarwal and Allamaneni 2005). This effect is mediated by immunological or cytotoxic mechanisms, not yet fully understood (Knopman, Papadopoulos et al. 2010). Some types of cancer such as testicular cancer and Hodgkin's lymphoma lead to lower counts of sperm, even before initiating cancer treatments. In the study by *van Casteren* and colleagues (van Casteren, Boellaard et al. 2010), conducted in 764 male cancer patients referred for semen cryopreservation prior to chemotherapy and radiotherapy, only one third of patients had normal semen parameters prior to cancer treatment. Patients with testicular germ-cell tumours and extra gonadal germ-cell tumours were reported as having the highest risk for impaired semen quality and gonadal dysfunction at the time of semen cryopreservation. These results were confirmed by a retrospective observational study by Auger and colleagues (Auger, Sermondade et al. 2016) which identified normozoospermia in only half of testicular cancer patients, and in 40 % or less for leukaemia and brain tumour patients, compared to more than 93 % in healthy men. Another published study found that even before treatment, men with Hodgkin and non-Hodgkin lymphomas had altered semen characteristics and higher sperm aneuploidy rates than the control group (Martinez, Walschaerts et al. 2017). Non-published data from male cancer patients consulted in the *Centre for Fertility Preservation* (CFP) confirm that sperm from patients with non-Hodgkin Lymphoma (n=17) and testicular cancer (n=114) present increased rates of azoospermia and teratozoospermia, as compared to patients with Hodgkin Lymphoma (n=50) and other type of cancers (n=49) (Barbosa D, Sousa AP et al. 2015). The definite mechanisms behind these effects have not been identified but may include endocrine and nutritional alterations, and the induction of a hypermetabolic state (Sabanegh and Ragheb 2009).

With regard to female fertility, the results of several published studies indicate that cancer has no negative effect on ovarian function. According to a review of the literature published in 2013, no significant change were found in baseline ovarian function parameters or in ovarian

response to stimulation, in terms of oocyte yield, in patients diagnosed with various types of cancer as compared with control subjects (Levin and Almog 2013). Other published retrospective study that compared OR and ovarian stimulation outcomes in patients with a new diagnosis of BC (n=191) and patients undergoing elective fertility preservation (n=398) concluded that a breast cancer diagnosis does not impact gonadal function (Quinn, Cakmak et al. 2017). Similarly, an unpublished retrospective analysis of response to ovarian stimulation in patients with a BC diagnosis (n=80) and healthy women (n=53) found no significant differences in the number of both harvested and mature oocytes (Subtil S, Pires R et al. 2018).

2.3. Effects of cancer treatments

2.3.1. Surgery

Various types of surgical procedures involving reproductive organs may reduce fertility or actually yield sterility.

In men with testicular cancer, surgical procedures like orchiectomy may affect fertility if the remaining testicle does not have a normal function. Many times, fortunately, the remaining testicle continues to produce sperm in a sufficient amount to preserve reproductive function. In men with advanced prostate cancer it may be necessary to remove both testicles to limit the production of testosterone and slow the growth of malignant cells. This bilateral orchiectomy precludes men from fathering children unless they cryopreserve sperm before the surgery. Additionally, prostate surgery to remove the prostate gland and seminal vesicles leaves men with no semen production. During prostate surgery, nerve damage may also occur, causing erectile dysfunction. In these cases, conception through sexual intercourse will not be possible. The same happens after radical cystectomy to treat some bladder cancers, where the bladder is removed along with the prostate and seminal vesicles.

A few other types of cancer surgery can damage nerves that are involved in the ejaculatory function and, therefore, will interfere with the normal reproductive function. These include abdominal lymphadenectomy, which may be part of the surgical procedures for testicular cancer and some colorectal cancers (American Cancer Society 2016).

In females, surgeries like hysterectomy or oophorectomy to treat cervical or ovarian cancer can have a significant impact in reproductive function, limiting their ability to conceive and/or to pursue a pregnancy. In early stage ovarian or cervical cancers, it may be possible to preserve the uterus and one of the ovaries, by using conservative surgical procedures (American Cancer Society 2016).

2.3.2. Radiation therapy

The testis is an exquisitely radiosensitive tissue, with even very low doses causing significant impairment of function. Radiation to the testes (for example, to treat some types of testicular cancer and childhood leukaemia), or to nearby pelvic areas (in seminoma, abdominal or pelvic tumours) can affect male's fertility. Radiation at high doses kills the stem cells that produce sperm and infertility may be irreversible (American Cancer Society 2016). With respect to damage to Leydig cell formation and testosterone production, pre-pubertal males and adults display different gonadal sensitivity with boys showing greater sensitivity to high doses of radiation (Meistrich 2009). In prostate cancer, external radiation therapy often causes permanent infertility, even if the testes are shielded. Brachytherapy effects are not so aggressive and many men will remain fertile or recover sperm production after this treatment (American Cancer Society 2016). Radiation directed to the brain may disrupt the pituitary-hypothalamus axis and also affects fertility by decreasing sperm production. Additionally, even when spermatogenesis remains functional after radiation therapy, sperm cells may get damaged by radiation so conception must be delayed for a period ranging from 6 months to 2 years after treatment is completed (Sabanegh and Ragheb 2009).

As for female patients, most women getting pelvic radiation will lose their fertility but the damage can be reduced if the ovaries are moved further from the target area in a minor surgery called ovarian transposition, before radiation begins. The oocyte is generally extremely sensitive to radiation therapy. Although primordial follicles are thought to be more radio-resistant than maturing follicles, even a small dose of radiation directed to the ovaries may result in early menopause (Wo and Viswanathan 2009). Radiation directed to the abdomen or to the vagina may also destroy ovarian follicles and affect fertility (American Cancer Society 2016). Radiation to the uterus can cause scarring, which decreases the blood flow to the uterus limiting its normal function. Women who have had radiation to the uterus have an increased risk of miscarriage, low birth-weight infants, and premature births which are most likely in women who had radiation before puberty (Teh, Stern et al. 2014). Similarly to what is observed in males, radiation directed to the brain may damage the pituitary or the hypothalamus inducing hormonal changes and interfering with the normal regulation mechanisms of ovulation, in women (Wo and Viswanathan 2009).

2.3.3. Conditioning regimens for stem cell or bone marrow transplant

A bone marrow or stem cell transplant usually requires high doses of chemotherapy and sometimes radiation to the whole body before the transplant takes place. Alkylating agents

such as busulphan, cyclophosphamide and melphalan, often in combination, remain the mainstay of conditioning chemotherapy regimens (Gyurkocza and Sandmaier 2014).

In male patients, hypogonadism is common after hematopoietic stem cell transplant (HCT). Impaired spermatogenesis, erectile dysfunction, low testosterone, and low libido occur in male patients (Inamoto and Lee 2017). High-dose conditioning regimens have been associated with azoospermia rates exceeding 90% (Joshi, Savani et al. 2014). Azoospermia occurred in 70% of male patients conditioned with cyclophosphamide alone but 90% of them recovered spermatogenesis. In patients conditioned with cyclophosphamide plus busulfan or thiotepa, only 50% of patients recovered spermatogenesis and in patients conditioned with total body irradiation (TBI) only 17% (Anserini, Chiodi et al. 2002).

In female patients, ovarian failure after HCT has been observed in 65–84% of transplant recipients (Joshi, Savani et al. 2014). The risk of premature ovarian failure (POF) increases with age and in the case of conditioning treatment with total body irradiation (Jadoul and Donnez 2012). Ovarian failure has occurred in more than 90% of female patients after HCT and recovered in 92% of patients conditioned with cyclophosphamide alone, but only in 24% of patients conditioned with cyclophosphamide and TBI (Inamoto and Lee 2017). Even where gonadal recovery and pregnancy occur, it is important that the patient is aware that their OR may be reduced by conditioning or pre-HCT chemo-radiotherapy and that premature menopause remains probable (Joshi, Savani et al. 2014).

2.3.4. Molecular targeted therapies

The field of oncology has recently entered the era of molecular targeted therapies (MTT), with the development of numerous targeted agents that inhibit various pathways responsible for the growth and survival of cancer cells. Despite their high selectivity, these agents also affect signal transduction in normal cells and tissues, causing a wide range of previously unknown on-target and off-target side effects. Whereas the fertility risk of cytotoxic agents for both men and women is well-recognized, the fertility risks and teratogenic potential associated with molecular targeted therapies are not established. Few preclinical studies have assessed the impact of MTT on fertility, and prospective clinical trials are not yet available (Lorenzi, Simonelli et al. 2016). An overview of the available data concerning gonadotoxicity of several available MTT is presented in Table i.1.

Table i.1 Impact of the various classes of molecular targeted therapies on male and female fertility. Adapted from: (Lorenzi, Simonelli et al. 2016, Walter, Xu et al. 2016).

Class and drugs of MTT	Effects on male fertility	Effects on female fertility
BCR-ABL, SCF/c-kit, and PDGFR signalling inhibitors Imatinib, nilotinib, and dasatinib	Imatinib does not impair male gonadal function. Data on nilotinib and dasatinib appear to show that gonadal function is not altered; however, the number of observations is too low for firm conclusions.	Imatinib does not impair female gonadal function. Data on nilotinib and dasatinib appear to show that gonadal function is not altered; however, the number of observations is too low for firm conclusions.
Angiogenesis inhibitors Sunitinib, sorafenib, pazopanib, bevacizumab	Preclinical findings indicate male fertility maybe only mildly compromised by treatment with sunitinib.	Preclinical findings indicate female fertility maybe only mildly compromised by treatment with sunitinib. Preclinical studies with bevacizumab have documented inhibition of maturation of ovarian follicles and a decrease/absence of *corpora lutea*.
mTOR-Inhibitors Everolimus	Animal models show a reversible reduction of fertility parameters when treated with everolimus at doses higher than those used in clinical setting.	Animal models show a reversible reduction of fertility parameters when treated with everolimus at doses higher than those used in clinical setting.
Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitors (TKIs) Erlotinib, gefitinib, lapatinib and afatinib	Pre-clinical models for EGFR TKIs, such as erlotinib and gefitinib, have shown a reduction in fertility parameters, but the effect on humans is unknown. The only clinical study of gefitinib on fertility parameters revealed suppression of androgen levels in both men and women during treatment.	Pre-clinical models for EGFR TKIs, such as erlotinib and gefitinib, have shown a reduction in fertility parameters, but the effect on humans is unknown. The only clinical study of gefitinib on fertility parameters revealed suppression of androgen levels in both men and women during treatment.

Class and drugs of MTT	Effects on male fertility	Effects on female fertility
Monoclonal Antibodies (mAb) vs EGFR/HER-2 Trastuzumab, pertuzumab, t-dm1 and cetuximab	Considering the lack of preclinical and clinical data, the effect of trastuzumab on spermatogenesis is uncertain. No data are reported on the impact of pertuzumab, t-dm1, or cetuximab on human fertility.	Considering the lack of preclinical and clinical data, the effect of trastuzumab on folliculogenesis is uncertain. No data are reported on the impact of pertuzumab, t-dm1, or cetuximab on human fertility.
ALK-inhibitors Crizotinib	Based on non-clinical safety findings, crizotinib may compromise both male and female fertility. Clinicians should monitor testosterone levels in every man who receives crizotinib, and identify those with low testosterone for discussion of the risks and benefits of testosterone therapy.	Based on non-clinical safety findings, crizotinib may compromise both male and female fertility.
PD1 – Inhibitors Pembrolizumab Nivolumab	No data available	No data available
CTLA4 – Inhibitors Ipilimumab	Preclinical studies in monkeys showed decreased testicular weights, but sperm showed no histopathological changes. A significant proportion of patients treated with ipilimumab exhibited persistent inflammation of the anterior hypophysis (11%), the portion responsible for gonadotropin production.	Preclinical studies in monkeys showed antibody binding specifically to ovary connective tissue, but no histopathological changes in ovum morphology. A significant proportion of patients treated with ipilimumab exhibited persistent inflammation of the anterior hypophysis (11%), the portion of the pituitary responsible for gonadotropin production.

Class and drugs of MTT	Effects on male fertility	Effects on female fertility
BRAF inhibitors Dabrafenib Vemurafenib	Dabrafenib has been shown to induce testicular degeneration in both male rats and dogs. Preclinical drug testing of vemurafenib in male and female rats and dogs were performed at doses significantly below the anticipated clinical exposure.	Dabrafenib has been shown to reduce corpora lutea in female rats. Preclinical drug testing of vemurafenib in male and female rats and dogs were performed at doses significantly below the anticipated clinical exposure.
MEK inhibitors Cobimetinib, trametinib	Trametinib use was not associated with testicular damage in animal studies at 13 weeks whereas cobimetinib caused testicular degeneration.	Both MEK inhibitors, cobimetinib and trametinib, have shown fertility toxicity in animal studies for female patients

Legend: ALK - anaplastic lymphoma kinase; BCR-ABL - gene formed when pieces of chromosomes 9 and 22 break off and trade places; the ABL gene from chromosome 9 joins to the BCR gene on chromosome 22, to form the BCR-ABL fusion gene; BRAF – gene that codes for the B-RAF protein; CTLA4 - Cytotoxic T-Lymphocyte Associated Protein 4; EGFR/HER-2 - Epidermal growth factor receptor/human epidermal growth factor receptor 2; MEK - mitogen-activated protein kinase; mTOR - Mammalian target of rapamycin; PD1 - Programmed cell death protein 1; PDGFR - Platelet-derived growth factor receptors; SCF/c-kit - stem cell factor/stem cell factor receptor; t-dm1 - Antibody-drug Conjugate Trastuzumab Emtansine.

2.3.5. Antineoplastic agents

All substances that inhibit or prevent the proliferation of neoplasms can be called antineoplastic agents. Mainly, these substances are cytotoxic (kill cells) or cytostatic (inhibit or prevent the proliferation of cells) (U.S. National Library of Medicine). The commonly used term of *chemotherapy* refers to the treatment of cancer with one or more antineoplastic cytotoxic agents, which mainly target the rapidly proliferating cancer cells (Sekar and Paulmurugan 2014). In modern oncology, many treatment regimens combine several chemotherapy drugs, the so called chemotherapy regimens or protocols, with the aim to maximize efficacy while minimising systemic toxicity through the delivery of lower doses (Pinto, Moreira et al. 2011).

2.3.5.1. Antineoplastic agents - Mechanisms of damage

2.3.5.1.1. Male gonadal toxicity

In males, exposure to systemic chemotherapy can cause long-term or permanent damage to the testis, the male gonads. These organs are composed of three main cell types: germ cells that develop into sperm, Sertoli cells that support and nurture developing germ cells and are also the site of production of the glycoprotein hormone inhibin, and Leydig cells that are responsible for testosterone synthesis. Germ cells that produce spermatozoa are more

sensitive to chemotherapy and radiation compared to Leydig cells that secrete testosterone, and endocrine dysfunction (e.g., testosterone reduction) only occurs in limited instances (Meistrich 2009). Alterations in sperm count include oligospermia, (i.e. a sperm density in the ejaculate of less than $20x10^6$/ml) or azoospermia (no sperm in the ejaculate). Damage to other aspects of sperm function, such as loss of motility or morphological abnormalities is less pronounced (Meistrich 2009).

The extent and reversibility of cytotoxic damage to the testes generally depends on the agent and cumulative dose received (Ginsberg 2012). In the process of spermatogenesis, the rapidly dividing differentiating spermatogonia in the early stage are much more sensitive to damage from chemotherapy than are the later stage germ cells (Figure i.2). Therefore, these later-stage germ cells progress along their differentiation pathway but in the mid-term they are not replaced by new cells that would have derived from the spermatogonia that were killed (Meistrich 2013). As a consequence of these differences in cell sensitivity, declines in sperm count may occur 1 to 2 months after exposure to antineoplastic gonadotoxic agents but azoospermia usually does not arise until after 2 months (Meistrich, Wilson et al. 1992, Meistrich, Wilson et al. 1997). When chemotherapy includes agents that do not kill stem spermatogonia, there is usually a return of normal sperm count within 12 weeks after the cessation of treatment. However, many combination antineoplastic regimens include treatment with agents that kill stem cells (e.g., alkylating agents). Even moderate doses of these agents, corresponding to partial stem cell killing, will produce azoospermia that may last for several years. After taking place, azoospermia may be transient or definitive depending on the survival of the spermatogonial stem cells, their ability to resume mitotic activity and their capacity to differentiate (Jahnukainen, Ehmcke et al. 2011). Many patients recover to normospermic levels although some may reach a plateau at oligospermia (Meistrich 2013).

Chemotherapy regimens do not have any marked effect on Leydig cell function, either in pre- or post-pubertal males (Meistrich 2009). Thus, infertility is a more common late effect of cancer therapy for male patients than impaired pubertal development or impaired sexual function. It is important to note that both pre-pubertal and pubertal testes are highly vulnerable to cytotoxic agents used in cancer therapy. In boys as in adults, alkylating agents and cisplatin are the most sterilizing agents and produce prolonged and sometimes permanent azoospermia.

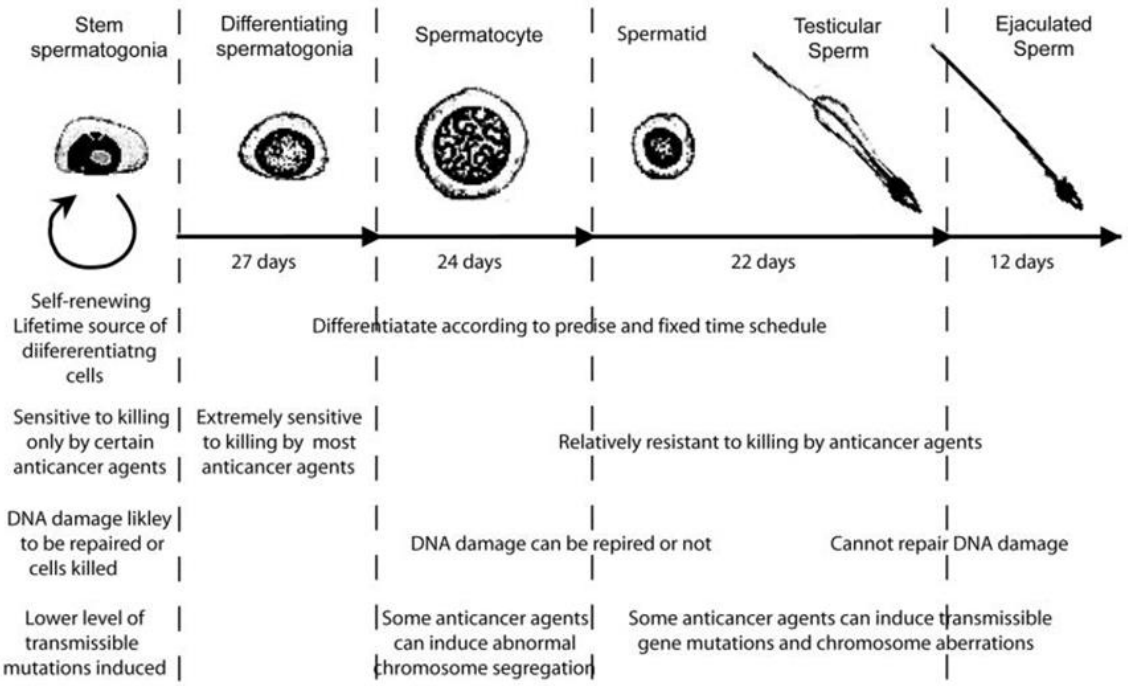

Figure i.2 Sequence of spermatogenic cells showing the cell morphology, kinetics, relative sensitivity to killing by anticancer agents, ability to accumulate and repair DNA damage, and sensitivity to induction of transmissible mutations (Meistrich 2009). *Reused with permission from John Wiley & Sons, Inc.*

In addition, toxic effects of antineoplastic therapy to Leydig cells may rarely occur, resulting in androgen insufficiency or need for testosterone replacement. When this dysfunction occurs prior to or during puberty, affected individuals will experience delayed and/or arrested pubertal maturation and lack of secondary sexual development. If the insult follows completion of normal pubertal development, observed symptoms include loss of libido, erectile dysfunction, decreased bone density and decreased muscle mass (Ginsberg 2012).

2.3.5.1.2. Female gonadal toxicity

In female patients, chemotherapeutic agents induce ovarian cell damage in a variety of ways not yet fully understood. In general, they exert their cytotoxic action by interrupting essential cell processes and arresting cellular proliferation (Fleischer, Vollenhoven et al. 2011). Nevertheless, other gonadotoxic effects involving a variety of pathophysiologic mechanisms have been identified. Chemotherapy-induced ovarian damage is not an *all or none* phenomenon, and depends on the woman's age, chemotherapeutic regimen, and existing number of primordial follicles (ovarian reserve) (Blumenfeld 2012). Also, ovarian age before treatment and genetic variability may explain some of the variation in reproductive impairment that is observed for a given treatment and chronologic age (Letourneau, Chan et al. 2013).

From what is known until now, gonadotoxicity of antineoplastic agents in ovarian function can be caused by one or more of three mechanisms (Ben-Aharon, Meizner et al. 2012, Morgan, Anderson et al. 2012, Codacci-Pisanelli, Del Pup et al. 2017), that will now be described.

I. Exhaustion of the ovarian reserve through direct damage to germ cells or somatic cells

Chemotherapy-induced premature ovarian ageing appears to result from a complex process involving both the germ and non-germ cell components of the ovary (Soleimani, Heytens et al. 2011). Ovarian follicles are composed of oocytes (germ cells) and their supporting somatic cells. They grow and develop in a process called folliculogenesis (Figure i.3), which typically leads to ovulation of one follicle approximately every 28 days, along with death of multiple other follicles. The death of ovarian follicles is called atresia, and can occur at any point during follicular development. Follicles progress from primordial to primary and then to secondary and tertiary stages prior to ovulation—with the oocyte inside the follicle remaining as a primary oocyte until right before ovulation. Primordial follicles have only a single flat layer of support cells, called granulosa cells, that surround the oocyte, and they can stay in this resting state for years—some until right before menopause (OpenStax 2013).

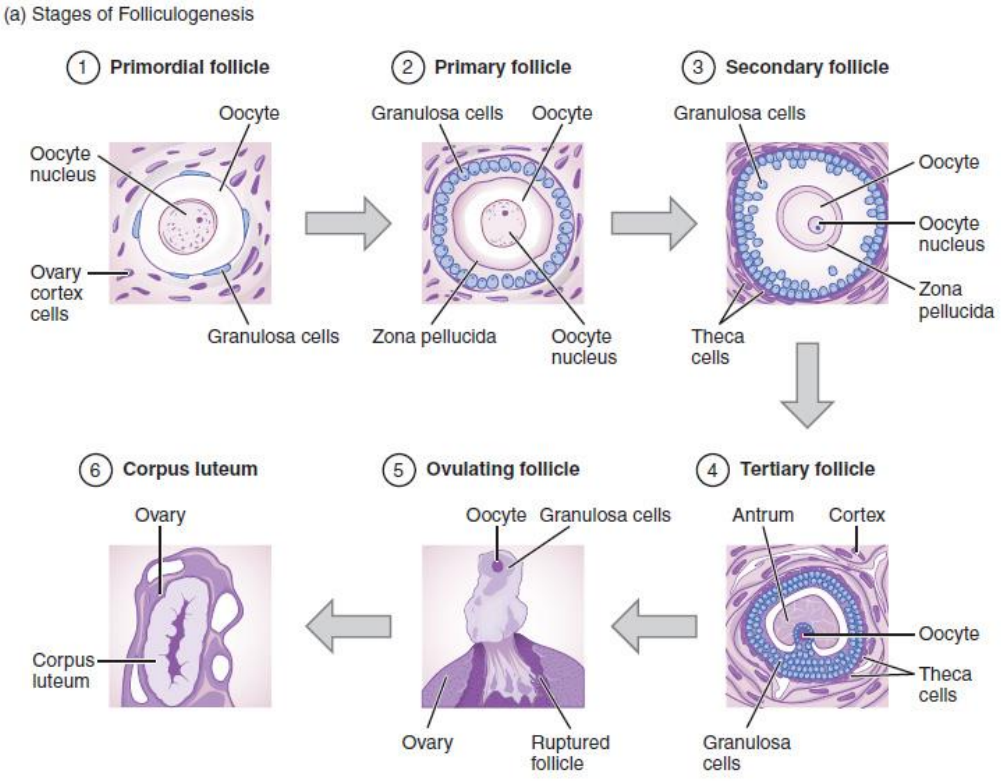

Figure i.3 Stages of follicle maturation. Accessible in https://opentextbc.ca/anatomyandphysiology/chapter/27-2-anatomy-and-physiology-of-the-female-reproductive-system/.

Oocytes and somatic cells will have different vulnerabilities to cytotoxic agents. Although rapidly growing in developing follicles, the maturing oocytes do not divide. In opposition, the somatic cells of such follicles have a high degree of proliferation (Morgan, Anderson et al. 2012). Some authors state that oocyte death by apoptosis is the main mechanism responsible for the loss of germ cells and premature ovarian failure (Bedoschi, Navarro et al. 2016) while for others it is more likely that somatic cells are the primary targets of chemotherapy drugs due to their high degree of proliferation (Morgan, Anderson et al. 2012).

It is not clear which specific stages of follicle development are more susceptible to chemotherapy-induced damage. Azarbaijani and colleagues found lower densities of intermediary, primary and secondary follicles and higher densities of atretic follicles in ovarian tissue samples from cancer patients collected after exposure to chemotherapy (Asadi Azarbaijani, Sheikhi et al. 2015). Several studies have identified toxic effects in the primordial follicle population and also in the pre-antral/antral follicles (Soleimani, Heytens et al. 2011, Morgan, Anderson et al. 2012, Yuksel, Bildik et al. 2015). In the work by *Yuksel*, using human and rat models, cyclophosphamide and cisplatin impacted both primordial and pre-antral/antral follicles whereas gemcitabine was detrimental only to pre-antral/antral follicles, suggesting that the targeted cells depend upon the specific mechanism of action of antineoplastic agents. The toxicity on the later stages of follicle development may explain the short-term effects, like temporary amenorrhea and declining AMH levels (Morgan, Anderson et al. 2012).

Concerning the specific mechanisms involved in cellular death, there is some evidence that apoptosis may be the cause of oocyte loss following chemotherapy (Perez, Knudson et al. 1997, Morita, Perez et al. 2000). Cell death by apoptosis may be mediated by DNA damage (Soleimani, Heytens et al. 2011) or reactive oxygen species and consequent oxidative stress (Tsai-Turton, Luong et al. 2007, Devine, Perreault et al. 2012, Bedoschi, Navarro et al. 2016).

II. Exhaustion of the ovarian reserve through increased follicle activation

An interesting explanation for the exhaustion of quiescent primordial follicles that are less sensitive to the effect of chemotherapy agents has been proposed (Meirow, Biederman et al. 2010). According to the “burnout” theory, reduction of the primordial pool can arise indirectly, via the loss of activated, growing follicles. Growing follicles produce factors, such as AMH which regulate the rate of follicle activation: thus acute loss of the growing follicle population is thought to result in increased recruitment of primordial follicles into the growing pool (Morgan, Anderson et al. 2012). This theory is supported by the results of several animal studies (Durlinger, Kramer et al. 1999, Kalich-Philosoph, Roness et al. 2013, Chang, Lim et al. 2015). The over-recruitment of primordial follicles might be due to a reduced production of

anti-Mullerian hormone (Durlinger, Kramer et al. 1999, Durlinger, Gruijters et al. 2002, Ben-Aharon and Shalgi 2012) or to a reduced number of LH receptors (Chang, Lim et al. 2015). The work by Chang et al demonstrated that cisplatin acts through this mechanism in mice, i.e. over-activating dormant primordial follicles by increasing the pool size of growing follicles (Chang, Lim et al. 2015).

III. Damage to the stroma or blood vessels in the ovary

The ovarian tissue is highly specialized tissue where it is possible to identify two main areas, the outer area of the cortex and the inner area of the medulla. They both consist of stroma, a unique type of highly vascular connective tissue that includes peculiar spindle-shaped stromal cells arranged into a characteristic whorled texture. Ovarian follicles are interspersed in the stroma of the ovarian cortex, while the medulla contains the larger blood vessels. Each ovarian follicle contains a single oocyte surrounded by granulosa cells that proliferate and differentiate into the theca cells, during follicle maturation.

Even if the most important cells in the ovary are the oocytes, these are supported and protected by stromal cells. Ovarian stromal tissue is susceptible to chemotherapy and local ischaemia may be a potential additional mechanism by which follicles are lost (Morgan, Anderson et al. 2012). Several studies done in ovaries previously exposed to chemotherapy have found evidence of stromal fibrosis (Meirow, Dor et al. 2007), impaired function of stromal cells (Oktem and Oktay 2007), microvascular damage (Meirow, Dor et al. 2007, Soleimani, Heytens et al. 2011). Another study also found signs of impaired ovarian blood flow and reduced ovarian size in transvaginal ultrasound (Ben-Aharon, Meizner et al. 2012). These effects appear not to be specific to a certain class of antineoplastic agents, as reported in the study by Oktem & Oktay. They showed that both alkylating and non-alkylating regimens affected ovarian stromal function in cancer patients, as quantified by oestradiol production (Oktem and Oktay 2007).

Clinically, the damage induced in ovarian cells or stroma through these mechanisms manifests as two distinct effects on ovarian function (Morgan, Anderson et al. 2012). The first is an immediate, although temporary, effect and presents as amenorrhea. This clinical outcome generally arises during treatment and results from the loss of the growing follicle population. In the best case scenario, sufficient primordial follicles remain in the ovary and the population of growing follicles will be replaced. In this case, menstruation will later resume. However, a second effect may arise if a significant proportion of primordial follicles are lost. This effect manifests as an ovarian failure and may occur shortly or several years after treatment, depending on the remaining ovarian reserve, leading to premature ovarian insufficiency and

premature menopause. So, even in women with temporary amenorrhea that recover menses after treatment, one cannot exclude the possibility of a shortened reproductive window, lower likelihood of pregnancy and risk of future infertility (Bedoschi, Navarro et al. 2016). Temporary amenorrhea at the time of treatment was identified as an indicator of early menopause (Partridge and Ruddy 2007). *Garcia et al* also found that women with regular menstrual cycles and FSH levels within the normal range presented significantly lower levels of AMH and AFC after exposure to chemotherapy compared to unexposed females of similar age (Gracia, Sammel et al. 2012).

In this context, the assessment of the impact of chemotherapy on female fertility cannot be based solely on the presence or absence of amenorrhea and markers of specific damage to the OR must be used.

2.3.5.2. Antineoplastic agents - Effects of specific agents and classes

2.3.5.2.1. Alkylating agents

The group of alkylating agents has in common the feature that they covalently bind to (alkylate) the nucleic acid bases of DNA and produce cellular death unless the damage is repaired (Colvin 2002)**.** Their cytotoxic effects are a result of reactions with DNA, mostly by interstrand cross-linking of DNA. They comprise several groups of chemically different agents, namely nitrogen mustards such as cyclophosphamide, ifosfamide, melphalan, and chlorambucil, aziridines, which are represented in current therapy by thiotepa, mitomycin C, and diaziquone, the alkyl sulfonates like busulfan, used as a component of bone marrow ablative regimens for bone marrow and stem cell transplantation, nitrosoureas such as carmustine and lomustine, used in the treatment of CNS tumours, lymphomas and myeloma, and triazenes and hydrazines, used in the treatment of Hodgkin's disease (procarbazine, dacarbazine), brain tumours (procarbazine), melanoma (dacarbazine) and glioma (temozolomide) (Colvin 2003).

Most alkylating agents, including chlorambucil, cyclophosphamide, procarbazine and melphalan, are known to produce prolonged or permanent azoospermia (Loren, Mangu et al. 2013, Meistrich 2013), especially when combined with gonadotoxic radiotherapy. The effect is dose dependent, with doses of 19 g/m^2 of cyclophosphamide and 4 g/m^2 of procarbazine, for instance, needed to produce significant long-term effects (Meistrich 2009). Also, the duration and permanence of the induced azoospermia depends on the additive effects of different agents. The nitrosoureas (carmustine, lomustine) can also cause prolonged or permanent azoospermia after treatment prior to puberty. Other alkylating agents like busulfan, ifosfamide

and nitrogen mustard and also actinomycin D, commonly used in the treatment of sarcomas, will probably also cause sustained azoospermia although they were studied only in combination with other agents known as highly sterilizing (Meistrich 2013). Regarding combination regimens, protocols commonly used in the treatment of Hodgkin's lymphoma like MOPP and BEACOPP or those for brain tumours (containing temozolomide or BCNU in association with cranial radiation) are also proven to cause prolonged or permanent azoospermia (Loren, Mangu et al. 2013).

In females, this widely used group of antineoplastic agents has proved to have extremely damaging effects on the ovary (Roness, Kalich-Philosoph et al. 2014) and is responsible for an age-adjusted odds ratio of ovarian failure of 3.98, the highest when compared with other antineoplastic agents like platinum compounds, cytotoxic antibiotics, plant alkaloids and antimetabolites (Meirow, Biederman et al. 2010). Due to their non-cell cycle specific mechanism, they may affect cells that are not actively dividing, like oocytes or primordial follicles. Destruction of follicles at all stages of development in a dose-dependent manner has been reported in preclinical studies and in human ovarian tissue as well (Ben-Aharon, Meizner et al. 2012, Morgan, Anderson et al. 2012).

Alkylating agents are recognized as the most gonadotoxic chemotherapy agents. Permanent azoospermia and ovarian failure are common effects of exposure, especially in higher doses or in regimens combining several agents or associating them with pelvic, gonadal or total body irradiation. Accordingly, alkylating agents are the only antineoplastic agents included in the *high risk* category in the risk categorization tables published by the American Society of Clinical Oncology (ASCO) in 2013 (Loren, Mangu et al. 2013), meaning that prolonged or permanent azoospermia is common and that over 70% of women will develop amenorrhea, upon exposure.

2.3.5.2.2. Antimetabolites

Antimetabolites can be defined as analogues of naturally occurring compounds that interfere with their formation or utilization, thus inhibiting essential metabolic routes. Although the enzymes inhibited by antimetabolites are also present in normal cells, some selectivity toward cancer cells is possible due to their faster division rates (Avendano and Menendez 2008). Antimetabolites can be further divided into several subclasses, including: folic acid analogues (methotrexate), purine analogues (6-mercaptopurine, fludarabine) and pyrimidine analogues (5-fluorouracil, capecitabine, cytarabine, gemcitabine) (WHO Collaborating Centre for Drug Statistics Methodology 2009).

Antimetabolites do not damage DNA and although data is limited, there are some indications that they do not impact on fertility. In males they only cause temporary reductions in sperm counts because most of them kill differentiating spermatogonia but do not appreciably affect the stem cells or their subsequent differentiation. Nevertheless, the association of some antimetabolites (e.g. cytarabine) with more gonadotoxic agents may have additive effects and cause prolonged azoospermia (Meistrich 2013).

In female breast cancer patients, the antimetabolites methotrexate and 5-fluorouracil (5-FU) in the CMF (cyclophosphamide, methotrexate and fluorouracil) regimen have not been associated with an increased rate of amenorrhea (Bines, Oleske et al. 1996). Similarly, in the study by Meirow that evaluated the occurrence of ovarian failure in 168 female cancer patients (Meirow, Biederman et al. 2010), an age-adjusted odds-ratio inferior to 1 was found for the group of antimetabolites.

2.3.5.2.3. Plant alkaloids

I. Vinca alkaloids

The Vinca alkaloids such as vincristine, vinblastine and vinorelbine, are naturally occurring or semisynthetic nitrogenous bases extracted from the plant *Catharanthus roseus*. Although they produce a wide range of biochemical effects in cells and tissues, the principal mechanisms of cytotoxicity relate to their interactions with tubulin and disruption of microtubule function, particularly of microtubules comprising the mitotic spindle apparatus, leading to metaphase arrest (Colvin 2003). This effect on the mitotic apparatus accounts for the cytotoxicity of these agents. However, other cellular functions depend on microtubular integrity as well (central nervous system function, neuromuscular transmission) and when affected account for the toxicity of these agents (Colvin 2002). Vincristine has a broad antitumor spectrum and is an important component of combination chemotherapy regimens to treat lymphocytic leukaemias, Hodgkin and non-Hodgkin lymphomas, Wilms' tumour, neuroblastoma, and rhabdomyosarcoma, multiple myeloma, lymphoblastic crisis of chronic myelogenous leukaemia, sarcomas, and small-cell lung carcinoma. Vinblastine has been an integral component of curative treatment regimens for testicular carcinoma and both Hodgkin and non-Hodgkin lymphomas. For Hodgkin lymphoma, vinblastine is often used in combination with doxorubicin, bleomycin, and dacarbazine (ABVD regimen). Vindesine is applied to treat melanoma, lung cancers, and (combined with other drugs) uterine cancers. Vinorelbine has exhibited significant antitumor activity in patients with breast cancer and lung cancer and anti-proliferation effects on osteosarcoma (Colvin 2003).

Vinca alkaloids are aneuploidy inducing, and animal studies show high levels of aneuploidy in oocytes exposed to vinblastine (Roness, Kalich-Philosoph et al. 2014). However, no increased risk of amenorrhea or ovarian failure was detected in patients treated with plant alkaloids (Meirow, Biederman et al. 2010, Zhou, Yin et al. 2010). Moreover, chemotherapy regimens for lymphoma, leukaemia, breast and lung cancer that include vincristine are classified as low risk by ASCO, meaning they have no effect on sperm production and that less than 20% of exposed women will develop post-treatment amenorrhea (Loren, Mangu et al. 2013). Nevertheless, microtubule inhibitors are reported to have additive effects when combined with more gonadotoxic agents like the alkylating drugs, causing prolonged azoospermia (Meistrich 2013).

II. Taxanes

Although taxanes equally affect microtubules, they are substantially different from the Vinca alkaloids in terms of their principal mechanisms of action, pharmacology, clinical indications, and toxicology (Colvin 2003). Paclitaxel and docetaxel are among the most important antineoplastic agents to be introduced into the clinic in the past 20 years. These agents, which cause cell cycle arrest and apoptosis consequent to microtubule stabilization, have established roles in the treatment of common cancers, such as breast, lung and ovary (Kruh 2005).

In pre-clinical studies, taxanes have shown to damage granulosa cells in the ovary and also to reduce the number of primordial follicles (Codacci-Pisanelli, Del Pup et al. 2017). In males, taxanes are identified as causing temporary reductions in sperm counts (Meistrich 2013). However, in a clinical study in male patients of reproductive age with solid tumours that were treated with docetaxel or paclitaxel, the authors found a significant decrease in serum inhibin B and testicular volume and an increase in serum FSH after completion of chemotherapy, although taxanes were combined with gemcitabine or cisplatin (Chatzidarellis, Makrilia et al. 2010).

Concerning female gonadotoxicity, the results from clinical studies are far from being consistent and, once more, the evaluation of the specific gonadotoxicity of taxanes is difficult due to their frequent use in combination regimens. Two published meta-analysis that used chemotherapy-induced amenorrhea as a clinical outcome present opposing conclusions: in the study by *Zhao* (Zhao, Liu et al. 2014), patients exposed to taxanes presented a significant increased risk (OR 1.24; p=0.02) while in the work by *Zavos* and colleagues (Zavos and Valachis 2016), treatment with taxanes was not found to be associated with a higher risk of post-treatment amenorrhea. Two other recently published studies that prospectively assessed AMH levels as a surrogate fertility marker, found that the addition of taxanes to an anthracyclines + alkylating-based regimen was significantly associated with a greater AMH decrease (p=0.007)

(Perdrix, Saint-Ghislain et al. 2017) and a lower probability of pregnancy (p=0.002) (Hamy, Porcher et al. 2016). However, in the adjuvant paclitaxel-trastuzumab (APT) trial, where women were exposed to a taxane-only CT regimen (weekly paclitaxel for 12 weeks), in association with trastuzumab, amenorrhea rates were lower than expected with standard multi-agent BC regimens (Ruddy, Guo et al. 2015).

2.3.5.2.4. Cytotoxic antibiotics and related substances

I. Anthracyclines

Anthracyclines are antibiotics that are derived from a species of fungus and have multi-modal mechanisms of action (Grant and Gourley 2015). They damage DNA by intercalating into and inhibiting the DNA–topoisomerase II complex, and generate free radicals that in turn damage cell membranes, proteins, and lipids. The prototype anthracycline is the pro-drug doxorubicin (also named adriamycin). Other drugs in the group are daunorubicin, idarubicin, epirubicin and the analogue mitoxantrone (Bardal, Waechter et al. 2011). Daunorubicin is important for induction in treating acute leukaemia. Doxorubicin and epirubicin are primary therapeutic agents in combination regimens for the treatment of lymphomas, breast cancer and other solid tumours. Idarubicin is used in the treatment of leukaemia (Colvin 2002).

Animal studies found that doxorubicin damages oocytes, directly or through granulosa cells injury (Morgan, Anderson et al. 2012, Codacci-Pisanelli, Del Pup et al. 2017) and may also affect ovarian vasculature, reducing blood flow (Ben-Aharon, Meizner et al. 2012). In male rats, testicular oxidative status of doxorubicin-treated individuals was severely compromised (Saalu, Osinubi et al. 2010) and significant and persistent damage to the endocrine and spermatogenic compartments of the testis was also described (Ward, Bardin et al. 1988). The clinical evaluation of their specific effects on fertility is difficult as they are often included in multi-agent regimens. Furthermore, the most common chemotherapy regimens for breast cancer (FEC, FAC, AC or TAC) and for non-Hodgkin lymphoma (CHOP, R-CHOP) also include the alkylating agent cyclophosphamide, recognized as one of the most gonadotoxic. In males, anthracyclines are reported to have additive effects with other antineoplastic agents causing prolonged azoospermia (Meistrich 2013). However, a retrospective clinical study found similar rates of parenthood in male and female lymphoma patients after treatment with CHOP, CHOEP or dose-dense CHOP-like regimens compared to the general population (Meissner, Tichy et al. 2014). Nonetheless, a succeeding analysis in the female group of survivors found a significant decrease in the age at menopause and low AMH levels in female survivors of non-Hodgkin lymphoma who had received CHOP or CHOEP (Meissner, Tichy et al. 2015). Other protocols for lymphoma, like ABVD are considered of low risk i.e. they are not expected to

affect sperm production and cause amenorrhea in less than 20% of exposed female patients (Loren, Mangu et al. 2013). The risk of chemotherapy-induced amenorrhea for breast cancer regimens including anthracyclines, like FEC, FAC or AC, is low or moderate, depending on their association with alkylating agents or the woman's age (Loren, Mangu et al. 2013, Zavos and Valachis 2016).

II. Bleomycin

Bleomycin is an antibiotic complex produced by fermentation from *Streptomyces verticillus*. It causes single- and double-strand DNA breaks through the formation of an intermediate iron complex. DNA synthesis, and to a lesser degree, RNA and protein synthesis are inhibited. Bleomycin is cell cycle phase-specific. This agent is used in combination with other drugs for treating many different types of cancer, such as Hodgkin's disease, lymphomas, head and neck cancers, or testicular cancer (Cancer Care Ontario-Medication Information Sheets Working Group 2012). There are no preclinical data on bleomycin's effects on fertility. As a single agent, bleomycin does not have a significant effect in male germ cells (Meistrich 2013). For combination regimens, the risk classification is dependent on the type of regimen where it is included. After treatment with the BEP protocol used for testicular cancer, permanent azoospermia is not common but may occur (Loren, Mangu et al. 2013). In the treatment of male or female's Hodgkin's lymphoma, the ABVD regimen is classified as low risk but BEACOPP is a higher risk regimen, especially in higher doses (more than 6 cycles) and older women (over 30 years) (Loren, Mangu et al. 2013), possibly due to combination with the alkylating agents procarbazine and cyclophosphamide.

2.3.5.2.5. Other antineoplastic agents

I. Platinum-based compounds

Platinum compounds (e.g., carboplatin, cisplatin, oxaliplatin) have been central to the practice of oncology for the last 40 years. They covalently bind to DNA causing intra-strand and inter-strand DNA adducts, restricting DNA replication and transcription and causing cell cycle arrest and programmed cell death (Morgan, Anderson et al. 2012). There also appears to be an effect on the intrinsic mitochondrial pathway and a component of endoplasmic reticulum stress which can both result in apoptosis (Grant and Gourley 2015). Cisplatin is an important chemotherapeutic agent for testicular cancer, including advanced forms of the disease. This agent is also effective in the treatment of ovarian, head and neck, and bladder cancers (Colvin 2002). Its analogue carboplatin is an equally effective agent, except for the treatment of germ

cell tumours. The platinum compound Oxaliplatin is particularly effective in the treatment of colorectal cancer, for which neither cisplatin nor carboplatin are beneficial (Chabner and Longo 2011).

In male patients, cisplatin may cause prolonged or permanent azoospermia in a minimum dose of 400-500 mg/m^2, especially when combined with gonadotoxic radiotherapy (Loren, Mangu et al. 2013, Meistrich 2013). In the ovary, cisplatin appears to act by targeting directly the oocyte (Morgan, Anderson et al. 2012) and platinum based compounds are classified as intermediate risk regarding effects on female fertility (Loren, Mangu et al. 2013, Roness, Kalich-Philosoph et al. 2014, Bedoschi, Navarro et al. 2016). Clinical data is, however, very limited and evidence has been published only with regard to cisplatin, showing mild to moderate rates of amenorrhea following cisplatin-based treatments (Ben-Aharon and Shalgi 2012).

2.3.6. Hormonal therapy

2.3.6.1. Tamoxifen

Tamoxifen is a selective oestrogen receptor modulator that binds to oestrogen receptors and inhibits their action on breast tissue. It is the first-line agent for premenopausal women diagnosed with early hormone-sensitive breast cancer, as it has been shown to significantly improve survival when taken daily for 5 to 10 years (Senkus, Kyriakides et al. 2015). Still, it remains unclear how tamoxifen influences ovarian toxicity (Torino, Barnabei et al. 2014). Tamoxifen is a known teratogen and women are strongly advised not to conceive during and until 2 months after treatment. Studies in rodent models have shown conflicting results regarding the effect of tamoxifen on ovarian reserve (Shandley, Spencer et al. 2017). Likewise clinical studies do not report consistent effects. In large prospective trials, the use of this drug has been significantly associated with an increased rate and/or duration of amenorrhea (Torino, Barnabei et al. 2014). Therefore, the presence of amenorrhea is an insufficient parameter to define menopausal status in patients receiving tamoxifen (Berliere, Duhoux et al. 2013). In a large cohort of breast cancer patients (Shandley, Spencer et al. 2017), patients exposed to tamoxifen were less likely to have a child but there was no evidence of decreased ovarian reserve. Moreover, no association of exposure to tamoxifen alone to an earlier age onset of menopause was found in a retrospective cohort (Chien, Duralde et al. 2015). Other large prospective studies (Su, Haunschild et al. 2014, Dezellus, Barriere et al. 2017, Trapp, Steidl et al. 2017) and a retrospective study in very young BC patients (Perdrix, Saint-Ghislain et al. 2017) have found no association of tamoxifen exposure with serum AMH levels measured 2-3 years post-treatment or timing of menses recovery. Contradictory evidence

comes from the work by Partridge (Partridge, Ruddy et al. 2010) who reported that breast cancer survivors using tamoxifen had lower AMH and AFC compared with survivors who were not using tamoxifen. However, no differences were found in the FSH levels and the small sample size precluded formal analysis.

2.3.6.2. Aromatase inhibitors (AI)

Aromatase inhibitors (AIs) block the enzyme that converts androgens to oestrogens. In premenopausal women, AI's as monotherapy are ineffective due to ongoing ovarian oestrogen production and are contra-indicated due to the suppression of peripheral aromatase that results in negative feedback to the hypothalamus which increases the secretion of GnRH and consequently stimulates ovarian function (Smyth and Hudis 2015).

For high-risk patients, which can be defined as those aged <35 or those with sufficient risk to warrant treatment with chemotherapy and who remain premenopausal following treatment, AI plus ovarian suppression (OS) is associated with a significant improvement in disease free survival over tamoxifen plus OS or tamoxifen alone (Early Breast Cancer Trialists' Collaborative Group 2015). So, the use of AIs in coordination with OS is a new adjuvant treatment option for premenopausal women with hormone-receptor-positive breast cancer and reduces the risk of recurrence (Burstein, Lacchetti et al. 2016).

The effects of AI in ovarian function have not been specifically assessed but several studies raise concerns about their use in post-treatment amenorrheic women due to reports of ovarian function recovery in patients with up to 50 years of age (Guerrero, Gavila et al. 2013, Henry, Xia et al. 2013, Krekow, Hellerstedt et al. 2016). In view of these results, it is not expected that treatment with AI would negatively affect ovarian function in breast cancer patients.

2.3.6.3. Gonadotropin-releasing hormone (GnRH) agonists

GnRH agonists act by downregulating pituitary GnRH receptors, thereby suppressing the release of luteinising hormone (LH) and follicle-stimulating hormone (FSH), which in turn reduce the main source of oestradiol production in the ovaries.

Treatment with GnRH agonists during chemotherapy has been proposed as a non-invasive, pharmacologic fertility protection method but this remains a much debated issue (Oktay and Bedoschi 2016, Oktay and Turan 2016, Donnez and Dolmans 2017, Taylan and Oktay 2017, von Wolff and Stute 2017). Although amenorrhea occurs in all patients treated with GnRH agonists, their effects in the ovarian reserve are far from being conclusive. As primordial follicles do not express FSH, LH, or GnRH receptors, many authors sustain that GnRH agonists could have no

direct effect on ovarian reserve (Oktay and Bedoschi 2016, Taylan and Oktay 2017). However, in the few available studies assessing this influence, changes in the levels of AMH are reported. In the study by Su *et al*, AMH levels increased up to 30% in healthy women after a single GnRH agonist administration. These changes in AMH occurred independently of gonadotropin levels and the authors hypothesize for a direct effect of GnRH agonists on granulosa cell expression of AMH or an indirect effect on the development and/or dynamics of the follicle pool (Su, Maas et al. 2013). On the contrary, other authors found a suppressive effect of GnRH agonist treatment in the levels of AMH of girls with precocious puberty, an effect that however reversed 6-12 months after treatment discontinuation (Hagen, Sorensen et al. 2012, Nam, Kim et al. 2017). Once again, the changes in the AMH levels did not correlate with changes in the gonadotropin or E2 levels during or after treatment (Nam, Kim et al. 2017). Only a few clinical studies have independently assessed the effects of exposure to GnRH agonists on measures of OR. In a prospective study published in 2006 (Anderson, Themmen et al. 2006, a group of nine premenopausal BC women were treated with goserelin for at least 1 year, almost always in combination with tamoxifen. The authors found that AMH levels declined in all women during treatment and until 12 months posttreatment but were only significantly different from pre-treatment levels at 6 months after treatment. On the contrary, no significant changes in either AFC or ovarian volume were found in the gonadotrophin suppression group (Anderson, Themmen et al. 2006). In a more recent work by Trapp and colleagues exposure to combined endocrine treatment (tamoxifen and OS by GnRH agonist) also resulted in significantly lower AMH levels two years after the end of chemotherapy, when compared to women without endocrine treatment. Importantly, patients that were treated with tamoxifen-only endocrine therapy had the same AMH-levels as patients who were not (Trapp, Steidl et al. 2017).

In males, therapy with GnRH agonists may be used to suppress the hypothalamic-pituitary-gonadal axis during chemotherapy in an attempt to protect the germinal epithelium. Some animal studies have suggested efficacy for this technique. However, only one of eight clinical trials was able to demonstrate protection or restoration of spermatogenesis after cytotoxic therapy (Meistrich and Shetty 2008).

2.3.6.4. Androgen suppression therapy

Androgen suppression therapies are mostly used in the context of palliative therapies in prostate cancer, which affects mainly older men. Nevertheless, there is a generally younger subpopulation in which hormone therapy can be used with curative intent, as an adjuvant treatment of prostatic radiotherapy. In these patients, this chemical castration almost

inevitably leads to azoospermia, which may become relevant if the patient has future parenting projects (Tran, Boissier et al. 2015, Almeida-Santos, Sousa et al. 2016).

2.4. Estimating the risk of infertility

In order to estimate the infertility risk in each cancer patient, several variables have to be taken into account, including patient, disease and treatment-related factors.

In males, the more important factors are treatment-related, although some cancer diseases their self are a cause of diminished sperm counts, as mentioned before. The type and dose of chemotherapy agent(s) is determinant for the occurrence of oligo or azoospermia and for the extent of effects (temporary or permanent) (Sabanegh and Ragheb 2009). In what concerns the effects of cancer treatments (radiotherapy and chemotherapy) in male germ cells, sensitivity is similar in pre-pubertal and adult patients. However, the toxic effects of radiation therapy in Leydig cells, and consequently on testosterone production, may be more pronounced in pre-pubertal boys (Meistrich 2009).

In women, age is of paramount importance due to the natural decay in the primordial follicle reserve and consequent decline in fertility, normally expected for the mid-30s (Kelsey, Anderson et al. 2012). Accordingly, older age is recognized as one of the most relevant factors for the occurrence of reduced ovarian function after cancer treatment (Overbeek, van den Berg et al. 2017). Nevertheless, this fertility decline associated with age displays a high inter-individual variability, due to the variability in the number of growing follicles within groups of women of similar age (La Marca, Grisendi et al. 2013). Like in males, infertility risk in female cancer patients is dependent on the intrinsic gonadotoxicity of each antineoplastic agent/chemotherapy regimen, their cumulative doses and treatment duration and/or the field and dose of radiation therapy. The estimation of risk is further complicated by the fact that multi-agent regimens are frequently administered, on one hand, and by the absence of data regarding male and/or female gonadotoxicity of the modern cancer treatment agents, on the other. In summary, estimating the risk of infertility in cancer patients can be a quite complex task, due to the multiplicity of disease, patient and treatment variables to consider, a high of inter-individual variability and high level of risk uncertainty for many multi-agent regimens. For instance, in the treatment of early BC, which is the most common type of female cancer and the leading cause of cancer-related deaths in young women (Ribnikar, Ribeiro et al. 2015), current clinical guidelines recommend a multitude of chemotherapy regimens combining anthracyclines, cyclophosphamide and/or taxanes in a variety of ways, complemented with hormonal therapy (tamoxifen, AI and/or GnRH agonist) in patients with hormone-responsive

tumours and/or targeted therapies (e.g. trastuzumab or pertuzumab) for those with Her2-positive tumours (Senkus, Kyriakides et al. 2015).

2.4.1. Tools for risk estimation

The American Society of Clinical Oncology includes, in the 2013 update of their guidelines on fertility preservation in cancer patients, a series of tables that categorize infertility risks of cancer treatments (Loren, Mangu et al. 2013). This categorization is made according to the risk of inducing post-treatment azoospermia, in male, or amenorrhea, in female patients. This tool is also available in a more interactive format as a web resource of the LIVESTRONG Foundation (https://www.teamlivestrong.org/we-can-help/fertility-services/risks/). In this tool to help risk estimation, treatments are classified in four levels of risk: high risk (prolonged or permanent azoospermia is common after male treatment and more than 70% of women will develop post-treatment amenorrhea), intermediate or moderate risk (prolonged or permanent azoospermia may occur after male treatment and 30-70% of women will develop post-treatment amenorrhea), low risk (treatment causes only temporary damage to sperm production and less than 20% of women will develop post-treatment amenorrhea) and very low or no risk. Besides considering the specific chemotherapy agents or regimens to which the patient is exposed, this tool takes into account other relevant patient and treatment-related variables, such as patient's age and the administered dose, for risk categorization. Concerning female risk estimation, the use of amenorrhea as the only surrogate marker for infertility is an important limitation of this risk assessment tool (as previously discussed).

Alkylating agents are the only antineoplastic agents included in the high risk category, particularly when administered in high doses, in older women or combined with pelvic, testicular, cranial or total body irradiation (Table i.2 and Table i.3). In women aged 30 to 40 years, doses of 5 g /m^2 of cyclophosphamide are categorized as causing an intermediate risk of amenorrhea. Platinum compounds, the FOLFOX (folinic acid, fluorouracil and oxaliplatin) regimen for colorectal cancer and some common BC treatment regimens are included in the intermediate risk group as well (Table i.3). In general terms, chemotherapy regimens without alkylating agents or those that use very low doses are considered of low risk. This is the case for many common treatment regimens for lymphoma and leukaemia (Table i.2 and Table i.3).

Table i.2 Effects of Different Anti-tumour Agents on Sperm Production.

Degree of Risk	Treatment Protocol	Patient and Dose Factors	Common Usage
High Risk Prolonged/permanent azoospermia common after treatment.	Any alkylating agent (e.g. busulfan, carmustine, cyclophosphamide, ifosfamide, lomustine, melphalan, procarbazine) + total body irradiation		Conditioning for HSCT for leukaemias, lymphomas, myelomas, Ewing's sarcoma, neuroblastoma
	Any alkylating agent + pelvic or testicular radiation		Sarcomas, testicular
	Total cyclophosphamide	< 7.5 g/m^2	Multiple cancers and conditioning for HSCT
	Protocols containing procarbazine: MOPP BEACOPP	> 3 cycles > 6 cycles	Hodgkin lymphoma
	Protocols containing temozolomide or BCNU + cranial radiation		Brain tumour
	Testicular radiation	> 2.5 Gy in men > 6 Gy in boys	Testicular, ALL, NHL, sarcoma, germ cell tumours
	Total Body Irradiation (TBI) doses		HSCT
	Cranial radiation	>40 Gy	Brain tumour
Intermediate Risk Prolonged/permanent azoospermia not common after treatment, but can occur	Protocols containing heavy metals: BEP Total Cisplatin Total Carboplatin	 2-4 cycles >400 mg/m^2 >2 g/m^2	Testicular

Degree of Risk	Treatment Protocol	Patient and Dose Factors	Common Usage
	Testicular radiation (due to scatter)	1-6 Gy	Wilms' tumour, neuroblastoma
Lower Risk Treatments typically cause only temporary damage to sperm production	Protocols containing non-alkylating agents (e.g., ABVD, CHOP, COP; multi-agent therapies for leukaemia)		Hodgkin lymphoma, NHL; leukaemia
	Testicular radiation	<0 .2 - 0.7 Gy	Testicular
	Anthracycline + cytarabine		AML
Very Low/No Risk No effect on sperm production	Multi-agent therapies using vincristine		Leukaemia, Lymphoma and Lung Cancer
	Radioactive iodine		Thyroid
	Testicular radiation (due to scatter)	<0.2 Gy	Multiple cancers
Unknown	Monoclonal Antibodies (e.g., Bevacizumab (Avastin), Cetuximab (Erbitux)		Colon, Non-small-cell lung Head and neck
	Tyrosine-kinase inhibitors (e.g., Erlotinib (Tarceva), Imatinib (Gleevec))		Non-small cell lung, pancreatic CML, GIST

Fertility Preservation for Patients with Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update (2013) (Loren, Mangu et al. 2013). Data Supplement # 5: Effects of Different Antitumor Agents on Sperm Production in Men
This table represents a compilation of clinical experience and publishes data about the effect of common cancer treatments on sperm production. Risks categorized for post-pubertal men, based on available literature, unless otherwise indicated by specific age range.

Table i.3 Risk of Amenorrhea in Women Treated with Modern Chemotherapy and Radiotherapy.

Degree of Risk	Treatment Protocol	Patient and Dose Factors	Common Usage
High Risk >70% of women develop amenorrhea post-treatment	Any alkylating agent (e.g. busulfan, carmustine, cyclophosphamide, ifosfamide, lomustine, melphalan, procarbazine) + total body irradiation		Conditioning for HSCT for leukaemias, lymphomas, myelomas, Ewing's sarcoma, neuroblastoma, choriocarcinoma

Degree of Risk	Treatment Protocol	Patient and Dose Factors	Common Usage
	Any alkylating agent + pelvic radiation		Sarcomas, ovarian
	Total cyclophosphamide	5 g/m^2 in women age > 40 7.5 g/m^2 in women and girls age <20	Multiple cancers: breast cancer, NHL, conditioning for HSCT
	Protocols containing procarbazine: MOPP BEACOPP	 > 3 cycles > 6 cycles	Hodgkin's lymphoma
	Protocols containing temozolomide or BCNU + cranial radiation		Brain tumour
	Whole abdominal or pelvic radiation doses	> 6 Gy in adult women > 10 Gy in post-pubertal girls > 15 Gy in pre-pubertal girls	Wilms' tumour, neuroblastoma, sarcomas, Hodgkin's lymphoma, ovarian
	Total Body Irradiation (TBI) doses		HSCT
	Cranial radiation	>40 Gy	Brain tumour
Intermediate Risk 30-70% of women develop amenorrhea post-treatment	Total cyclophosphamide	5 g/m^2 in women age 30-40	Multiple cancers, breast
	AC for breast cancer	x4 + Paclitaxel or Docetaxel in women age < 40	Breast
	FOLFOX4		Colon
	Protocols containing cisplatin		Cervical
	Abdominal/pelvic radiation	10-15 Gy in pre-pubertal girls 5-10 Gy in post-pubertal girls	Wilms' tumour, neuroblastoma, spinal tumours, brain tumour, relapsed ALL or NHL

Degree of Risk	Treatment Protocol	Patient and Dose Factors	Common Usage
Lower Risk <30% of women develop amenorrhea post-treatment	Protocols containing non-alkylating agents (e.g., ABVD, CHOP, COP; multi-agent therapies for leukaemia)		Hodgkin's and non-Hodgkin's lymphoma, leukaemia
	Protocols for breast cancer containing cyclophosphamide (e.g., CMF, CEF, or CAF)	Women < 30	Breast
	Anthracycline + cytarabine		AML
Very Low/No Risk Negligible - no effects on menses	Multi-agent therapies using vincristine		Leukaemia, Lymphoma, Breast and Lung Cancer
	Radioactive iodine		Thyroid
Unknown	Monoclonal Antibodies (e.g., Bevacizumab (Avastin), Cetuximab (Erbitux), Trastuzumab (Herceptin))		Colon, Non-small-cell lung Head and neck, Breast
	Tyrosine-kinase inhibitors (e.g., Erlotinib (Tarceva), Imatinib (Gleevec)		Non-small cell lung, pancreatic CML, GIST

Fertility Preservation for Patients with Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update (2013). (Loren, Mangu et al. 2013). Data Supplement #6: Risks of Permanent Amenorrhea in Women Treated With Modern Chemotherapy and Radiotherapy

This table represents a compilation of clinical experience and published data about the effect of common cancer treatments on menstruation. Other measures of reproductive capacity such as hormone levels, follicle counts and pregnancy outcomes are not reflected in this table.

Risks categorized for post-pubertal women, based on available literature, unless otherwise indicated by specific age range.

As mentioned, the risk categorization in female cancer patients is based solely on the presence or absence of post-treatment amenorrhea. Currently, no tool is available that categorizes the risk of infertility associated with cancer treatments according to their relative impact in hormonal and/or ultrasound measures like Anti-Mullerian Hormone or Antral Follicle Count which are, as already discussed, much more reliable markers of the ovarian reserve. Published data on this impact has been steadily increasing and some attempts of systematization have been made, with breast cancer patients being the most common focus (Peigne and Decanter 2014, Freour, Barriere et al. 2017). However, we are still far from having a similar risk calculator that organizes agents/regimens in risk categories or that compares the damage induced by different regimens for the same type of cancer.

Researchers have also tried to identify possible risk factors or variables – and corresponding cut-off values – that would predict the occurrence of female infertility after cancer treatment. Patient-related factors, like age and pre-treatment OR levels (as assessed by indirect measures like AMH levels), and treatment factors (like the dose of alkylating agents), have been identified and used to develop risk prediction tools or nomograms. However, none of these tools includes both patient and treatment-associated variables, which may limit their reliability. Furthermore, besides the exposure to alkylating agents, no other treatment-related factors are considered. Despite their limitations, these tools may be an additional support for infertility risk estimation, namely risk of post-treatment oligo/amenorrhea (Anderson, Rosendahl et al. 2013, D'Avila, Biolchi et al. 2015), probability of maintaining post-treatment ovarian activity (Barnabei, Strigari et al. 2015) or the estimated time until return of ovarian function (Su, Haunschild et al. 2014). The University of Pittsburgh has developed a *fertility risk calculator* (available online at http://fertilitypreservationpittsburgh.org/fertility-resources/fertility-risk-calculator/) based on the work from *Green et al* (Green, Nolan et al. 2014) that classifies infertility risk based on the exposure to, and equivalent dose of, alkylating agents.

3. Overcoming the effects of cancer and cancer treatments in human fertility

There are various options that may enable patients to prevent damage on fertility, or coping with that damage, in consequence of cancer treatments. The first line of action must be at the pre-treatment setting, when wider possibilities for fertility preservation and/or protection are available, allowing patients to have a *plan B* for future natural parenthood. For instance, techniques like ovarian transposition or gametes cryopreservation may allow for fertility protection or preservation and should be performed before exposure to gonadotoxic agents takes place, to achieve maximum efficacy. During treatment, patients may still have the option to perform a conservative surgery or to shield their gonads from radiation. Finally, after treatment is completed and if damage has occurred, assisted reproduction techniques (ART) might be helpful to achieve conception but only if the damage was partial or fertility preservation (embryo and/or gametes cryopreservation) was previously performed. If this is not the case patients can only become parents by using sperm, oocytes or embryos from donors or through child adoption. Importantly, many of these techniques for fertility protection or preservation are complementary or even synergistic and can be performed in association, for risk minimization and maximum success rates. For instance, ovarian stimulation for oocyte cryopreservation can be combined with cryopreservation of ovarian

tissue to increase success rates (Huober-Zeeb, Lawrenz et al. 2011, Dolmans, Marotta et al. 2014, Hourvitz, Yerushalmi et al. 2015).

The various options for fertility protection/preservation available at each time point are described in more detail below.

3.1. Options for fertility protection/preservation (FP) before cancer treatments

3.1.1. Male

3.1.1.1. Sperm cryopreservation

This FP technique can be used in post-pubertal male adolescents and adults. Sperm samples, obtained by masturbation or electro-ejaculation, are cryopreserved. Later, when the patient wishes, sperm can be used for embryo breeding, by intrauterine insemination (IUI), *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI)[1] .

Pregnancy rates are variable and depend, among other factors, on the technique of fertilization used. There are centres reporting pregnancy rates of 40 to 50% per cycle, with the use of cryopreserved sperm from cancer survivors and using IVF/ICSI techniques (van Casteren, van Santbrink et al. 2008, Selk, Belej-Rak et al. 2009). The collection and storage of spermatozoa for fertility preservation in male cancer patients of post-pubertal age is a simple, safe and well-established procedure.

It is recommended that three sperm collections should be performed, with a minimum of 48 hours of abstinence between each collection. Therefore, despite being a simple and quick procedure, patients should be referenced in a timely manner to maximize success rates (Almeida-Santos, Sousa et al. 2016).

3.1.1.2. Testicular sperm or testicular tissue cryopreservation

In adult males this technique is an alternative to sperm cryopreservation when ejaculation is not possible or the ejaculated semen does not contain sperm. Samples of testicular tissue are obtained through biopsy and then prepared and cryopreserved. After

[1] Intrauterine insemination (IUI) - sperm is placed into the uterus when the woman is ovulating and fertilization occurs in the uterus; *In Vitro* Fertilization (IVF) – the ovaries are stimulated to produce oocytes which are then fertilized in the laboratory using sperm from the male partner or from a donor; Intracytoplasmic Sperm Injection (ICSI) – refinement of the IVF technique in which the sperm is inserted directly into the oocyte.

finishing cancer treatments, the samples may be thawed and the spermatozoa used for fertilization by ICSI.

In pre-pubertal children and adolescents, cryopreservation of testicular tissue is the only option for preservation of fertility. However, at present this is an experimental technique with no successful clinical experience reported. Nevertheless, the possibility to perform the transplantation of cryopreserved / thawed tissue or the *in vitro* maturation of the spermatozoa is foreseen, in the near future (Dohle 2010). Success rates, although highly variable, are generally lower than those obtained with the use of cryopreserved sperm. Nevertheless, there are reports of pregnancy rates of 40% and 55.8% per cycle, using cryopreserved/thawed testicular sperm (Ulug, Bener et al. 2005, Kalsi, Thum et al. 2011). Concerning the cryopreservation of immature testicular tissue, the investigation is still limited to preclinical studies.

3.1.2. Female

3.1.2.1. Embryo cryopreservation

Embryo cryopreservation is a well-established procedure that can be used to preserve fertility in women of reproductive age with an available partner (or using donor sperm). This technique comprises an initial phase of hormonal stimulation, followed by follicular aspiration for collection of oocytes and posterior insemination by IVF or ICSI. The embryos obtained are then cryopreserved. When the couple so wishes, the embryos are thawed and transferred to the woman's uterus. A pregnancy rate of 35.6% per cycle in women with up to 35 years of age is described (Westphal and Massie 2012). As the woman's age increases, the probability of getting a pregnancy significantly decreases. Embryo cryopreservation requires a male partner or use of donor sperm, which raises ethical and legal concerns about the fate of the orphan embryos if the patient dies or if she and her partner separate (Donnez and Dolmans 2017). Also, it should be pointed out that cryopreserved embryos are joint property of the woman and her male partner in most countries, which might be an issue when they come to be used a number of years later (Donnez and Dolmans 2013).

3.1.2.2. Oocyte cryopreservation

In a similar way to embryo cryopreservation, the preservation of fertility through cryopreservation of oocytes comprises an initial phase of hormonal stimulation, followed by follicular aspiration and cryopreservation of the retrieved oocytes by vitrification (fast-freezing method). Oocyte vitrification has significantly improved their survival, fertilization rates and

proportion of high quality embryos as compared to slow freezing, and can even produce results comparable to fresh oocytes (Cobo and Diaz 2011). When the woman so desires, the preserved gametes are thawed and fertilized using IVF or ICSI techniques. The embryos obtained are then transferred to the woman's uterus.

Cryopreservation of mature oocytes has become an established technique in 2012 (Practice Committees of American Society for Reproductive Medicine and Society for Assisted Reproductive Technology 2013) and can circumvent the main concerns associated with embryo storage, preserving a woman's ability to procreate with a chosen partner in the future (Donnez and Dolmans 2017). Also, this technique is an option for patients who do not have a male partner, do not wish to use donor sperm, or have religious or ethical objections to embryo freezing (Loren, Mangu et al. 2013).

The birth rate is significantly dependent on the number of vitrified oocytes and the woman's age (Donnez and Dolmans 2017). Kato has summarized the live births reported in cancer patients who preserved their fertility by oocyte cryopreservation and have returned for pregnancy. In a series of 12 cases, the percentage of live births per slow-frozen/ thawed or vitrified/warmed oocyte varied between 4.5 and 33.3% (Kato 2016). Repeating the procedure in multiple cycles is advantageous as more oocytes can be cryopreserved, potentially leading to greater success rates.

In cancer patients, one of the main concerns related to embryo or oocyte cryopreservation is the need to postpone treatment initiation for several weeks. However, with the use of more flexible ovarian stimulation protocols for oocyte collection, timing no longer depends on the menstrual cycle in most cases, and stimulation can be initiated with less delay compared with old protocols. Oocyte harvesting for the purpose of oocyte or embryo cryopreservation is now possible on a cycle day–independent schedule (Loren, Mangu et al. 2013). Other significant concern is the exposure of patients with oestrogen-sensitive malignancies (usually breast cancer) to increased oestrogen levels, during ovarian stimulation. To minimize this risk, ovarian stimulation protocols using the aromatase inhibitor letrozole have been developed and long term prospective data do not indicate increased risk of breast cancer recurrence (Kim, Turan et al. 2016).

3.1.2.3. Ovarian tissue cryopreservation

Cryopreservation of ovarian tissue is still considered an experimental procedure, although experts are anticipating that it will become labelled as non-experimental in a short time, due to cumulative evidence for restoration of ovarian function and spontaneous pregnancies after orthotopic transplantation (Martinez and International Society for Fertility Preservation–

ESHRE–ASRM Expert Working Group 2017, Oktay, Harvey et al. 2018). This technique remains the only option for fertility preservation in pre-pubertal girls and in women who cannot delay the start of chemotherapy.

A fragment from or the whole ovary is collected by laparoscopic surgery. The ovarian tissue is properly prepared and fragments of the cortex are isolated and cryopreserved. When the woman so desires, the fragments are thawed and may be grafted onto the remaining ovary (orthotopic transplantation) or to another location (heterotopic transplantation). There is an advantage in removing the whole ovary as it allows for more tissue to be cryopreserved and the repetition of the transplantation, if the first graft is not successful (Rosendahl, Schmidt et al. 2011). After transplantation, the ovarian tissue may restore endocrine function and fertility, enabling a natural conception (Dolmans, Jadoul et al. 2013, Schmidt, Nyboe Andersen et al. 2013). It is not possible to calculate the success rate of ovarian tissue transplantation since the total number of performed transplants is not known. However, several centres worldwide have reported cryopreservation and subsequent ovarian transplantation experiments, with promising results. In a series of cases described by Donnez in 2013 (Donnez, Dolmans et al. 2013), a total of 60 patients underwent orthotopic ovarian tissue transplant and 11 were able to become pregnant. Moreover, the majority (93%) of these patients recovered ovarian function. In a Belgian centre with over 15 years of experience, 582 cryopreservations of ovarian tissue were performed of which resulted 11 successful transplants and five live births, four of which as a result of natural conception (Dolmans, Jadoul et al. 2013). In patients using this FP technique the total number of live births reported as of June 2017 has exceeded 130, worldwide (Donnez and Dolmans 2017). After transplantation, maintenance of ovarian function is dependent on the amount of transplanted tissue and on the age of the woman at the time of the procedure. On average, the ovarian remains functional for four to five years (Donnez and Dolmans 2013) but there were cases where it persisted for more than 7 years (Andersen, Silber et al. 2012).

Regarding the theoretical risk of cryopreserving, and subsequently transplanting, ovarian tissue with neoplastic cells, several histological techniques like PCR (Polymerase Chain Reaction), flow cytometry and xenotransplantation, that help to exclude such possibility, are being developed. Nevertheless, the recently updated guideline on fertility preservation from ASCO (Oktay, Harvey et al. 2018) states that further investigation is needed to confirm whether it is safe in patients with leukaemia. The transplantation of isolated follicles after *in vitro* maturation or through the "artificial ovary" may help overcome this risk in the future (Donnez and Dolmans 2013). A successful birth report resulting from *in vitro* maturation of oocytes obtained after oophorectomy in a patient with ovarian cancer has been published

(Prasath, Chan et al. 2014). The risk of ovarian metastases according to the type of cancer has been categorized: it is high in situations of leukaemia, neuroblastoma and Burkitt's lymphoma; moderate for stage IV or lobular subtype breast cancer, colon cancer, adenocarcinoma of the uterine cervix, non-Hodgkin's lymphoma and Ewing's sarcoma; and low for BC in stages I-II or the ductal subtype, squamous cell carcinoma of the cervix, Hodgkin's disease, osteogenic carcinoma, non-genital rhabdomyosarcoma and Wilms' tumour (Dolmans, Luyckx et al. 2013).

3.1.2.4. Ovarian transposition

Oophoropexy is a surgical procedure that relocates the ovaries away from the radiation field and can be considered in all patients for which pelvic irradiation is planned. At the conclusion of treatment, the ovaries are returned to their original position to allow return of reproductive function. Success rates range from 60 – 89% and are largely dependent on patient age, radiation dose, site, type of treatment and whether or not chemotherapy is also integrated in the treatment plan. Transposition should be performed just before radiation therapy to prevent ovarian remigration (Hudson, Stanley et al. 2017).

3.1.2.5. Conservative surgery

Whenever possible, conservative gynaecologic surgeries like trachelectomy for cervical carcinoma (surgical removal of the cervix while preserving the uterus) should be performed to preserve female fertility.

3.1.2.6. Other potential protection options

Agents which can prevent or attenuate the toxic effects of cancer treatments on female fertility would provide significant advantages over the existing FP techniques, and would allow patients to retain their natural fertility without the necessity for costly, invasive and risky procedures (Roness, Kashi et al. 2016). Moreover, as the evidence to support the effectiveness of GnRH agonists in female fertility preservation is still not conclusive, other preventive strategies to reduce the gonadotoxic effects need to be developed (Donnez and Dolmans 2017). Preliminary studies have identified a number of agents acting on the molecular pathways involved in the cellular response to cytotoxic treatments which could prevent or reduce ovarian follicle loss (Table i.4). Future advances in this area will require the demonstration of the non-interference of these agents with the anti-cancer activity of the chemotherapy drugs and the conduction of well-designed robust clinical studies that use adequate reproductive outcomes (Roness, Kalich-Philosoph et al. 2014).

Another potential approach for the development of agents that protect fertility (fertoprotective agents) is the study of new drug formulations such as drug encapsulation in nanoparticles or liposomes, in order to reduce their toxic effects. This strategy has already led to the development of pegylated-doxorubicin that has proved to be less cardiotoxic and a recent study examined the possibility that nano-encapsulation of arsenic trioxide (used in the treatment of hematologic cancers) would increase its efficacy, whilst reducing the toxic effects on the ovary. In a murine lymphoma model, this new formulation was much less deleterious to ovarian function than the parent drug (Ahn, Barrett et al. 2013).

Table i.4 Characterization of agents with potential to prevent or reduce ovarian follicle loss. Adapted from (Roness, Kashi et al. 2016).

General mechanism	Agent	Specific mechanism	Outcomes	Type of study
Agents preventing over-recruitment of primordial follicles	**AS101**	Modulation of the PI3K/PTEN/Akt pathway	Reduced loss of primordial follicles; reduced apoptosis in granulosa cells of growing follicles; improved reproductive outcomes	Preclinical (mice)
	Anti-Mullerian Hormone	Negative regulation of follicle activation	Increased number of primordial follicles in AMH-treated animals than in controls	Preclinical (mice)
Agents inhibiting follicle apoptosis	**Imatinib**	c-Abl kinase inhibitor in the apoptotic pathway	Reduced primordial follicle loss, improved fertility and reproductive outcomes	Preclinical (mice)
	Sphingosine-1-phosphate	Inhibitor of the ceramide-promoted apoptotic pathway	Reduced follicle apoptosis; preservation of both primordial and growing follicles	Preclinical (human ovarian tissue xenografts in mice)

General mechanism	Agent	Specific mechanism	Outcomes	Type of study
Agents inhibiting vascular effects	**Granulocyte colony-stimulating factor (G-CSF)**	*Not yet identified*	Reduced destruction of primordial follicles; preventing damage to the micro-vessels, reducing DNA damage in growing follicles	Preclinical (mice)
Other	**Bortezomib**	Prevents doxorubicin nuclear accumulation	Prevented doxorubicin-induced DNA damage and the activation of apoptotic pathways	Preclinical (mice)
	Retrovirus-mediated transduction	Reduces the uptake of chemotherapy into granulosa cells	Increased granulosa cell survival following treatment with either doxorubicin or paclitaxel	Preclinical (*in vitro* cell lines)

Legend: PI3K/PTEN/Akt - Phosphatidylinositol 3-kinase/ Phosphatase and tensin homolog/v-akt murine thymoma viral oncogene homolog.

3.2. Options for fertility protection or preservation during cancer treatments

3.2.1. Ovarian suppression

It is hypothesized that GnRH agonists reduce ovarian toxicity by down-regulating the secretion of FSH and LH from the pituitary and consequently creating a hypo-gonadotropic milieu, in which follicular recruitment is inhibited and fewer primordial follicles attain the chemotherapy-sensitive stages of proliferation and follicle maturation (Blumenfeld and von Wolff 2008). However, the efficacy of this non-invasive, pharmacologic fertility protection method remains a much debated issue (Oktay and Bedoschi 2016, Oktay and Turan 2016, Donnez and Dolmans 2017, Taylan and Oktay 2017, von Wolff and Stute 2017). Many new results have recently come to light, including a randomized controlled trial with a 5 year follow-up in lymphoma patients (Demeestere, Brice et al. 2016) and several meta-analyses (Del Mastro, Ceppi et al. 2014, Elgindy, Sibai et al. 2015, Lambertini, Ceppi et al. 2015, Munhoz, Pereira et al. 2016) but conclusions remain conflicting. Some authors believe that the opposing results may not be contradictory as long they are interpreted in different perspectives (von Wolff and Stute 2017). Some of these recent studies favour the use of GnRH agonist as they

have effectively increased the rate of menses recovery (Munhoz, Pereira et al. 2016) and reduced the risk of Premature Ovarian Insufficiency (Del Mastro, Ceppi et al. 2014, Lambertini, Ceppi et al. 2015) while other studies have found no evidence of benefit (Vitek, Shayne et al. 2014, Elgindy, Sibai et al. 2015, Demeestere, Brice et al. 2016). Nevertheless, substantial weaknesses in conducted trials are pointed (Oktay and Bedoschi 2016, Taylan and Oktay 2017) and substantial heterogeneity is acknowledged in the published meta-analysis (Del Mastro, Ceppi et al. 2014, Munhoz, Pereira et al. 2016). The debate is far from ending: some authors still very reluctant in accepting a protective effect of GnRH agonists in OR, as primordial follicles do not express gonadotropin or GnRH receptors (Taylan and Oktay 2017) whereas others rose the hypothesis that GnRH agonists display a differential protective effect on fertility, depending upon the specific chemotherapy-induced mechanism of ovarian injury (Hasky, Uri-Belapolsky et al. 2015). Controversy is also present in published clinical guidelines: the oldest, and eventually outdated, from ASRM (Practice Committee of American Society for Reproductive Medicine 2013) and ESMO (Peccatori, Azim et al. 2013) do not recommend the use of GnRH agonists (at least without considering additional FP options), while some of the most recent guidelines recommend their use for FP, although they do not entirely agree on the proposed indications (Coates, Winer et al. 2015, Lambertini, Del Mastro et al. 2016, Lambertini, Cinquini et al. 2017). The most recently updated guideline on FP from ASCO (Oktay, Harvey et al. 2018) states that evidence is conflicting and does not recommend this FP strategy unless other proven methods (such as oocyte, embryo, or ovarian tissue cryopreservation) are not feasible, and exclusively in young women with BC.

3.2.2. Radiation shielding of reproductive organs

Gonadal and/or uterus shielding during radiation therapy is also recommended as a standard option for FP (Lee, Schover et al. 2006). Use of shielding will reduce the dose of radiation therapy delivered to the testis or to female reproductive organs (uterus, ovaries).

3.2.3. Administration of less gonadotoxic chemotherapy regimens

As previously detailed, the gonadotoxicity of chemotherapy is related with the specific effects of the antineoplastic agents being used. It is recognized that the group of alkylating agents, especially in higher doses or in regimens combining more than one agent, are associated with the higher risk of post-treatment azoospermia and amenorrhea (Meirow, Biederman et al. 2010, Ben-Aharon and Shalgi 2012, Loren, Mangu et al. 2013). The less gonadotoxicity of CT regimens without alkylating agents is evident in the risk categorization tables published by ASCO, where for instance the BEACOPP regimen (bleomycin, etoposide, doxorubicin,

cyclophosphamide, vincristine, procarbazine and prednisone) and the ABVD regimen (doxorubicin [adriamycin], bleomycin, vinblastine, and dacarbazine), both for treatment of Hodgkin lymphoma, are included in the higher risk and lower risk categories, respectively. Accordingly, the results of a large study assessing fertility outcomes in Hodgkin lymphoma survivors concluded that chemotherapy-induced gonadal toxicity was highest after six to eight cycles of escalated BEACOPP(-14) in both female and male survivors, as evidenced by reduced ovarian reserve and amenorrhea in the majority of women, and a relevant impairment of spermatogenesis in the majority of men (Behringer, Mueller et al. 2013). In the case of breast cancer, almost all first-line regimens include cyclophosphamide but limited results from the APT trial, where patients were treated with the adjuvant paclitaxel-trastuzumab regimen, reveal a lower amenorrhea rate as compared to the usual rate after the standard anthracycline-alkylating regimens (Ruddy, Guo et al. 2015). In the setting of breast cancer, more research is needed that compare the various available regimens in terms of their impact on ovarian reserve markers and on the incidence of POI.

3.3. Options for parenthood after cancer treatments

After cancer treatment, patients may attempt to conceive and/or get pregnant naturally, unless they have medical contraindications. However, they are advised to wait six months to two years (male patients) or a minimum of six months (female patients), after the end of treatment to minimize the risk of genetic mutations in the available sperm or oocytes. If their reproductive function is significantly affected, they can attempt to produce offspring by using their cryopreserved embryos, oocytes or sperm in the setting of assisted reproduction techniques (ART) like IUI, IVF or ICSI or by transplanting their ovarian tissue. Not all patients will have the opportunity to preserve their gametes before treatment nor will all patients want to undergo such procedures. In these circumstances, and if natural conception and/or pregnancy is not possible, other parenthood options are available. For couples in which the male has no sperm in the ejaculate, the TESE/TESA[2] procedures (Testicular/Epididymal Sperm Aspiration or Extraction) may sometimes be able to extract sperm cells directly from the testicular tissue. These sperm cells can then be used with ICSI to create embryos and achieve a pregnancy. If the male has become infertile, there is also the option of using sperm from a donor and perform IUI or IVF. For couples in which the female is no longer able of producing oocytes, they can also be obtained from donors and used to achieve pregnancy. Gestational

[2] Testicular Sperm Aspiration (TESA) - A needle is inserted in the testicle and tissue/sperm are aspirated. Testicular Sperm Extraction (TESE) - involves making a small incision in the testis and examining the tubules for the presence of sperm which is then aspirated.

surrogacy is an option for women who do not want to or cannot carry out a pregnancy, for instance when the uterus has been severely damaged by radiotherapy. The embryo is produced through assisted reproduction techniques, using oocyte and sperm from the couple and then transferred to the surrogate mother's uterus. The child is the genetic offspring of the couple. If both elements of the couple are infertile, there is still the possibility for them to be parents by using embryos from another couple (donor) and, if needed, a gestational carrier. Adoption is an additional viable option that can be considered by anyone seeking parenthood. Importantly, these options always have to be considered in the light of specific country laws and regulations and may sometimes raise significant ethical issues.

4. Providing support for an informed and shared decision-making process in the context of (in)fertility risk in cancer patients

4.1. Fertility preservation counselling as a shared decision-making process

Shared decision making (SDM) is a process whereby health professionals and patients work together to make healthcare choices. Informed shared decisions are shared by doctor and patient and informed by the best evidence, not only about risks and benefits but also patient specific characteristics and values (Towle and Godolphin 1999). SDM is most useful for decisions in which there is more than one medically reasonable option, and the choice of which option is best for a given patient depends on his/her preferences and values. When patients are more involved in health decisions, they are more likely to experience confidence in and satisfaction with treatment decisions along with increased trust in their care providers (Kane, Halpern et al. 2014). Also, outcomes of care and adherence to treatment regimens improve (Towle and Godolphin 1999).

In the process of SDM, four steps can be distinguished (Stiggelbout, Pieterse et al. 2015):

1. The professional informs the patient that a decision is to be made and that the patient's opinion is important;
2. The professional explains the options and their *pros* and *cons*;
3. The professional and the patient discuss the patient's preferences and the professional supports the patient in deliberation;
4. The professional and the patient discuss the patient's wish to make the decision, they make or defer the decision, and discuss follow-up.

Therefore, in order to make or participate in a "good" decision, patients should be well informed about their treatment options, including the risks, benefits, and uncertainties

associated with each option (including choosing not to get treatment at a certain time) (Kane, Halpern et al. 2014). Several patient-related factors contribute to a quality decision-making process, including patients' values and attitudes, support from family and friends and the acquisition of information (Michie, Dormandy et al. 2002, Woolf, Chan et al. 2005). In addition, the knowledge and recommendations from the health professional are essential to SDM (Makoul and Clayman 2006). Health professionals are involved in every step of the decision-making process, from identifying that a decision needs to be made, presenting the evidence and counselling the patient to implement a strategy with which both parties feel comfortable (Legare and Thompson-Leduc 2014).

In the cancer setting, several factors have been pointed by the Institute of Medicine (IOM) to justify the need of a patient-centred approach and shared decision making (Committee on Improving the Quality of Cancer Care: Addressing the Challenges of an Aging, Board on Health Care et al. 2013):

1. Cancer care can be extremely complex, and patients' treatment choices have serious implications for their health outcomes and quality of life;
2. Evidence supporting many decisions in cancer care is limited or incomplete;
3. Individuals differ in how they weigh the trade-offs of different choices.

Decisions regarding FP, especially for female cancer patients, are complex and preference-sensitive i.e. the best strategy for an individual is unclear (O'Connor, Legare et al. 2003) and there is a need to consider patients' values for benefits and harms plus the scientific uncertainties across options. Such conditions are ideal for SDM (Elwyn, O'Connor et al. 2009). The first and most important step for SDM in preference sensitive decisions is explaining to the patient that there is no best choice, that a decision has to be made and that doing nothing or keeping the *status quo* is also an option (Stiggelbout, Van der Weijden et al. 2012). After having laid out the options, the next step is to discuss the benefits and harms of each, as well as their respective probabilities of success. Next, patients' ideas, concerns, and expectations about the options, their benefits, and their harms should be explained, and the patient should be supported in the process of deliberation (O'Connor, Legare et al. 2003).

All FP options come with risks and success rates and, very often, decisions concerning FP have to be made in a short time frame (between diagnosis and start of the chemotherapy treatment), along with other treatment decisions, in a period of a great emotional distress for the patients. Although this decision-making process occurs in a particularly difficult context, after a recent cancer diagnosis, patients recognize the importance of being able to play an active role in this decision (Ussher, Parton et al. 2018), in a context particularly marked by lack of control. According to a published systematic review investigating the impact of FP

counselling on overall psychological outcomes in women with cancer, FP counselling is perceived as critical, regardless of age or parity. Furthermore, it reduces long-term regret and dissatisfaction concerning fertility and is associated with improved physical quality of life. Moreover, the possibility of FP was instrumental to improved coping (Deshpande, Braun et al. 2015). While counselling by the oncology team is vital, additional benefit is seen patients are referred to a reproductive endocrinologist and have access to the opportunity to preserve fertility (Letourneau, Ebbel et al. 2012). Other study in Australian cancer patients concludes that discussion with a health care professional about fertility concerns, and satisfaction with the discussion, was associated with lower patient distress, greater knowledge and understanding of the consequences of cancer/cancer treatments on fertility, involvement in the decision making process about FP, and satisfaction with health care (Ussher, Parton et al. 2018).

The FP decision-making process may be influenced by a wide range of factors, including the provision and timing of FP information and internal factors such as the women's overestimation of the risks associated with pursuing FP (Jones, Hughes et al. 2017). A study conducted in the Netherlands, reinforce these results: FP decision-making in young cancer women was found to be based mainly on weighing two issues: the intensity of the wish to conceive a child in the future and the expected burden of undergoing FP treatment (Baysal, Bastings et al. 2015).

4.2. Information strategies in fertility preservation counselling

The need of female cancer patients for prompt, standardized, and written information addressing specific oncofertility issues is now an evidence (Deshpande, Braun et al. 2015). Several studies reported that patients' who have access to specialized information concerning FP have an improved knowledge of the available options (Garvelink, ter Kuile et al. 2014) and fewer decisional conflicts (Peate, Meiser et al. 2011). A web-based, anonymous survey sent to young adult female cancer survivors also found that patients with limited awareness or knowledge of their risk for premature menopause and FP options reported higher levels of decisional conflict about future FP (Benedict, Thom et al. 2016).

Various studies confirm that information resources conceived to support decisions in healthcare such as decision aids (Stacey, Samant et al. 2008, Stacey, Bennett et al. 2011) or decision trees (Hunink, Weinstein et al. 2014) provide a more clear understanding of the available options, facilitate discussions with healthcare professionals and increase patients' and professionals' involvement in the decision-making process. Patient decision aids (DA) are defined by the *International Patient Decision Aid Standards* (IPDAS) *Collaboration* as ''tools

designed to help people participate in decision making about health care options. They provide information about the options, and help patients to construct, clarify, and communicate the personal values they associate with the different features of the options. Furthermore, they provide structured guidance in the steps of decision making (International Patient Decision Aid Standards (IPDAS) Collaboration 2017). A well-designed DA must be able to: provide up-to-date and accurate information about the health condition, the treatment options, the potential benefits and harms associated with each option and corresponding probabilities, and any uncertainties; help patients to clarify the value they place on different health care outcomes; and offer structure and guidance for the decision-making steps (Kane, Halpern et al. 2014). DA differ from traditional health education materials by focusing on the treatment decision and the personalized patient connection with the treatment options being considered. A recent review of the published evidence concludes that patients feel more knowledgeable, better informed, and clearer about their values, and they probably have a more active role in decision making and more accurate risk perceptions, when exposed to DA as compared to usual care (Stacey, Legare et al. 2017). Various studies report very positive results on the development and evaluation of DA conceived to support FP decisions, whether in print format (Peate, Meiser et al. 2011, Peate, Meiser et al. 2012) or electronically available (Garvelink, ter Kuile et al. 2013, Garvelink, ter Kuile et al. 2014, Woodard, Hoffman et al. 2018). In general, patients and health care professionals assessed DA as relevant, understandable and useful sources of information. These tools may be presented in printed, electronic or video format but decision-support websites are increasingly used as they are more interactive and allow for individualized information (Cox, Wysham et al. 2015).

Decision trees are another type of decision tool that describe the path of patient decision-making, portraying the consequences of each separate decision, and the multiple ways in which a patient may arrive at the final outcome. They have proven to help patients accurately weigh the outcomes associated with a given decision, and lead to more informed clinical judgments (Aleem, Jalal et al. 2009). Decision trees can also be used to help health care providers to communicate in a systematic and logical way. Decision trees for FP options have been developed by an interdisciplinary team of researchers at the Northwestern University (Gardino, Jeruss et al. 2010). These tools are intended for providers to use when counselling female and male cancer patients and provide a visual map of the sequence of events that a patient must undergo. Each tool captures the unique aspects and options for female and male patients and special attention is paid to each “decision point” in which the patient can change the course of his/her trajectory.

4.3. Oncofertility information needs

Several international cancer care organizations such as ESMO and ASCO, state that healthcare providers caring for adult and pediatric patients with cancer should address the possibility of infertility as early as possible before treatment starts. Moreover, they must be prepared to refer potential patients to reproductive medicine specialists. To preserve the full range of options, FP approaches should also be discussed as early as possible (Peccatori, Azim et al. 2013, Oktay, Harvey et al. 2018). However, FP services remain underutilised in many developed countries: the proportion of eligible women who undergo some type of FP is of 12% at the most, and sperm banking also remains an underused procedure, despite being simple, effective and medically uncomplicated (Schover, van der Kaaij et al. 2014).

In accordance with these numbers, several international studies indicate that not every oncologist is following these orientations (Schover, Brey et al. 2002, Quinn, Vadaparampil et al. 2007, Quinn, Vadaparampil et al. 2009, Forman, Anders et al. 2010, Adams, Hill et al. 2013, Shimizu, Bando et al. 2013). In addition, a recently published systematic review of qualitative and quantitative studies confirms that oncofertility support is often not delivered to the standard of current guidelines (Logan, Perz et al. 2018). Moreover, this author found that fertility discussions and the use of FP services are negatively influenced by a variety of factors including patient-related (e.g. low infertility risk, pre-pubertal status, female gender, poor prognosis, urgency to initiate treatment), clinician-related (medical specialties like surgery, low clinician knowledge and training) and institutional-related factors like time constraints. In our country, the results of a locally applied questionnaire to a sample of 37 oncologists from two hospitals in the central region, have pointed to significant information needs, with most oncologists highly rating the importance of receiving more information on several topics concerning both infertility risks and FP options (Silva, Almeida-Santos et al. 2015). Concerning other barriers that could impact FP practices of Portuguese oncologists, published data indicates that the barriers “communication skills” and “patient-related factors” were related to a lower frequency of informing about both the risk of cancer-related infertility and about FP (Melo, Fonseca et al. 2018).

In addition, when we look at the published studies assessing cancer patients’ needs, the results corroborate the mentioned gaps in care with regard to fertility issues. Around 50% of patients report not to have received fertility information or engaged in discussions about FP with their doctors (Logan, Perz et al. 2018, Ussher, Parton et al. 2018), although it seems to exist a trend towards increasing provision of fertility information (Logan, Perz et al. 2018). The results of several surveys of reproductive-age cancer patients and survivors disclose gaps in the information received about the opportunity of preserving fertility, the options available or the

possibility of consultation with a reproductive medicine specialist (Schover, Brey et al. 2002, Partridge, Gelber et al. 2004, Peate, Meiser et al. 2009, Wilkes, Coulson et al. 2010). Moreover, an online survey to 217 North American and Canadian young adult cancer patients and survivors demonstrate a high demand (65.7%) for information and assistance regarding FP options but also shows that these needs were unmet in 40–50% of the patients inquired (Zebrack 2008). Another study in four oncology clinics in the USA (n=104) found that one-third of young pre-menopausal women with cancer were dissatisfied with the quality and length of oncologist discussions about the impact of cancer treatment on reproductive health. Furthermore, this work concludes that in women aged over 40 years counselling on the effect of treatment on fertility was inadequate (Scanlon, Blaes et al. 2012). A large study in Sweden, in which 484 survivors completed a postal questionnaire, also shows marked sex differences regarding the receipt of fertility-related information and use of FP resources, with more pronounced gaps in female counselling (Armuand, Rodriguez-Wallberg et al. 2012). Regarding information strategies, female cancer patients aged 18-45 years reporting decisional conflict with regard to FP (n=155), rated specialised websites and leaflets as the most helpful decision-support tools (Muller, Urech et al. 2017). Evidence from a number of studies also indicates the need for evidence-based, straightforward written information materials. Patients prefer to be informed after diagnosis and prior to treatment initiation (Logan, Perz et al. 2018).

4.4. National and international recommendations

The first published guideline concerning FP in cancer patients was published more than ten years ago, in 2006, by the *American Society of Clinical Oncology*. Since then, several international cancer, reproductive medicine and fertility preservation societies and organizations have published guidance and recommendations on this subject.

In Europe, both the *European Society of Medical Oncology* (ESMO) and the *European Menopause and Andropause Society* (EMAS) have published guidelines in 2013 (Peccatori, Azim et al. 2013, Mintziori, Lambrinoudaki et al. 2014), including very similar recommendations concerning FP. Additionally, the *Ferti*-PROTEKT network, a physicians and biologists network specialized in FP based in Germany, Austria and parts of Switzerland, has recently updated its recommendations, which are now more practically orientated with a great emphasis on topics relevant for decision support (Schuring, Fehm et al. 2018, von Wolff, Germeyer et al. 2018). In an attempt to address remaining controversial issues, to detail aspects concerning FP techniques and to update the recommendations from ESMO and ASCO, other guidelines have also been published by international consensus of European and North-

American societies (Martinez and International Society for Fertility Preservation–ESHRE–ASRM Expert Working Group 2017) and expert meetings (Lambertini, Del Mastro et al. 2016).

In the USA, the American Society for Reproductive Medicine (ASRM) has also given important contributions for the definition of FP guidelines, complementing those from ASCO with more scientific, technical and ethical perspectives on FP (Ethics Committee of American Society for Reproductive 2013, Practice Committee of American Society for Reproductive Medicine 2013, Practice Committees of American Society for Reproductive Medicine and Society for Assisted Reproductive Technology 2013). It was also an initiative of this society, jointly with the Society for Assisted Reproductive Technology, to withdraw, in 2012, the experimental label from the technique of oocyte cryopreservation (Practice Committee of American Society for Reproductive Medicine 2013). This document was endorsed in 2014 by the American College of Obstetricians and Gynaecologists (ACOG). An international meeting that joined experts from ASRM, the European Society for Human Reproduction and Embryology (ESHRE) and the International Society of Fertility Preservation (ISFP), held in Barcelona, in 2015, also delivered recommendations on several topics concerning FP, with a special focus on indications, current outcomes and future perspectives (Martinez and International Society for Fertility Preservation–ESHRE–ASRM Expert Working Group 2017).

In Portugal, it was not until recently that national recommendations on FP in cancer patients were published (Almeida-Santos, Sousa et al. 2016). This document was an outcome of two previous national oncofertility meetings in 2015 and 2016, organized by the Portuguese Society of Reproductive Medicine (SPMR). The Portuguese recommendations were reviewed and endorsed by several national medical societies, namely the SPMR, the Portuguese Oncology Society (SPO), the Portuguese Haematology Society (SPH), the Portuguese Andrology, Sexual Medicine and Reproduction Society (SPAMSR) and the Portuguese Gynaecology Society (SPG). The Portuguese Recommendations for the Preservation of the Reproductive Potential in Cancer Patients (full contents in Chapter II of the results) present the general indications, contraindications and clinical recommendations for protection and preservation of the reproductive potential, in general terms and according to specific types of cancer and types of cancer treatment. In detail, it is recommended that every post-pubertal male patient collects and cryopreserves sperm and that all women who wish to preserve their reproductive potential should be referred to a reproductive medicine service, before the initiation of any systemic treatment. Specific clinical recommendations are made for preservation of the reproductive potential in the more incident cancer types in adolescent and young adult patients.

These documents and their recommendations can be used as additional decision support tools for healthcare professionals involved in cancer care and reproductive medicine.

References

Adams, E., E. Hill and E. Watson (2013). "Fertility preservation in cancer survivors: a national survey of oncologists' current knowledge, practice and attitudes." Br J Cancer **108**(8): 1602-1615.

Agarwal, A. and S. S. Allamaneni (2005). "Disruption of spermatogenesis by the cancer disease process." J Natl Cancer Inst Monogr(34): 9-12.

Ahn, R. W., S. L. Barrett, M. R. Raja, J. K. Jozefik, L. Spaho, H. Chen, M. B. Bally, A. P. Mazar, M. J. Avram, J. N. Winter, L. I. Gordon, L. D. Shea, T. V. O'Halloran and T. K. Woodruff (2013). "Nano-encapsulation of arsenic trioxide enhances efficacy against murine lymphoma model while minimizing its impact on ovarian reserve in vitro and in vivo." PLoS One **8**(3): e58491.

Aleem, I. S., H. Jalal, I. S. Aleem, A. A. Sheikh and M. Bhandari (2009). "Clinical decision analysis: Incorporating the evidence with patient preferences." Patient Prefer Adherence **3**: 21-24.

Almeida-Santos, A. T., G. Sousa, A. Teixeira, P. Cardoso, C. Melo, A. Teixeira, J. Andrade, C. Silva, E. Gouveia, I. Bravo, I. Augusto, J. Magalhães, N. Louro, B. Pereira, R. Ramalho, V. Patrício and S. Pinelo (2016). "Recommendations for the preservation of the reproductive potential in cancer patients." Revista Portuguesa de Oncologia **2**(I): 5-24.

American Cancer Society. (2016, June 28, 2017). "How Cancer Treatments Can Affect Fertility in Men." Retrieved January 2018, from https://www.cancer.org/treatment/treatments-and-side-effects/physical-side-effects/fertility-and-sexual-side-effects/fertility-and-men-with-cancer/how-cancer-treatments-affect-fertility.html.

American Cancer Society. (2016, June 28, 2017). "How Cancer Treatments Can Affect Fertility in Women." Retrieved January 2018, from https://www.cancer.org/treatment/treatments-and-side-effects/physical-side-effects/fertility-and-sexual-side-effects/fertility-and-women-with-cancer/how-cancer-treatments-affect-fertility.html.

Anders, C. K., R. Johnson, J. Litton, M. Phillips and A. Bleyer (2009). "Breast cancer before age 40 years." Semin Oncol **36**(3): 237-249.

Andersen, C. Y., S. J. Silber, S. H. Bergholdt, J. S. Jorgensen and E. Ernst (2012). "Long-term duration of function of ovarian tissue transplants: case reports." Reprod Biomed Online **25**(2): 128-132.

Anderson, R. A., M. Rosendahl, T. W. Kelsey and D. A. Cameron (2013). "Pretreatment anti-Mullerian hormone predicts for loss of ovarian function after chemotherapy for early breast cancer." Eur J Cancer **49**(16): 3404-3411.

Anderson, R. A., A. P. Themmen, A. Al-Qahtani, N. P. Groome and D. A. Cameron (2006). "The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer." Hum Reprod **21**(10): 2583-2592.

Anderson, R. A. and W. H. Wallace (2013). "Antimullerian hormone, the assessment of the ovarian reserve, and the reproductive outcome of the young patient with cancer." Fertil Steril **99**(6): 1469-1475.

Anserini, P., S. Chiodi, S. Spinelli, M. Costa, N. Conte, F. Copello and A. Bacigalupo (2002). "Semen analysis following allogeneic bone marrow transplantation. Additional data for evidence-based counselling." Bone Marrow Transplant **30**(7): 447-451.

Armuand, G. M., K. A. Rodriguez-Wallberg, L. Wettergren, J. Ahlgren, G. Enblad, M. Hoglund and C. Lampic (2012). "Sex differences in fertility-related information received by young adult cancer survivors." J Clin Oncol **30**(17): 2147-2153.

Asadi Azarbaijani, B., M. Sheikhi, I. C. Oskam, M. Nurmio, T. Laine, H. Tinkanen, S. Makinen, T. G. Tanbo, O. Hovatta and K. Jahnukainen (2015). "Effect of Previous Chemotherapy on the Quality of Cryopreserved Human Ovarian Tissue In Vitro." PLoS One **10**(7): e0133985.

Auger, J., N. Sermondade and F. Eustache (2016). "Semen quality of 4480 young cancer and systemic disease patients: baseline data and clinical considerations." Basic Clin Androl **26**: 3.

Avendano, C. and J. C. Menendez (2008). Medicinal Chemistry of Anticancer Drugs, Elsevier B.V.

Barbosa D, Sousa AP and Almeida-Santos T (2015). Sperm cryopreservation in cancer patients. Unpublished work, FMUC.

Bardal, S., J. Waechter and D. Martin (2011). Applied Pharmacology. Anthracyclines, Elsevier Inc. **Chapter 20. Neoplasia**.

Barnabei, A., L. Strigari, P. Marchetti, V. Sini, L. De Vecchis, S. M. Corsello and F. Torino (2015). "Predicting Ovarian Activity in Women Affected by Early Breast Cancer: A Meta-Analysis-Based Nomogram." Oncologist **20**(10): 1111-1118.

Barnhart, K. T. (2014). "Live birth is the correct outcome for clinical trials evaluating therapy for the infertile couple." Fertil Steril **101**(5): 1205-1208.

Barton, S. E., J. S. Najita, E. S. Ginsburg, W. M. Leisenring, M. Stovall, R. E. Weathers, C. A. Sklar, L. L. Robison and L. Diller (2013). "Infertility, infertility treatment, and achievement of pregnancy in female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study cohort." Lancet Oncol **14**(9): 873-881.

Baysal, O., L. Bastings, C. C. Beerendonk, S. A. Postma, J. IntHout, C. M. Verhaak, D. D. Braat and W. L. Nelen (2015). "Decision-making in female fertility preservation is balancing the expected burden of fertility preservation treatment and the wish to conceive." Hum Reprod **30**(7): 1625-1634.

Bedoschi, G., P. A. Navarro and K. Oktay (2016). "Chemotherapy-induced damage to ovary: mechanisms and clinical impact." Future Oncol **12**(20): 2333-2344.

Behringer, K., H. Mueller, H. Goergen, I. Thielen, A. D. Eibl, V. Stumpf, C. Wessels, M. Wiehlputz, J. Rosenbrock, T. Halbsguth, K. S. Reiners, T. Schober, J. H. Renno, M. von Wolff, K. van der Ven, M. Kuehr, M. Fuchs, V. Diehl, A. Engert and P. Borchmann (2013). "Gonadal function and fertility in survivors after Hodgkin lymphoma treatment within the German Hodgkin Study Group HD13 to HD15 trials." J Clin Oncol **31**(2): 231-239.

Ben-Aharon, I., I. Meizner, T. Granot, S. Uri, N. Hasky, S. Rizel, R. Yerushalmi, A. Sulkes and S. M. Stemmer (2012). "Chemotherapy-induced ovarian failure as a prototype for acute vascular toxicity." Oncologist **17**(11): 1386-1393.

Ben-Aharon, I. and R. Shalgi (2012). "What lies behind chemotherapy-induced ovarian toxicity?" Reproduction **144**(2): 153-163.

Benedict, C., B. Thom, N. F. D, D. Diotallevi, M. P. E, J. R. N and J. F. Kelvin (2016). "Young adult female cancer survivors' unmet information needs and reproductive concerns contribute to decisional conflict regarding posttreatment fertility preservation." Cancer **122**(13): 2101-2109.

Berliere, M., F. P. Duhoux, F. Dalenc, J. F. Baurain, L. Dellevigne, C. Galant, A. Van Maanen, P. Piette and J. P. Machiels (2013). "Tamoxifen and ovarian function." PLoS One **8**(6): e66616.

Bines, J., D. M. Oleske and M. A. Cobleigh (1996). "Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer." J Clin Oncol **14**(5): 1718-1729.

Blumenfeld, Z. (2012). "Chemotherapy and fertility." Best Pract Res Clin Obstet Gynaecol **26**(3): 379-390.

Blumenfeld, Z. and M. von Wolff (2008). "GnRH-analogues and oral contraceptives for fertility preservation in women during chemotherapy." Hum Reprod Update **14**(6): 543-552.

Braakhekke, M., E. I. Kamphuis, E. A. Dancet, F. Mol, F. van der Veen and B. W. Mol (2014). "Ongoing pregnancy qualifies best as the primary outcome measure of choice in trials in reproductive medicine: an opinion paper." Fertil Steril **101**(5): 1203-1204.

Broekmans, F. J., D. de Ziegler, C. M. Howles, A. Gougeon, G. Trew and F. Olivennes (2010). "The antral follicle count: practical recommendations for better standardization." Fertil Steril **94**(3): 1044-1051.

Broekmans, F. J., M. R. Soules and B. C. Fauser (2009). "Ovarian aging: mechanisms and clinical consequences." Endocr Rev **30**(5): 465-493.

Burstein, H. J., C. Lacchetti, H. Anderson, T. A. Buchholz, N. E. Davidson, K. E. Gelmon, S. H. Giordano, C. A. Hudis, A. J. Solky, V. Stearns, E. P. Winer and J. J. Griggs (2016). "Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update on Ovarian Suppression." J Clin Oncol **34**(14): 1689-1701.

Cancer Care Ontario-Medication Information Sheets Working Group. (2012). "Bleomycin Medication Information Sheet." Retrieved January 2018, from https://www.cancercareontario.ca/en/node/44211.

Chabner, B. A. and D. L. Longo (2011). Cancer Chemotherapy and Biotherapy: Principles and Practice, Lippincott Williams & Wilkins.

Chang, E. M., E. Lim, S. Yoon, K. Jeong, S. Bae, D. R. Lee, T. K. Yoon, Y. Choi and W. S. Lee (2015). "Cisplatin Induces Overactivation of the Dormant Primordial Follicle through PTEN/AKT/FOXO3a Pathway which Leads to Loss of Ovarian Reserve in Mice." PLoS One **10**(12): e0144245.

Chatzidarellis, E., N. Makrilia, L. Giza, E. Georgiadis, C. Alamara and K. N. Syrigos (2010). "Effects of taxane-based chemotherapy on inhibin B and gonadotropins as biomarkers of spermatogenesis." Fertil Steril **94**(2): 558-563.

Chien, A. J., E. Duralde, R. Hwang, K. Tsung, C. N. Kao, H. S. Rugo, M. E. Melisko, L. J. Esserman, P. N. Munster, M. Cedars, K. Kerlikowske, C. E. McCulloch and M. P. Rosen (2015). "Association of tamoxifen use and ovarian function in patients with invasive or pre-invasive breast cancer." Breast Cancer Res Treat **153**(1): 173-181.

Chow, E. J., K. L. Stratton, W. M. Leisenring, K. C. Oeffinger, C. A. Sklar, S. S. Donaldson, J. P. Ginsberg, L. B. Kenney, J. M. Levine, L. L. Robison, M. Shnorhavorian, M. Stovall, G. T. Armstrong and D. M. Green (2016). "Pregnancy after chemotherapy in male and female survivors of childhood cancer treated between 1970 and 1999: a report from the Childhood Cancer Survivor Study cohort." Lancet Oncol **17**(5): 567-576.

Coates, A. S., E. P. Winer, A. Goldhirsch, R. D. Gelber, M. Gnant, M. Piccart-Gebhart, B. Thurlimann and H. J. Senn (2015). "Tailoring therapies--improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015." Ann Oncol **26**(8): 1533-1546.

Cobo, A. and C. Diaz (2011). "Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials." Fertil Steril **96**(2): 277-285.

Codacci-Pisanelli, G., L. Del Pup, M. Del Grande and F. A. Peccatori (2017). "Mechanisms of chemotherapy-induced ovarian damage in breast cancer patients." Crit Rev Oncol Hematol **113**: 90-96.

Colvin, M. (2003). Holland-Frei Cancer Medicine. P. R. Kufe DW, Weichselbaum RR, et al, BC Decker.

Colvin, O. M. (2002). Encyclopedia of Cancer. J. R. Bertino, Elsevier.

Committee on Improving the Quality of Cancer Care: Addressing the Challenges of an Aging, P., S. Board on Health Care and M. Institute of (2013). Patient-centered communication and Shared decision making Delivering High-Quality Cancer Care: Charting a New Course for a System in Crisis. L. Levit, E. Balogh, S. Nass and P. A. Ganz. Washington (DC), National Academies Press (US)

Cox, C. E., N. G. Wysham, B. Walton, D. Jones, B. Cass, M. Tobin, M. Jonsson, J. M. Kahn, D. B. White, C. L. Hough, C. L. Lewis and S. S. Carson (2015). "Development and usability testing of a Web-based decision aid for families of patients receiving prolonged mechanical ventilation." Ann Intensive Care **5**: 6.

D'Avila, A. M., V. Biolchi, E. Capp and H. Corleta (2015). "Age, anti-mullerian hormone, antral follicles count to predict amenorrhea or oligomenorrhea after chemotherapy with cyclophosphamide." J Ovarian Res **8**: 82.

Del Mastro, L., M. Ceppi, F. Poggio, C. Bighin, F. Peccatori, I. Demeestere, A. Levaggi, S. Giraudi, M. Lambertini, A. D'Alonzo, G. Canavese, P. Pronzato and P. Bruzzi (2014). "Gonadotropin-releasing hormone analogues for the prevention of chemotherapy-induced premature ovarian failure in cancer women: systematic review and meta-analysis of randomized trials." Cancer Treat Rev **40**(5): 675-683.

Demeestere, I., P. Brice, F. A. Peccatori, A. Kentos, J. Dupuis, P. Zachee, O. Casasnovas, E. Van Den Neste, J. Dechene, V. De Maertelaer, D. Bron and Y. Englert (2016). "No Evidence for the Benefit of Gonadotropin-Releasing Hormone Agonist in Preserving Ovarian Function and Fertility in Lymphoma Survivors Treated With Chemotherapy: Final Long-Term Report of a Prospective Randomized Trial." J Clin Oncol **34**(22): 2568-2574.

Deshpande, N. A., I. M. Braun and F. L. Meyer (2015). "Impact of fertility preservation counseling and treatment on psychological outcomes among women with cancer: A systematic review." Cancer **121**(22): 3938-3947.

Devine, P. J., S. D. Perreault and U. Luderer (2012). "Roles of reactive oxygen species and antioxidants in ovarian toxicity." Biol Reprod **86**(2): 27.

Dewailly, D., C. Y. Andersen, A. Balen, F. Broekmans, N. Dilaver, R. Fanchin, G. Griesinger, T. W. Kelsey, A. La Marca, C. Lambalk, H. Mason, S. M. Nelson, J. A. Visser, W. H. Wallace and R. A. Anderson (2014). "The physiology and clinical utility of anti-Mullerian hormone in women." Hum Reprod Update **20**(3): 370-385.

Dezellus, A., P. Barriere, M. Campone, C. Lemanski, L. Vanlemmens, L. Mignot, T. Delozier, C. Levy, C. Bendavid, M. Debled, T. Bachelot, C. Jouannaud, C. Loustalot, M. A. Mouret-Reynier, A. Gallais-Umbert, D. Masson and T. Freour (2017). "Prospective evaluation of serum anti-Mullerian hormone dynamics in 250 women of reproductive age treated with chemotherapy for breast cancer." Eur J Cancer **79**: 72-80.

Dillon, K. E., M. D. Sammel, M. Prewitt, J. P. Ginsberg, D. Walker, J. E. Mersereau, Y. Gosiengfiao and C. R. Gracia (2013). "Pretreatment antimullerian hormone levels determine rate of posttherapy ovarian reserve recovery: acute changes in ovarian reserve during and after chemotherapy." Fertil Steril **99**(2): 477-483.

Dohle, G. R. (2010). "Male infertility in cancer patients: Review of the literature." Int J Urol **17**(4): 327-331.

Dolleman, M., W. M. Verschuren, M. J. Eijkemans, M. E. Dolle, E. H. Jansen, F. J. Broekmans and Y. T. van der Schouw (2013). "Reproductive and lifestyle determinants of anti-Mullerian hormone in a large population-based study." J Clin Endocrinol Metab **98**(5): 2106-2115.

Dolmans, M. M., P. Jadoul, S. Gilliaux, C. A. Amorim, V. Luyckx, J. Squifflet, J. Donnez and A. Van Langendonckt (2013). "A review of 15 years of ovarian tissue bank activities." J Assist Reprod Genet **30**(3): 305-314.

Dolmans, M. M., V. Luyckx, J. Donnez, C. Y. Andersen and T. Greve (2013). "Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue." Fertil Steril **99**(6): 1514-1522.

Dolmans, M. M., M. L. Marotta, C. Pirard, J. Donnez and O. Donnez (2014). "Ovarian tissue cryopreservation followed by controlled ovarian stimulation and pick-up of mature oocytes does not impair the number or quality of retrieved oocytes." J Ovarian Res **7**: 80.

Donnez, J. and M. M. Dolmans (2013). "Fertility preservation in women." Nat Rev Endocrinol **9**(12): 735-749.

Donnez, J. and M. M. Dolmans (2017). "Fertility Preservation in Women." N Engl J Med **377**(17): 1657-1665.

Donnez, J., M. M. Dolmans, A. Pellicer, C. Diaz-Garcia, M. Sanchez Serrano, K. T. Schmidt, E. Ernst, V. Luyckx and C. Y. Andersen (2013). "Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation." Fertil Steril **99**(6): 1503-1513.

Durlinger, A. L., M. J. Gruijters, P. Kramer, B. Karels, H. A. Ingraham, M. W. Nachtigal, J. T. Uilenbroek, J. A. Grootegoed and A. P. Themmen (2002). "Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary." Endocrinology **143**(3): 1076-1084.

Durlinger, A. L., P. Kramer, B. Karels, F. H. de Jong, J. T. Uilenbroek, J. A. Grootegoed and A. P. Themmen (1999). "Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary." Endocrinology **140**(12): 5789-5796.

Early Breast Cancer Trialists' Collaborative Group (2015). "Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials." Lancet **386**(10001): 1341-1352.

Elgindy, E., H. Sibai, A. Abdelghani and M. Mostafa (2015). "Protecting Ovaries During Chemotherapy Through Gonad Suppression: A Systematic Review and Meta-analysis." Obstet Gynecol **126**(1): 187-195.

Elwyn, G., A. M. O'Connor, C. Bennett, R. G. Newcombe, M. Politi, M. A. Durand, E. Drake, N. Joseph-Williams, S. Khangura, A. Saarimaki, S. Sivell, M. Stiel, S. J. Bernstein, N. Col, A. Coulter, K. Eden, M. Harter, M. H. Rovner, N. Moumjid, D. Stacey, R. Thomson, T. Whelan, T. van der Weijden and A. Edwards (2009). "Assessing the quality of decision support technologies using the International Patient Decision Aid Standards instrument (IPDASi)." PLoS One **4**(3): e4705.

Ethics Committee of American Society for Reproductive, M. (2013). "Fertility preservation and reproduction in patients facing gonadotoxic therapies: a committee opinion." Fertil Steril **100**(5): 1224-1231.

Fleischer, R. T., B. J. Vollenhoven and G. C. Weston (2011). "The effects of chemotherapy and radiotherapy on fertility in premenopausal women." Obstet Gynecol Surv **66**(4): 248-254.

Forman, E. J., C. K. Anders and M. A. Behera (2010). "A nationwide survey of oncologists regarding treatment-related infertility and fertility preservation in female cancer patients." Fertil Steril **94**(5): 1652-1656.

Freour, T., P. Barriere and D. Masson (2017). "Anti-mullerian hormone levels and evolution in women of reproductive age with breast cancer treated with chemotherapy." Eur J Cancer **74**: 1-8.

Gardino, S. L., J. S. Jeruss and T. K. Woodruff (2010). "Using decision trees to enhance interdisciplinary team work: the case of oncofertility." J Assist Reprod Genet **27**(5): 227-231.

Garvelink, M. M., M. M. ter Kuile, M. J. Fischer, L. A. Louwe, C. G. Hilders, J. R. Kroep and A. M. Stiggelbout (2013). "Development of a Decision Aid about fertility preservation for women with breast cancer in The Netherlands." J Psychosom Obstet Gynaecol **34**(4): 170-178.

Garvelink, M. M., M. M. ter Kuile, A. M. Stiggelbout and M. de Vries (2014). "Values clarification in a decision aid about fertility preservation: does it add to information provision?" BMC Med Inform Decis Mak **14**: 68.

Gerstl, B., E. Sullivan, A. Ives, C. Saunders, H. Wand and A. Anazodo (2018). "Pregnancy Outcomes After a Breast Cancer Diagnosis: A Systematic Review and Meta-analysis." Clin Breast Cancer **18**(1): e79-e88.

Ginsberg, J. P. (2012). "Gonadotoxicity of Cancer Therapies in Pediatric and Reproductive-Age Males." 15-23.

Gosiengfiao, Y. and V. Gomez-Lobo. (2015). "Assessing Ovarian Reserve." Retrieved January 2018, from https://oncofertility.northwestern.edu/resources/assessing-ovarian-reserve.

Gracia, C. R., M. D. Sammel, E. Freeman, M. Prewitt, C. Carlson, A. Ray, A. Vance and J. P. Ginsberg (2012). "Impact of cancer therapies on ovarian reserve." Fertil Steril **97**(1): 134-140 e131.

Grant, C. H. and C. Gourley (2015). Cancer Treatment and the Ovary R. A. Anderson and N. Spears, Elsevier Inc.

Green, D. M., T. Kawashima, M. Stovall, W. Leisenring, C. A. Sklar, A. C. Mertens, S. S. Donaldson, J. Byrne and L. L. Robison (2009). "Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study." J Clin Oncol **27**(16): 2677-2685.

Green, D. M., V. G. Nolan, P. J. Goodman, J. A. Whitton, D. Srivastava, W. M. Leisenring, J. P. Neglia, C. A. Sklar, S. C. Kaste, M. M. Hudson, L. R. Diller, M. Stovall, S. S. Donaldson and L. L. Robison (2014). "The cyclophosphamide equivalent dose as an approach for quantifying alkylating agent exposure: a report from the Childhood Cancer Survivor Study." Pediatr Blood Cancer **61**(1): 53-67.

Guerrero, A., J. Gavila, E. Folkerd, B. Ortiz, F. Martinez, A. Garcia, M. A. Climent, V. Guillem and A. Ruiz (2013). "Incidence and predictors of ovarian function recovery (OFR) in breast cancer (BC) patients with chemotherapy-induced amenorrhea (CIA) who switched from tamoxifen to exemestane." Ann Oncol **24**(3): 674-679.

Gunnes, M. W., R. T. Lie, T. Bjorge, S. Ghaderi, E. Ruud, A. Syse and D. Moster (2016). "Reproduction and marriage among male survivors of cancer in childhood, adolescence and young adulthood: a national cohort study." Br J Cancer **114**(3): 348-356.

Gyurkocza, B. and B. M. Sandmaier (2014). "Conditioning regimens for hematopoietic cell transplantation: one size does not fit all." **124**(3): 344-353.

Hagen, C. P., K. Sorensen, R. A. Anderson and A. Juul (2012). "Serum levels of antimullerian hormone in early maturing girls before, during, and after suppression with GnRH agonist." Fertil Steril **98**(5): 1326-1330.

Hamy, A. S., R. Porcher, S. Eskenazi, C. Cuvier, S. Giacchetti, F. Coussy, H. Hocini, B. Tournant, F. Perret, S. Bonfils, P. Charveriat, J. M. Lacorte and M. Espie (2016). "Anti-Mullerian hormone in breast cancer patients treated with chemotherapy: a retrospective evaluation of subsequent pregnancies." Reprod Biomed Online **32**(3): 299-307.

Hansen, K. R., G. M. Hodnett, N. Knowlton and L. B. Craig (2011). "Correlation of ovarian reserve tests with histologically determined primordial follicle number." Fertil Steril **95**(1): 170-175.

Hasky, N., S. Uri-Belapolsky, K. Goldberg, I. Miller, H. Grossman, S. M. Stemmer, I. Ben-Aharon and R. Shalgi (2015). "Gonadotrophin-releasing hormone agonists for fertility preservation: unraveling the enigma?" Hum Reprod **30**(5): 1089-1101.

Henry, N. L., R. Xia, M. Banerjee, C. Gersch, D. McConnell, D. Giacherio, A. F. Schott, M. Pearlman, V. Stearns, A. H. Partridge and D. F. Hayes (2013). "Predictors of recovery of ovarian function during aromatase inhibitor therapy." Ann Oncol **24**(8): 2011-2016.

Hourvitz, A., G. M. Yerushalmi, E. Maman, H. Raanani, S. Elizur, M. Brengauz, R. Orvieto, J. Dor and D. Meirow (2015). "Combination of ovarian tissue harvesting and immature oocyte collection for fertility preservation increases preservation yield." Reprod Biomed Online **31**(4): 497-505.

Hudson, J. N., N. B. Stanley, L. Nahata, M. Bowman-Curci and G. P. Quinn (2017). "New Promising Strategies in Oncofertility." Expert Rev Qual Life Cancer Care **2**(2): 67-78.

Hunink, M. G. M., M. C. Weinstein, E. Wittenberg, U. o. Y. Michael F. Drummond, J. S. Pliskin, J. B. Wong and P. P. Glasziou (2014). Decision Making in Health and Medicine, Cambridge University Press.

Huober-Zeeb, C., B. Lawrenz, R. M. Popovici, T. Strowitzki, A. Germeyer, P. Stute and M. von Wolff (2011). "Improving fertility preservation in cancer: ovarian tissue cryobanking followed by ovarian stimulation can be efficiently combined." Fertil Steril **95**(1): 342-344.

Inamoto, Y. and S. J. Lee (2017). "Late effects of blood and marrow transplantation." Haematologica **102**(4): 614-625.

International Patient Decision Aid Standards (IPDAS) Collaboration. (2017). "What are Patient Decision Aids? ." Retrieved January 2018, from http://www.ipdas.ohri.ca/what.html.

Jadoul, P. and J. Donnez (2012). "How does bone marrow transplantation affect ovarian function and fertility?" Curr Opin Obstet Gynecol **24**(3): 164-171.

Jahnukainen, K., J. Ehmcke, M. Hou and S. Schlatt (2011). "Testicular function and fertility preservation in male cancer patients." Best Pract Res Clin Endocrinol Metab **25**(2): 287-302.

Jones, G., J. Hughes, N. Mahmoodi, E. Smith, J. Skull and W. Ledger (2017). "What factors hinder the decision-making process for women with cancer and contemplating fertility preservation treatment?" Hum Reprod Update **23**(4): 433-457.

Joshi, S., B. N. Savani, E. J. Chow, M. H. Gilleece, J. Halter, D. A. Jacobsohn, J. Pidala, G. P. Quinn, J. Y. Cahn, A. A. Jakubowski, N. R. Kamani, H. M. Lazarus, J. D. Rizzo, H. C. Schouten, G. Socie, P. Stratton, M. L. Sorror, A. B. Warwick, J. R. Wingard, A. W. Loren and N. S. Majhail (2014). "Clinical guide to fertility preservation in hematopoietic cell transplant recipients." Bone Marrow Transplant **49**(4): 477-484.

Jung, M., H. J. Shin, S. Y. Rha, H. C. Jeung, S. Hong, Y. W. Moon, H. S. Kim, K. J. Oh, W. I. Yang, J. K. Roh and H. C. Chung (2010). "The clinical outcome of chemotherapy-induced amenorrhea in premenopausal young patients with breast cancer with long-term follow-up." Ann Surg Oncol **17**(12): 3259-3268.

Kalich-Philosoph, L., H. Roness, A. Carmely, M. Fishel-Bartal, H. Ligumsky, S. Paglin, I. Wolf, H. Kanety, B. Sredni and D. Meirow (2013). "Cyclophosphamide triggers follicle activation and "burnout"; AS101 prevents follicle loss and preserves fertility." Sci Transl Med **5**(185): 185ra162.

Kalsi, J., M. Y. Thum, A. Muneer, J. Pryor, H. Abdullah and S. Minhas (2011). "Analysis of the outcome of intracytoplasmic sperm injection using fresh or frozen sperm." BJU Int **107**(7): 1124-1128.

Kane, H. L., M. T. Halpern, L. B. Squiers, K. A. Treiman and L. A. McCormack (2014). "Implementing and evaluating shared decision making in oncology practice." CA Cancer J Clin **64**(6): 377-388.

Kato, K. (2016). "Vitrification of embryos and oocytes for fertility preservation in cancer patients." Reprod Med Biol **15**(4): 227-233.

Kelsey, T. W., R. A. Anderson, P. Wright, S. M. Nelson and W. H. Wallace (2012). "Data-driven assessment of the human ovarian reserve." Mol Hum Reprod **18**(2): 79-87.

Kim, J., V. Turan and K. Oktay (2016). "Long-Term Safety of Letrozole and Gonadotropin Stimulation for Fertility Preservation in Women With Breast Cancer." J Clin Endocrinol Metab **101**(4): 1364-1371.

Knopman, J. M., E. B. Papadopoulos, J. A. Grifo, M. E. Fino and N. Noyes (2010). "Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood." Lancet Oncol **11**(5): 490-498.

Krekow, L. K., B. A. Hellerstedt, R. P. Collea, S. Papish, S. M. Diggikar, R. Resta, S. J. Vukelja, F. A. Holmes, P. K. Reddy, L. Asmar, Y. Wang, P. S. Fox, S. R. Peck and J. O'Shaughnessy (2016). "Incidence and Predictive Factors for Recovery of Ovarian Function in Amenorrheic Women in Their 40s Treated With Letrozole." J Clin Oncol **34**(14): 1594-1600.

Kruh, G. D. (2005). "Ins and outs of taxanes." Cancer Biology & Therapy **4**(9): 1030-1032.

La Marca, A., V. Grisendi and G. Griesinger (2013). "How Much Does AMH Really Vary in Normal Women?" Int J Endocrinol **2013**: 959487.

Lambertini, M., M. Ceppi, F. Poggio, F. A. Peccatori, H. A. Azim, Jr., D. Ugolini, P. Pronzato, S. Loibl, H. C. Moore, A. H. Partridge, P. Bruzzi and L. Del Mastro (2015). "Ovarian suppression using luteinizing hormone-releasing hormone agonists during chemotherapy to preserve ovarian function and fertility of breast cancer patients: a meta-analysis of randomized studies." Ann Oncol **26**(12): 2408-2419.

Lambertini, M., M. Cinquini, I. Moschetti, F. A. Peccatori, P. Anserini, M. Valenzano Menada, M. Tomirotti and L. Del Mastro (2017). "Temporary ovarian suppression during chemotherapy to preserve ovarian function and fertility in breast cancer patients: A GRADE approach for evidence evaluation and recommendations by the Italian Association of Medical Oncology." Eur J Cancer **71**: 25-33.

Lambertini, M., L. Del Mastro, M. C. Pescio, C. Y. Andersen, H. A. Azim, Jr., F. A. Peccatori, M. Costa, A. Revelli, F. Salvagno, A. Gennari, F. M. Ubaldi, G. B. La Sala, C. De Stefano, W. H. Wallace, A. H. Partridge and P. Anserini (2016). "Cancer and fertility preservation: international recommendations from an expert meeting." BMC Med **14**: 1.

Lee, S. J., L. R. Schover, A. H. Partridge, P. Patrizio, W. H. Wallace, K. Hagerty, L. N. Beck, L. V. Brennan, K. Oktay and O. American Society of Clinical (2006). "American Society of Clinical Oncology recommendations on fertility preservation in cancer patients." J Clin Oncol **24**(18): 2917-2931.

Legare, F. and P. Thompson-Leduc (2014). "Twelve myths about shared decision making." Patient Educ Couns **96**(3): 281-286.

Letourneau, J., S. W. Chan and M. P. Rosen (2013). "Accelerating ovarian age: cancer treatment in the premenopausal woman." Semin Reprod Med **31**(6): 462-468.

Letourneau, J. M., E. E. Ebbel, P. P. Katz, A. Katz, W. Z. Ai, A. J. Chien, M. E. Melisko, M. I. Cedars and M. P. Rosen (2012). "Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer." Cancer **118**(6): 1710-1717.

Letourneau, J. M., E. E. Ebbel, P. P. Katz, K. H. Oktay, C. E. McCulloch, W. Z. Ai, A. J. Chien, M. E. Melisko, M. I. Cedars and M. P. Rosen (2012). "Acute ovarian failure underestimates age-specific reproductive impairment for young women undergoing chemotherapy for cancer." Cancer **118**(7): 1933-1939.

Levin, I. and B. Almog (2013). "Effect of cancer on ovarian function in patients undergoing in vitro fertilization for fertility preservation: a reappraisal." Curr Oncol **20**(1): e1-3.

Logan, S., J. Perz, J. Ussher, M. Peate and A. Anazodo (2018). "Clinician provision of oncofertility support in cancer patients of a reproductive age: A systematic review." Psychooncology **27**(3): 748-756.

Loren, A. W., P. B. Mangu, L. N. Beck, L. Brennan, A. J. Magdalinski, A. H. Partridge, G. Quinn, W. H. Wallace, K. Oktay and O. American Society of Clinical (2013). "Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update." J Clin Oncol **31**(19): 2500-2510.

Lorenzi, E., M. Simonelli and A. Santoro (2016). "Infertility risk and teratogenicity of molecularly targeted anticancer therapy: A challenging issue." Crit Rev Oncol Hematol **107**: 1-13.

Makoul, G. and M. L. Clayman (2006). "An integrative model of shared decision making in medical encounters." Patient Educ Couns **60**(3): 301-312.

Martinez, F. and International Society for Fertility Preservation–ESHRE–ASRM Expert Working Group (2017). "Update on fertility preservation from the Barcelona International Society for Fertility Preservation-ESHRE-ASRM 2015 expert meeting: indications, results and future perspectives." Hum Reprod **32**(9): 1802-1811.

Martinez, G., M. Walschaerts, M. Le Mitouard, R. Borye, C. Thomas, J. Auger, I. Berthaut, F. Brugnon, M. Daudin, N. Moinard, C. Ravel, J. Saias, E. Szerman, N. Rives, S. Hennebicq and L. Bujan (2017). "Impact of Hodgkin or non-Hodgkin lymphoma and their treatments on sperm aneuploidy: a prospective study by the French CECOS network." Fertil Steril **107**(2): 341-350.e345.

Meirow, D., H. Biederman, R. A. Anderson and W. H. Wallace (2010). "Toxicity of chemotherapy and radiation on female reproduction." Clin Obstet Gynecol **53**(4): 727-739.

Meirow, D., J. Dor, B. Kaufman, A. Shrim, J. Rabinovici, E. Schiff, H. Raanani, J. Levron and E. Fridman (2007). "Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms of ovarian injury." Hum Reprod **22**(6): 1626-1633.

Meissner, J., D. Tichy, S. Dietrich, T. Schmitt, M. Ziepert, E. Kuhnt, T. Rixecker, M. Witzens-Harig, M. Pfreundschuh and A. D. Ho (2014). "Parenthood in long-term survivors after CHOP with or without etoposide treatment for aggressive lymphoma." Br J Haematol **166**(4): 612-615.

Meissner, J., D. Tichy, V. Katzke, T. Kuhn, S. Dietrich, T. Schmitt, M. Ziepert, E. Kuhnt, T. Rixecker, M. Zorn, M. Witzens-Harig, M. Pfreundschuh and A. D. Ho (2015). "Long-term ovarian function in women treated with CHOP or CHOP plus etoposide for aggressive lymphoma." Ann Oncol **26**(8): 1771-1776.

Meistrich, M. L. (2009). "Male gonadal toxicity." Pediatr Blood Cancer **53**(2): 261-266.

Meistrich, M. L. (2013). "Effects of chemotherapy and radiotherapy on spermatogenesis in humans." Fertil Steril **100**(5): 1180-1186.

Meistrich, M. L. and G. Shetty (2008). "Hormonal suppression for fertility preservation in males and females." Reproduction **136**(6): 691-701.

Meistrich, M. L., G. Wilson, B. W. Brown, M. F. da Cunha and L. I. Lipshultz (1992). "Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas." Cancer **70**(11): 2703-2712.

Meistrich, M. L., G. Wilson, K. Mathur, L. M. Fuller, M. A. Rodriguez, P. McLaughlin, J. E. Romaguera, F. F. Cabanillas, C. S. Ha, L. I. Lipshultz and F. B. Hagemeister (1997). "Rapid recovery of spermatogenesis after mitoxantrone, vincristine, vinblastine, and prednisone chemotherapy for Hodgkin's disease." J Clin Oncol **15**(12): 3488-3495.

Melo, C., A. Fonseca, C. Silva, T. Almeida-Santos and M. C. Canavarro (2018). "Portuguese oncologists' practices regarding female fertility preservation: Which barriers most relate to these practices?" Eur J Cancer Care (Engl) **27**(2): e12812.

Michie, S., E. Dormandy and T. M. Marteau (2002). "The multi-dimensional measure of informed choice: a validation study." Patient Educ Couns **48**(1): 87-91.

Mintziori, G., I. Lambrinoudaki, I. Ceausu, H. Depypere, C. T. Erel, F. R. Perez-Lopez, K. Schenck-Gustafsson, Y. T. van der Schouw, T. Simoncini, F. Tremollieres, B. C. Tarlatzis, M. Rees and D. G. Goulis (2014). "EMAS position statement: Fertility preservation." Maturitas **77**(1): 85-89.

Morgan, S., R. A. Anderson, C. Gourley, W. H. Wallace and N. Spears (2012). "How do chemotherapeutic agents damage the ovary?" Hum Reprod Update **18**(5): 525-535.

Morita, Y., G. I. Perez, F. Paris, S. R. Miranda, D. Ehleiter, A. Haimovitz-Friedman, Z. Fuks, Z. Xie, J. C. Reed, E. H. Schuchman, R. N. Kolesnick and J. L. Tilly (2000). "Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy." Nat Med **6**(10): 1109-1114.

Muller, M., C. Urech, J. Boivin and V. Ehrbar (2017). "Addressing decisional conflict about fertility preservation: helping young female cancer survivors' family planning decisions."

Munhoz, R. R., A. A. Pereira, A. D. Sasse, P. M. Hoff, T. A. Traina, C. A. Hudis and R. J. Marques (2016). "Gonadotropin-Releasing Hormone Agonists for Ovarian Function Preservation in Premenopausal Women Undergoing Chemotherapy for Early-Stage Breast Cancer: A Systematic Review and Meta-analysis." JAMA Oncol **2**(1): 65-73.

Nam, H. K., H. R. Kim, Y. J. Rhie and K. H. Lee (2017). "Serum Anti-Mullerian Hormone Levels in Precocious Puberty Girls according to Stage of GnRH Agonist Treatment." J Korean Med Sci **32**(3): 475-479.

Nelson, S. M. (2013). "Biomarkers of ovarian response: current and future applications." Fertil Steril **99**(4): 963-969.

Northwestern University. (2011). "Fertility." Repropedia Retrieved January 2018, from http://www.repropedia.org/fertility.

Northwestern University. (2011). "Ovarian follicle." Repropedia Retrieved January 2018, from http://www.repropedia.org/ovarian-follicle.

Northwestern University. (2011). "Spermatogenesis." Repropedia Retrieved January 2018, from http://www.repropedia.org/spermatogenesis.

O'Connor, A. M., F. Legare and D. Stacey (2003). "Risk communication in practice: the contribution of decision aids." Bmj **327**(7417): 736-740.

Oktay, K. and G. Bedoschi (2016). "Appraising the Biological Evidence for and Against the Utility of GnRHa for Preservation of Fertility in Patients With Cancer." J Clin Oncol **34**(22): 2563-2565.

Oktay, K., B. E. Harvey, A. H. Partridge, G. P. Quinn, J. Reinecke, H. S. Taylor, W. H. Wallace, E. T. Wang and A. W. Loren (2018). "Fertility Preservation in Patients With Cancer: ASCO Clinical Practice Guideline Update." J Clin Oncol: Jco2018781914.

Oktay, K. and V. Turan (2016). "Failure of Ovarian Suppression With Gonadotropin-Releasing Hormone Analogs to Preserve Fertility: An Assessment Based on the Quality of Evidence." JAMA Oncol **2**(1): 74-75.

Oktem, O. and K. Oktay (2007). "Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function." Cancer **110**(10): 2222-2229.

OpenStax (2013). Anatomy and Physiology of the Female Reproductive System. Anatomy and Physiology, OpenStax.

Overbeek, A., M. H. van den Berg, F. E. van Leeuwen, G. J. Kaspers, C. B. Lambalk and E. van Dulmen-den Broeder (2017). "Chemotherapy-related late adverse effects on ovarian function in female survivors of childhood and young adult cancer: A systematic review." Cancer Treat Rev **53**: 10-24.

Partridge, A. H., S. Gelber, J. Peppercorn, E. Sampson, K. Knudsen, M. Laufer, R. Rosenberg, M. Przypyszny, A. Rein and E. P. Winer (2004). "Web-based survey of fertility issues in young women with breast cancer." J Clin Oncol **22**(20): 4174-4183.

Partridge, A. H. and K. J. Ruddy (2007). "Fertility and adjuvant treatment in young women with breast cancer." Breast **16 Suppl 2**: S175-181.

Partridge, A. H., K. J. Ruddy, S. Gelber, L. Schapira, M. Abusief, M. Meyer and E. Ginsburg (2010). "Ovarian reserve in women who remain premenopausal after chemotherapy for early stage breast cancer." Fertil Steril **94**(2): 638-644.

Peate, M., B. Meiser, B. C. Cheah, C. Saunders, P. Butow, B. Thewes, R. Hart, K. A. Phillips, M. Hickey and M. Friedlander (2012). "Making hard choices easier: a prospective, multicentre study to assess the efficacy of a fertility-related decision aid in young women with early-stage breast cancer." Br J Cancer **106**(6): 1053-1061.

Peate, M., B. Meiser, M. Friedlander, C. Saunders, R. Martinello, C. E. Wakefield and M. Hickey (2011). "Development and pilot testing of a fertility decision aid for young women diagnosed with early breast cancer." Breast J **17**(1): 112-114.

Peate, M., B. Meiser, M. Hickey and M. Friedlander (2009). "The fertility-related concerns, needs and preferences of younger women with breast cancer: a systematic review." Breast Cancer Res Treat **116**(2): 215-223.

Peccatori, F. A., H. A. Azim, Jr., R. Orecchia, H. J. Hoekstra, N. Pavlidis, V. Kesic, G. Pentheroudakis and E. G. W. Group (2013). "Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up." Ann Oncol **24 Suppl 6**: vi160-170.

Peigne, M. and C. Decanter (2014). "Serum AMH level as a marker of acute and long-term effects of chemotherapy on the ovarian follicular content: a systematic review." Reprod Biol Endocrinol **12**: 26.

Perdrix, A., M. Saint-Ghislain, M. Degremont, M. David, Z. Khaznadar, A. Loeb, M. Leheurteur, F. Di Fiore and F. Clatot (2017). "Influence of adjuvant chemotherapy on anti-Mullerian hormone in women below 35 years treated for early breast cancer." Reprod Biomed Online **35**(4): 468-474.

Perez, G. I., C. M. Knudson, L. Leykin, S. J. Korsmeyer and J. L. Tilly (1997). "Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction." Nat Med **3**(11): 1228-1232.

Pinto, A. C., J. N. Moreira and S. Simões (2011). Combination Chemotherapy in Cancer: Principles, Evaluation and Drug Delivery Strategies. Current Cancer Treatment - Novel Beyond Conventional Approaches. P. O. Ozdemir, InTech.

Practice Committee of American Society for Reproductive Medicine (2013). "Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion." Fertil Steril **100**(5): 1214-1223.

Practice Committee of the American Society for Reproductive, M. (2012). "Testing and interpreting measures of ovarian reserve: a committee opinion." Fertil Steril **98**(6): 1407-1415.

Practice Committee of the American Society for Reproductive Medicine (2015). "Testing and interpreting measures of ovarian reserve: a committee opinion." Fertil Steril **103**(3): e9-e17.

Practice Committees of American Society for Reproductive Medicine and Society for Assisted Reproductive Technology (2013). "Mature oocyte cryopreservation: a guideline." Fertil Steril **99**(1): 37-43.

Prasath, E. B., M. L. Chan, W. H. Wong, C. J. Lim, M. D. Tharmalingam, M. Hendricks, S. F. Loh and Y. N. Chia (2014). "First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient." Hum Reprod **29**(2): 276-278.

Quinn, G. P., S. T. Vadaparampil, C. K. Gwede, C. Miree, L. M. King, H. B. Clayton, C. Wilson and P. Munster (2007). "Discussion of fertility preservation with newly diagnosed patients: oncologists' views." J Cancer Surviv **1**(2): 146-155.

Quinn, G. P., S. T. Vadaparampil, L. King, C. A. Miree, C. Wilson, O. Raj, J. Watson, A. Lopez and T. L. Albrecht (2009). "Impact of physicians' personal discomfort and patient prognosis on discussion of fertility preservation with young cancer patients." Patient Educ Couns **77**(3): 338-343.

Quinn, M. M., H. Cakmak, J. M. Letourneau, M. I. Cedars and M. P. Rosen (2017). "Response to ovarian stimulation is not impacted by a breast cancer diagnosis." Hum Reprod **32**(3): 568-574.

Ribnikar, D., J. M. Ribeiro, D. Pinto, B. Sousa, A. C. Pinto, E. Gomes, E. C. Moser, M. J. Cardoso and F. Cardoso (2015). "Breast cancer under age 40: a different approach." Curr Treat Options Oncol **16**(4): 16.

Roness, H., L. Kalich-Philosoph and D. Meirow (2014). "Prevention of chemotherapy-induced ovarian damage: possible roles for hormonal and non-hormonal attenuating agents." Hum Reprod Update **20**(5): 759-774.

Roness, H., O. Kashi and D. Meirow (2016). "Prevention of chemotherapy-induced ovarian damage." Fertil Steril **105**(1): 20-29.

Rosendahl, M., K. T. Schmidt, E. Ernst, P. E. Rasmussen, A. Loft, A. G. Byskov, A. N. Andersen and C. Y. Andersen (2011). "Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique." Reprod Biomed Online **22**(2): 162-171.

Ruddy, K. J., H. Guo, W. Barry, C. T. Dang, D. A. Yardley, B. Moy, P. K. Marcom, K. S. Albain, H. S. Rugo, M. J. Ellis, I. Shapira, A. C. Wolff, L. A. Carey, B. A. Overmoyer, C. Hudis, I. E. Krop, H. J. Burstein, E. P. Winer, A. H. Partridge and S. M. Tolaney (2015). "Chemotherapy-related amenorrhea after adjuvant paclitaxel-trastuzumab (APT trial)." Breast Cancer Res Treat **151**(3): 589-596.

Ruddy, K. J. and A. H. Partridge (2012). "Fertility (male and female) and menopause." J Clin Oncol **30**(30): 3705-3711.

Saalu, L. C., A. A. Osinubi and J. A. Olagunju (2010). "Early and Delayed Effects of Doxorubicin on Testicular Oxidative Status and Spermatogenesis in Rats." International Journal of Cancer Research **6**: 1-9.

Sabanegh, E. S., Jr. and A. M. Ragheb (2009). "Male fertility after cancer." Urology **73**(2): 225-231.

Scanlon, M., A. Blaes, M. Geller, N. S. Majhail, B. Lindgren and T. Haddad (2012). "Patient Satisfaction with Physician Discussions of Treatment Impact on Fertility, Menopause and Sexual Health among Pre-menopausal Women with Cancer." J Cancer **3**: 217-225.

Schmidt, K. T., A. Nyboe Andersen, T. Greve, E. Ernst, A. Loft and C. Yding Andersen (2013). "Fertility in cancer patients after cryopreservation of one ovary." Reprod Biomed Online **26**(3): 272-279.

Schover, L. R. (2005). "Motivation for parenthood after cancer: a review." J Natl Cancer Inst Monogr(34): 2-5.

Schover, L. R., K. Brey, A. Lichtin, L. I. Lipshultz and S. Jeha (2002). "Knowledge and experience regarding cancer, infertility, and sperm banking in younger male survivors." J Clin Oncol **20**(7): 1880-1889.

Schover, L. R., K. Brey, A. Lichtin, L. I. Lipshultz and S. Jeha (2002). "Oncologists' attitudes and practices regarding banking sperm before cancer treatment." J Clin Oncol **20**(7): 1890-1897.

Schover, L. R., M. van der Kaaij, E. van Dorst, C. Creutzberg, E. Huyghe and C. E. Kiserud (2014). "Sexual dysfunction and infertility as late effects of cancer treatment." EJC Suppl **12**(1): 41-53.

Schuring, A. N., T. Fehm, K. Behringer, M. Goeckenjan, P. Wimberger, M. Henes, J. Henes, M. F. Fey and M. von Wolff (2018). "Practical recommendations for fertility preservation in women by the FertiPROTEKT network. Part I: Indications for fertility preservation." Arch Gynecol Obstet **297**(1): 241-255.

Sekar, T. V. and R. Paulmurugan (2014). Cancer Theranostics. Chapter 6 – Bioluminescence Imaging of Cancer Therapy. Xiaoyuan Chen and Stephen Wong, Elsevier Inc.

Selk, A., T. Belej-Rak, H. Shapiro and E. Greenblatt (2009). "Use of an oncology sperm bank: a Canadian experience." Can Urol Assoc J **3**(3): 219-222.

Senkus, E., S. Kyriakides, S. Ohno, F. Penault-Llorca, P. Poortmans, E. Rutgers, S. Zackrisson and F. Cardoso (2015). "Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up." Ann Oncol **26 Suppl 5**: v8-30.

Shandley, L. M., J. B. Spencer, A. Fothergill, A. C. Mertens, A. Manatunga, E. Paplomata and P. P. Howards (2017). "Impact of tamoxifen therapy on fertility in breast cancer survivors." Fertil Steril **107**(1): 243-252 e245.

Shimizu, C., H. Bando, T. Kato, Y. Mizota, S. Yamamoto and Y. Fujiwara (2013). "Physicians' knowledge, attitude, and behavior regarding fertility issues for young breast cancer patients: a national survey for breast care specialists." Breast Cancer **20**(3): 230-240.

Silva, C., T. Almeida-Santos, C. Melo and A. C. R. Rama (2015). Decision on fertility preservation in cancer patients: development of information resources for healthcare professionals. Unpublished work.

Smith, M. A., S. F. Altekruse, P. C. Adamson, G. H. Reaman and N. L. Seibel (2014). "Declining childhood and adolescent cancer mortality." Cancer **120**(16): 2497-2506.

Smyth, L. and C. Hudis (2015). "Adjuvant hormonal therapy in premenopausal women with breast cancer." Indian J Med Paediatr Oncol **36**(4): 195-200.

Soleimani, R., E. Heytens, Z. Darzynkiewicz and K. Oktay (2011). "Mechanisms of chemotherapy-induced human ovarian aging: double strand DNA breaks and microvascular compromise." Aging (Albany NY) **3**(8): 782-793.

Stacey, D., C. L. Bennett, M. J. Barry, N. F. Col, K. B. Eden, M. Holmes-Rovner, H. Llewellyn-Thomas, A. Lyddiatt, F. Legare and R. Thomson (2011). "Decision aids for people facing health treatment or screening decisions." Cochrane Database Syst Rev(10): Cd001431.

Stacey, D., F. Legare, K. Lewis, M. J. Barry, C. L. Bennett, K. B. Eden, M. Holmes-Rovner, H. Llewellyn-Thomas, A. Lyddiatt, R. Thomson and L. Trevena (2017). "Decision aids for people facing health treatment or screening decisions." Cochrane Database Syst Rev **4**: Cd001431.

Stacey, D., R. Samant and C. Bennett (2008). "Decision making in oncology: a review of patient decision aids to support patient participation." CA Cancer J Clin **58**(5): 293-304.

Steiner, A. Z., D. Pritchard, F. Z. Stanczyk, J. S. Kesner, J. W. Meadows, A. H. Herring and D. D. Baird (2017). "Association Between Biomarkers of Ovarian Reserve and Infertility Among Older Women of Reproductive Age." Jama **318**(14): 1367-1376.

Stensheim, H., M. Cvancarova, B. Moller and S. D. Fossa (2011). "Pregnancy after adolescent and adult cancer: a population-based matched cohort study." Int J Cancer **129**(5): 1225-1236.

Stiggelbout, A. M., A. H. Pieterse and J. C. De Haes (2015). "Shared decision making: Concepts, evidence, and practice." Patient Educ Couns **98**(10): 1172-1179.

Stiggelbout, A. M., T. Van der Weijden, M. P. De Wit, D. Frosch, F. Legare, V. M. Montori, L. Trevena and G. Elwyn (2012). "Shared decision making: really putting patients at the centre of healthcare." Bmj **344**: e256.

Su, H. C., C. Haunschild, K. Chung, S. Komrokian, S. Boles, M. D. Sammel and A. DeMichele (2014). "Prechemotherapy antimullerian hormone, age, and body size predict timing of return of ovarian function in young breast cancer patients." Cancer **120**(23): 3691-3698.

Su, H. I., K. Maas, P. M. Sluss, R. J. Chang, J. E. Hall and H. Joffe (2013). "The impact of depot GnRH agonist on AMH levels in healthy reproductive-aged women." J Clin Endocrinol Metab **98**(12): E1961-1966.

Subtil S, Pires R, Couto D and Almeida-Santos T (2018). Fertility Preservation in Women with Breast Cancer. Unpublished work.

Tal, R. and D. B. Seifer (2017). "Ovarian reserve testing: a user's guide." Am J Obstet Gynecol **217**(2): 129-140.

Taylan, E. and K. H. Oktay (2017). "Current state and controversies in fertility preservation in women with breast cancer." World J Clin Oncol **8**(3): 241-248.

Teh, W. T., C. Stern, S. Chander and M. Hickey (2014). "The impact of uterine radiation on subsequent fertility and pregnancy outcomes." Biomed Res Int **2014**: 482968.

The Oncofertility Consortium. (2015). "Semen Testing." Retrieved January 2018, from https://oncofertility.northwestern.edu/resources/semen-testing.

Torino, F., A. Barnabei, L. De Vecchis, V. Sini, F. Schittulli, P. Marchetti and S. M. Corsello (2014). "Chemotherapy-induced ovarian toxicity in patients affected by endocrine-responsive early breast cancer." Crit Rev Oncol Hematol **89**(1): 27-42.

Towle, A. and W. Godolphin (1999). "Framework for teaching and learning informed shared decision making." Bmj **319**(7212): 766-771.

Tran, S., R. Boissier, J. Perrin, G. Karsenty and E. Lechevallier (2015). "Review of the Different Treatments and Management for Prostate Cancer and Fertility." Urology **86**(5): 936-941.

Trapp, E., J. Steidl, B. Rack, M. S. Kupka, U. Andergassen, J. Juckstock, A. Kurt, T. Vilsmaier, A. de Gregorio, N. de Gregorio, M. Tzschaschel, C. Lato, A. Polasik, H. Tesch, A. Schneeweiss, M. W. Beckmann, P. A. Fasching, W. Janni and V. Muller (2017). "Anti-Mullerian hormone (AMH) levels in premenopausal breast cancer patients treated with taxane-based adjuvant chemotherapy - A translational research project of the SUCCESS A study." Breast **35**: 130-135.

Tsai-Turton, M., B. T. Luong, Y. Tan and U. Luderer (2007). "Cyclophosphamide-induced apoptosis in COV434 human granulosa cells involves oxidative stress and glutathione depletion." Toxicol Sci **98**(1): 216-230.

U.S. National Library of Medicine. "MeSH Database - Antineoplastic Agents." Retrieved January 2018, from https://www.ncbi.nlm.nih.gov/mesh/68000970.

UK Cancer Research. (2013, 11 May 2016). "Breast cancer survival statistics by age." Retrieved January 2018, from http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/survival#heading-One.

Ulug, U., F. Bener, L. Karagenc, N. Ciray and M. Bahceci (2005). "Outcomes in couples undergoing ICSI: comparison between fresh and frozen-thawed surgically retrieved spermatozoa." Int J Androl **28**(6): 343-349.

Ussher, J. M., C. Parton and J. Perz (2018). "Need for information, honesty and respect: patient perspectives on health care professionals communication about cancer and fertility." Reprod Health **15**(1): 2.

van Casteren, N. J., W. P. Boellaard, J. C. Romijn and G. R. Dohle (2010). "Gonadal dysfunction in male cancer patients before cytotoxic treatment." Int J Androl **33**(1): 73-79.

van Casteren, N. J., E. J. van Santbrink, W. van Inzen, J. C. Romijn and G. R. Dohle (2008). "Use rate and assisted reproduction technologies outcome of cryopreserved semen from 629 cancer patients." Fertil Steril **90**(6): 2245-2250.

Vitek, W. S., M. Shayne, K. Hoeger, Y. Han, S. Messing and C. Fung (2014). "Gonadotropin-releasing hormone agonists for the preservation of ovarian function among women with breast cancer who did not use tamoxifen after chemotherapy: a systematic review and meta-analysis." Fertil Steril **102**(3): 808-815.e801.

von Wolff, M., A. Germeyer, J. Liebenthron, M. Korell and F. Nawroth (2018). "Practical recommendations for fertility preservation in women by the FertiPROTEKT network. Part II: fertility preservation techniques." Arch Gynecol Obstet **297**(1): 257-267.

von Wolff, M. and P. Stute (2017). "Judging the Fertility Protective Effect of GnRH Agonists in Chemotherapy-It Is a Matter of Perspective." Front Endocrinol (Lausanne) **8**: 69.

Walter, J. R., S. Xu, A. S. Paller, J. N. Choi and T. K. Woodruff (2016). "Oncofertility considerations in adolescents and young adults given a diagnosis of melanoma: Fertility risk of Food and Drug Administration-approved systemic therapies." J Am Acad Dermatol **75**(3): 528-534.

Ward, J. A., C. W. Bardin, M. Knight, J. Robinson, G. Gunsalus and I. D. Morris (1988). "Delayed effects of doxorubicin on spermatogenesis and endocrine function in rats." Reprod Toxicol **2**(2): 117-126.

Westphal, L. M. and J. A. M. Massie (2012). Embryo and Oocyte Banking. Oncofertility Medical Practice: Clinical Issues and Implementation. C. Gracia and T. K. Woodruff, Springer Science+Business Media.

WHO Collaborating Centre for Drug Statistics Methodology (2009). The Anatomical Therapeutic Chemical (ATC) Classification System

Wilkes, S., S. Coulson, A. Crosland, G. Rubin and J. Stewart (2010). "Experience of fertility preservation among younger people diagnosed with cancer." Hum Fertil (Camb) **13**(3): 151-158.

Wo, J. Y. and A. N. Viswanathan (2009). "Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients." Int J Radiat Oncol Biol Phys **73**(5): 1304-1312.

Woodard, T. L., A. S. Hoffman, L. A. Covarrubias, D. Holman, L. Schover, A. Bradford, D. B. Hoffman, A. Mathur, J. Thomas and R. J. Volk (2018). "The Pathways fertility preservation decision aid website for women with cancer: development and field testing." J Cancer Surviv **12**(1): 101-114.

Woodruff, T. K. (2015). "Oncofertility: a grand collaboration between reproductive medicine and oncology." Reproduction **150**(3): S1-10.

Woolf, S. H., E. C. Chan, R. Harris, S. L. Sheridan, C. H. Braddock, 3rd, R. M. Kaplan, A. Krist, A. M. O'Connor and S. Tunis (2005). "Promoting informed choice: transforming health care to dispense knowledge for decision making." Ann Intern Med **143**(4): 293-300.

Yuksel, A., G. Bildik, F. Senbabaoglu, N. Akin, M. Arvas, F. Unal, Y. Kilic, I. Karanfil, B. Eryilmaz, P. Yilmaz, C. Ozkanbas, C. Taskiran, S. Aksoy, Y. Guzel, B. Balaban, U. Ince, A. Iwase, B. Urman and O. Oktem (2015). "The magnitude of gonadotoxicity of chemotherapy drugs on ovarian follicles and granulosa cells varies depending upon the category of the drugs and the type of granulosa cells." Hum Reprod **30**(12): 2926-2935.

Zavos, A. and A. Valachis (2016). "Risk of chemotherapy-induced amenorrhea in patients with breast cancer: a systematic review and meta-analysis." Acta Oncol **55**(6): 664-670.

Zebrack, B. (2008). "Information and service needs for young adult cancer patients." Support Care Cancer **16**(12): 1353-1360.

Zegers-Hochschild, F., G. D. Adamson, S. Dyer, C. Racowsky, J. de Mouzon, R. Sokol, L. Rienzi, A. Sunde, L. Schmidt, I. D. Cooke, J. L. Simpson and S. van der Poel (2017). "The International Glossary on Infertility and Fertility Care, 2017." Hum Reprod **32**(9): 1786-1801.

Zhao, J., J. Liu, K. Chen, S. Li, Y. Wang, Y. Yang, H. Deng, W. Jia, N. Rao, Q. Liu and F. Su (2014). "What lies behind chemotherapy-induced amenorrhea for breast cancer patients: a meta-analysis." Breast Cancer Res Treat **145**(1): 113-128.

Zhou, W. B., H. Yin, X. A. Liu, X. M. Zha, L. Chen, J. C. Dai, A. D. Tao, L. Chen, J. J. Ma, L. J. Ling and S. Wang (2010). "Incidence of chemotherapy-induced amenorrhea associated with epirubicin, docetaxel and navelbine in younger breast cancer patients." BMC Cancer **10**: 281.

OBJECTIVES

Objectives

The current research had the following general and specific objectives:

1. To provide significant contributions to a shared decision-making process concerning fertility preservation

1.1. To develop evidence-based, relevant and useful information resources directed for cancer patients, to promote and support their participation in decisions in the setting of infertility risks and fertility preservation.

1.2. To develop evidence-based, relevant and useful information resources directed for oncologists and other healthcare professionals, to increase their awareness of infertility risks and fertility preservation options and to promote discussion of these subjects with cancer patients.

1.3. To cooperate in the development of national clinical guidance and in the successful accomplishment of other national information and education initiatives concerning infertility risks and fertility preservation options in cancer patients.

2. To support a more accurate infertility risk assessment in young female patients with breast cancer

2.1. To systematically identify patient and treatment-related factors associated with the recovery of ovarian function in young premenopausal female breast cancer patients, after exposure to chemotherapy.

2.2. To assess the impact of modern treatment combinations for breast cancer (chemotherapy and/or targeted therapy and/or hormonal therapy) on reliable markers of ovarian reserve.

2.3. To evaluate the reproductive health outcomes of young premenopausal patients with breast cancer exposed to modern treatment combinations (chemotherapy and/or targeted therapy and/or hormonal therapy) and to identify patient and treatment-related factors that help to predict those outcomes.

METHODS

Methods

Detailed information about the methods used in this investigation is included in each chapter of the Results section.

RESULTS

Chapter I

Development of information resources to support decisions concerning fertility preservation in cancer patients

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1.1 Development of information resources for patients

1.1.1 Antineoplastic agents and (in)fertility: informing patients to improve decisions

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Abstract

Purpose: Infertility is a potential adverse effect of cancer treatment, and future fertility is an important issue for cancer patients**.** In Portugal, the Centre for Fertility Preservation of CHUC, EPE, conducted a project to develop and disseminate oncofertility information resources. Here, we report the results of the specific component of this program, which intended to produce information resources that promote patients' awareness of the subject and to support decisions concerning fertility preservation.

Methods: Guidance for writing health information for patients and criteria for developing decision aids were gathered. Information needs were assessed (literature review and locally applied questionnaire). Resources were pre-tested with a sample of patients and professionals. Their readability, presentation quality and ability to support decisions were evaluated.

Results: General information handouts on infertility risk and decision aids about fertility preservation options were developed and positively evaluated. The resources are currently being distributed in collaboration with several national organizations.

Conclusions: Through our multidisciplinary information program, reproductive-age cancer patients now have access to relevant information resources that will support timely, shared decision-making concerning fertility preservation.

Introduction

Infertility is a potential adverse effect of antineoplastic cancer therapy. The degree of gonadal toxicity from chemotherapy is influenced by several factors, such as patient age, total dose administered and the nature of antineoplastic agents.[1, 2] The possibility of an early diagnosis and considerable advances in cancer treatment, along with a rising incidence of cancer in teenagers and young adults, has led to an increase in the number of cancer survivors of reproductive age. In addition to facing the consequences of the disease, these patients will have to address the consequences of cancer treatments for their fertility.[3] It is recognized that future fertility is an important issue for these patients; [4] therefore, shared decisions concerning fertility preservation (FP) must occur at the time of diagnosis.

In this context, oncofertility, a term coined in 2006 by Teresa Woodruff, has recently emerged as a multidisciplinary field with the purpose of fulfilling the needs of cancer patients regarding

their reproductive potential.[5] Several professional organizations in the field of oncology have published recommendations regarding FP, advising health professionals to discuss infertility risks and FP options with all cancer patients of reproductive age.[6, 7] However, a number of published studies indicate that not every oncologist is following these orientations and that a considerable proportion of cancer patients is still not informed about the risks and possibilities regarding FP.[8-13] Moreover, surveys of reproductive-age cancer patients and survivors disclose gaps in the information received about the opportunity of preserving fertility, the techniques available or the possibility of consultation with a reproductive medicine specialist.[14-17]

Decisions regarding FP, especially for female cancer patients, are complex and preference-sensitive, i.e., they need to consider patients' values for benefits and harms across options. [18] All options come with risks and success rates and decisions concerning FP often have to be made in a short time frame, along with other treatment decisions and in a period of a great emotional distress. Several patient-related factors contribute to a quality decision-making process, including patients' values and attitudes, support from family and friends and the acquisition of information.[19, 20] Information resources conceived to support decisions in healthcare such as decision aids [21, 22] or decision trees [23] provide a more clear understanding of the available options, facilitate discussions and increase patients' and professionals' involvement in the decision-making process.

In the specific setting of infertility risk in cancer patients, the access to specialized information concerning FP improved patients' knowledge of the available options [24] and reduced decisional conflicts.[25]

In Portugal, oncofertility is of increasing importance in the context of quality of life in cancer survival. Recently, several Portuguese scientific societies, including the *Sociedade Portuguesa de Medicina da Reprodução* (Portuguese Society of Reproductive Medicine, SPMR) and the *Sociedade Portuguesa de Oncologia* (Portuguese Society of Oncology, SPO), in cooperation with the national hematology and andrology professional societies, published and endorsed the "Portuguese Recommendations for Preserving the Reproductive Potential of Cancer Patients".[26] This document was the final outcome of the 1st and 2nd Portuguese Oncofertility Meetings in 2015 and 2016. The Portuguese *Centro de Preservação da Fertilidade* (Centre for Fertility Preservation, CFP) of Coimbra Hospital and University Centre (CHUC), EPE, was a leader in this process and has been working actively since 2012 to promote local and national awareness of oncofertility. In cooperation with the *Liga Portuguesa Contra o Cancro* **(**Portuguese League Against Cancer, LPCC), a Portuguese nonprofit cancer patients

organization, the CFP conducted a project to develop and disseminate oncofertility information resources, directed both to health professionals and cancer patients. Here, we report the results of the specific component of this information program, which aimed to produce information resources for cancer patients to promote their informed participation in decisions in the context of infertility risks and FP.

2. Methods

2.1. Assessment of information needs

A literature search on Medline, through PubMed, was conducted to identify primary quantitative studies evaluating cancer patients' information needs or gaps in knowledge concerning infertility risks and FP options. The search equation was built using the following MeSH terms: *Patient Education as Topic; Consumer Health Information/methods; Health education; Antineoplastic Agents/adverse effects; Infertility, Female; Infertility, Male; Sperm Banks; Cryopreservation; Reproductive Techniques, Assisted;* and *Fertility Preservation*. The eligible articles were retrieved, and their results were gathered and organized. Additionally, a questionnaire directed to cancer survivors that had been diagnosed in reproductive age was applied locally[1]. These self-reported, anonymous questionnaires were distributed by clinicians in follow-up consultations at several clinical departments of CHUC, EPE. Demographic, reproductive and clinical information was requested. Participants were asked to rate, on a 5-point Likert scale (ranging from *Not important* to *Extremely important*), their self-perceived importance of discussing specific information topics regarding infertility risks and FP before cancer treatment initiation. An additional question queried patients on the usefulness of several information strategies (from *Not useful* to *Extremely useful*). The study received approval from the local ethical committee, and the questionnaire was previously tested in a small group of cancer survivors. Patients were informed of the objectives and methods of the study, and all participants signed written consent.

2.2. Development of information resources

The information resources were designed to target cancer patients of reproductive age (18 to 40 years) with a recent cancer diagnosis (any type of cancer) before treatment initiation. To

[1]This self-reported questionnaire was developed and administered in collaboration with Cláudia Melo, as the responsible researcher of a PhD project on Health Psychology about fertility preservation.

include relevant, yet specific, information for each gender (for instance, about fertility markers), distinct resources were developed for male and female patients.

Bearing in mind that the risk of infertility is not acknowledged by many cancer patients[27-31] and the complexity of the decision-making process regarding FP, particularly in women, two different types of written patient information resources were planned: 1) general information handouts with the aim of raising awareness of the effects of cancer and cancer treatments on fertility; 2) decision aids with the aim of supporting decisions in the context of FP.

With the purpose of producing quality written health information materials, searches were conducted in Medline, through PubMed, to find general guidance for writing health information for patients. For the specific production of the decision aids, the criteria included in the DISCERN instrument[32] (http://www.discern.org.uk/index.php) were taken into account. DISCERN consists of 16 key questions intending to evaluate the reliability of the publication, the information provided about treatment choices and its overall quality. This tool was designed to help users of consumer health information judge the quality of written information about treatment choices but can also be used as a checklist for authors and producers of written consumer health information.

Published evidence on infertility risks and FP options in cancer patients was identified and the most current evidence-based knowledge on clinical indications, time requisites, success rates, risks and advantages/disadvantages of each FP technique was gathered.

2.3. Evaluation of information resources

Readability

Readability is a measure of the facility with which a text is read, according to the length of words and sentences. Preliminary versions of the information resources were tested for readability using the *Fernandez-Huerta index*, a modified version of the *Flesch Reading Ease score* for the Spanish language.[33] In the *Flesch Reading Ease score*, the results range from 0 (the worst level, very difficult to read) to 100 (the best readability level). Usually, a reading ease of 60-70 is considered standard (Table 1.1).[34]

The number of words and syllables was estimated using the software *TextMeter*, an application of text statistics for the Portuguese language. After the first readability results, improvements were made by using alternative, shorter words and building less complex sentences.

Table 1.1 Flesch Reading Ease scores and corresponding readability and school levels.

Flesch Reading Ease score	Readability level	School level (Easy to read for…)
0-30	Very difficult	College graduate (University degree)
30-50	Difficult	College (University student)
50-60	Fairly difficult	10th-12th grade (High school graduate)
60-70	Standard	8th-9th grade
70-80	Fairly easy	7th grade
80-90	Easy	6th grade
90-100	Very easy	5th grade

Pre-test

The first drafts of both the general information handouts and the decision aids were provided, along with an evaluation form, to reproductive-age cancer patients and survivors by oncologists and psychologists in fertility preservation and follow up consultations. They were also evaluated by a variety of healthcare professionals with direct or indirect involvement in the care of reproductive-age cancer patients (Table 1.2). These groups evaluated the content, language and layout of both information resources. Additionally, the ability of the decision aids to support shared decisions was assessed by asking if the different options were presented in a balanced way; if the information on each option was sufficient; and if the information would increase the knowledge about the options, help patients to discuss the options with their oncologist and promote their participation in decisions.

Quality

The quality of the information resources was assessed using EQIP (Ensuring Quality Information for Patients), a tool designed to measure the presentation quality of all types of written health care information,[35] and the above-mentioned DISCERN instrument.[32]

Table 1.2 Evaluation criteria and evaluation groups used to pre-test the information resources.

Evaluation criteria	Evaluation groups
GENERAL INFORMATION HANDOUTS • Content (usefulness, completeness, organization) • Language • Layout (colors, titles, highlights, fonts)	**Patients** • Female cancer patients (n=3) • Female cancer survivors (n=6) • Male cancer survivors (n=3) **Cancer care professionals** • Psychologists (n=1) • Hospital pharmacists (n=2) • Oncologists (n=2) • Hematologists (n=1) • Gynecologists (n=2) **Human reproduction professionals** • Reproductive medicine specialists (n=2) • Nurses (n=2) **Other healthcare professionals** • Community pharmacists (n=2)
DECISION AIDS • Content (usefulness, completeness, organization) • Language • Layout (colors, titles, highlights, fonts) • Ability to support decisions • Usefulness for clinical practice (health professionals)	**Patients** • Female cancer patients (n=3) • Female cancer survivors (n=6) • Male cancer survivors (n=3) **Cancer care professionals** • Psychologists (n=1) • Hospital pharmacists (n=2) • Oncologists (n=2) • Hematologists (n=1) • Gynecologists (n=2) **Reproductive health professionals** • Reproductive medicine specialists (n=2) • Embryologist (n=1) • Nurses (n=2)

A flow diagram showing the sequence of steps in the development process can be seen in Figure 1.1.

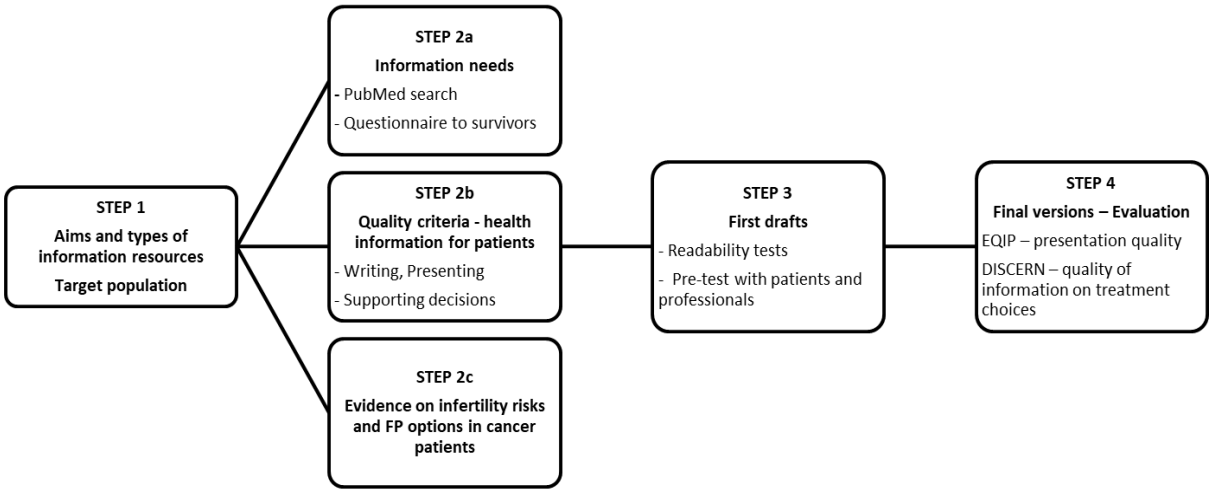

Figure 1.1 Flow diagram showing the sequential steps in the development of information resources.

3. Results

3.1. Information needs

In accordance with the defined eligibility criteria, ten published articles were selected and analyzed.[27-31; 36-40] Data on methods and results on reported patient information needs or gaps in knowledge were collected from each individual article and are presented in Table 3.

Table 1.3 Data on studies identifying cancer patients' information needs or gaps in knowledge concerning infertility risks and FP options.

Reference	Title	Methods (sample)	Information needs / gaps in knowledge identified
Armuand GM. J Clin Oncol 2012; 30:2147-53[27]	Sex differences in fertility-related information received by young adult cancer survivors	Postal questionnaire sent to cancer survivors identified in population-based registers in Sweden (n=484)	• Effects of cancer treatments on fertility • Effects of cancer treatments on future children

Reference	Title	Methods (sample)	Information needs / gaps in knowledge identified
Balthazar U. Fertil Steril 2011; 95:1913–6[36]	Fertility preservation: a pilot study to assess previsit patient knowledge quantitatively	Consecutive new FP patients seen at the University of North Carolina completed a pre-consultation questionnaire (n=41)	• Success rates of FP techniques • Effect of FP in cancer recurrence • Effects of cancer treatments on future children • Costs of FP • FP options before and after cancer treatment • Established *versus* experimental FP options
Balthazar U. Hum Rep 2012; 27:2413–19[37]	The current fertility preservation consultation model: are we adequately informing cancer patients of their options?	Web-based survey at academic IVF centers, including women aged 18–43 years seen for comprehensive FP consultation	• Time requirements for FP • Pregnancy rates after FP • Effect of FP on cancer recurrence • Age as a very important factor for FP success • Maximum time of embryo/oocyte cryopreservation • Effects of oophorectomy in future fertility
Jukkala AM, Fertil Steril 2010; 94:2396-8[28]	Self-assessed knowledge of treatment and fertility preservation in young women with breast cancer	Online assessment of knowledge in women (18 to 50 years) with history of breast cancer (n=106)	• Effects of cancer and cancer treatments on fertility • Infertility treatments • FP options
Karen M. Oncol Nurs Forum 2010; 37:191-7[38]	Development of the Fertility and Cancer Project: An Internet Approach to Help Young Cancer Survivors	Internet survey to assess fertility knowledge of young survivors of breast cancer from eight countries (n=106)	• Infertility treatments • FP options

Reference	Title	Methods (sample)	Information needs / gaps in knowledge identified
Peate M. J Clin Oncol 2011; 29:1670-7[39]	It's Now or Never: Fertility-Related Knowledge, Decision-Making Preferences, and Treatment Intentions in Young Women with Breast Cancer—An Australian Fertility Decision Aid Collaborative Group Study	Survey of women diagnosed with early breast cancer and reporting incomplete families (n=111)	• Effects of hormonal therapy on fertility • Established versus experimental FP options • Effect of pregnancy on cancer recurrence • Success rates of FP techniques • Time requirements for FP
Scanlon M. J Cancer 2012; 3: 217-25[29]	Patient Satisfaction with Physician Discussions of Treatment Impact on Fertility, Menopause and Sexual Health among Pre-menopausal Women with Cancer	Questionnaire applied to pre-menopausal women with cancer diagnosis in 2 time points (at enrollment and at 1-year follow-up) (n=104)	• Effects of cancer and cancer treatments on fertility • Effects of cancer and cancer treatments on sexual function
Schover LR. J Clin Oncol 2002;20:1880-9[30]	Knowledge and Experience Regarding Cancer, Infertility, and Sperm Banking in Younger Male Survivors	Postal survey sent to men with a new diagnosis of cancer at 14-40 years of age (n=201)	• Effects of cancer and cancer treatments on sperm quality • Infertility risk in boys *versus* girls • Amount of sperm needed for infertility treatments • Risk of cancer in future children

Reference	Title	Methods (sample)	Information needs / gaps in knowledge identified
Thewes B. J Clin Oncol 2005; 23:5155-65 [40]	Fertility- and Menopause-Related Information Needs of Younger Women with a Diagnosis of Early Breast Cancer	Mail questionnaire sent to women with a diagnosis of early-stage breast cancer aged ≤ 40 years at diagnosis (n=228)	• Contraception • Possibility of pregnancy after cancer treatment • Effects of pregnancy on cancer recurrence • Risks/benefits of having children after cancer • Effects of cancer treatments on future children • Statistics on infertility risks • Onset of infertility after cancer treatments
Zebrack B. Supp Care Cancer 2008;16:1353–60 [31]	Information and service needs for young adult cancer patients	Online survey of young adults aged 18–40 years and diagnosed with cancer between the ages of 15–35 (n=217)	• Infertility risks • Infertility treatments/services

In relation to the identification of local information needs, a sample of 31 cancer survivors answered and returned the questionnaire. It was not possible to calculate the response rate, as the total number of questionnaires distributed to patients by clinicians is not known. The mean age (± SD) of the participants was of 34.4 years (± 6.5), corresponding to a mean age of 26.6 years (± 7.5) at diagnosis. Most participants were females (n=27), and the most frequent cancer diagnoses were lymphoma (n=9), breast cancer (n=8) and osteosarcoma (n=8). The majority of survivors (n=23) had been treated with systemic chemotherapy. Almost one third (n=10) reported effects of cancer treatments on fertility, and 15 answered that they did not know or were unsure of those effects. Two patients used a fertility preservation technique before treatment initiation, and three patients had children after cancer treatment. All topics were rated as *Extremely important to be discussed* or *Very important to be discussed* by a significant majority of participants (Table 1.4). Concerning the strategies that are useful to inform cancer patients on these topics, consultation with a reproductive medicine specialist

and the supply of information through written information resources or the Internet were the most valued.

Table 1.4 List of information topics ordered according to the number of cancer survivors rating them as *Extremely important* or *Very important* to discuss before treatment initiation (n=31).

Information topics	Extremely important to discuss (n)	Very important to discuss (n)	Total (n)
Risks of effects of cancer treatments for future reproductive function/fertility	17	11	28
Risk of malformation in the offspring due to cancer treatments	23	5	28
Possibility of having children after cancer	18	10	28
Type of effects of cancer treatments on reproductive function/fertility	20	8	28
In women, risk of cancer recurrence due to pregnancy	21	6	27
Risk of genetic transmission of cancer to the offspring	19	8	27
FP options before and during treatments	15	12	27
Available FP techniques	13	14	27
Advantages of FP techniques	14	13	27
Disadvantages of FP techniques	16	11	27
Interference of FP in cancer treatment	16	11	27
Duration of effects of cancer treatments on reproductive function/fertility	18	8	26
Success rates	14	12	26
Availability of FP specialists	14	12	26
In women, risk of early menopause due to cancer treatments	17	7	24
Costs	15	9	24
How long the gametes can stay cryopreserved	15	9	24

FP – fertility preservation

3.2. Information resources

The contents of the information resources were developed with our previously mentioned purposes in mind (section 2.2) and the information needs most frequently identified in the international literature and/or reported by the local sample of survivors.

General criteria for writing health information for patients were collected from several published guidance documents[41-42] and organized according to the following themes: content (e.g., clearly defined aim), language (e.g., avoid paternalism and value judgements, use active voice, avoid technical terms), organization (e.g., use bullets and write short, single idea paragraphs), layout and graphics (e.g., avoid uppercase and italic, align text to the left), illustrations (e.g., use only to improve understanding) and learning and motivation (include interactive materials). These criteria supported the process of writing and organizing the information content (Table 1.5).

Table 1.5 – List of adopted quality topics and criteria for writing information for patients.

Quality topic	Criteria
Content	Clearly defined aim
	Focus on behavior changes
Language	Avoid paternalism and value judgements
	Readability level appropriate for 7th grade, at most
	Avoid technical terms, abbreviations, complex words
	Use active voice and conventional speech
Organization	Present the most relevant message first
	Use subtitles
	Use bullets
	Organize related information in lists
	Include five items, at maximum, in each list
	Write short, single idea paragraphs
	Summarize main ideas at the end of section/handout
Layout and Graphics	Use font size 12, at minimum, and sans serif type
	Avoid uppercase and italic
	Use bold only to highlight
	Contrast font with background
	Align text to the left
	Choose two different font types, at most
Illustrations	Used only to improve understanding
	Avoid complex line drawings

Quality topic	Criteria
	Use legends
Learning and Motivation	Include interactive materials

In the general handouts, the information was organized in the format of *Questions & Answers* as a form of dividing text and making it more attractive to read.[42] In the decision aids, after a brief introduction discussing the relevance of shared decision-making regarding FP, contents were structured according to the two main decision points: 1) the decision to use or not use a FP technique; 2) when applicable, the decision of which FP technique to choose. In each decision point, the positive and negative aspects of each option were presented. Moreover, in the second decision point, detailed information on the procedures and target populations for each FP technique was included. A third section was designed with a set of three q*uestions & answers* regarding general issues such as costs, maximum length of cryopreservation and fate of the non-used cryopreserved cells/tissues. Interactive components to increase learning and motivation (i.e., a box that patients can use to write questions and a small knowledge quiz at the end) were developed for all information resources. In the final section, other relevant sources of information were presented, including the address of the CFP's website and contact numbers of national telephone helplines on cancer and oncofertility.

Table 1.6 and Table 1.7 display images of the front pages and briefly outline the contents included in the handouts and decision aids, respectively. As the local sample of survivors reported that provision of information through the Internet would also be a useful strategy, the information contents were also adapted to be digitally displayed on the CFP's website.

Table 1.6 Front pages, titles and information contents of the handouts informing of the possible effects of cancer (and cancer treatments) on male and female fertility.

Front-page image	Title	Questions & Answers
	**Fertility in Men/Women with Cancer: Know the Risks**	▪ How to know if a woman/man is fertile? ▪ Is it possible to have children after cancer? ▪ When should the discussion with the doctor about the possible effects of cancer in fertility occur? ▪ How to know if fertility can be affected? ▪ How do cancer treatments affect fertility? Surgery, Radiotherapy, Chemotherapy…

Table 1.7 Front pages, titles and information contents of the decision aids for male and female cancer patients to support shared decision-making about FP.

Front-page image	Title	Information contents
	**Fertility in Men with Cancer: Know the Fertility Preservation Options**	▪ What is "fertility preservation"? ▪ What are the available options? ▪ Comprehensive information on procedures, indications, success rates and risks of each FP technique; ▪ Answers to frequent questions regarding costs, conservation and disposal of the cryopreserved material; ▪ Other sources of information: websites, telephone helplines; ▪ Interactive components: box to write questions; knowledge quiz.
	**Fertility in Women with Cancer: Know the Fertility Preservation Options**	▪ What is "fertility preservation"? ▪ What are the available options? ▪ 1st decision – to preserve or not fertility – positive and negative aspects of each option ▪ 2nd decision – which FP technique to choose - comprehensive information on procedures, indications, success rates and risks of each FP technique; ▪ Answers to general questions regarding costs, maintenance and disposal of non-used cryopreserved material; ▪ Other sources of information: websites, telephone helplines; ▪ Interactive components: box to write questions; knowledge quiz.

3.2.1. Evaluation of the information resources

Readability

The final versions of the handouts informing of the possible effects of cancer (and cancer treatments) on male and female fertility were rated by the Fernandez-Huerta readability index

as *fairly difficult* (score of 51). Readability of the decision aids to support FP decisions was classified as *difficult*, with scores of 46 for the male and 49 for the female decision aids.

Pre-test

In general, both cancer patients and healthcare professionals rated the information resources as easy to read, with contents that are relevant, complete and well organized. Only a few minor changes were necessary, mainly of language and sentence structure. Additionally, decision aids were considered by all participants as useful for shared decision-making and clinical practice.

Quality assessment

All information resources scored high on presentation quality, with EQUIP scores varying between 77 and 89%. Consistent with the recommendations from this tool, the resources produced are "ready for distribution and should be reviewed in two to three years". According to the criteria from the DISCERN instrument, the overall quality of the two decision aids developed was high (4 or 5 scores in all questions). This rating means that the information materials "are useful and appropriate sources of information about treatment choices and have the ability to support the patient's decisions".

3.2.2. Publication and dissemination

The handouts informing of cancer and cancer treatment effects on male and female fertility were published in 2015 by the LPCC, which is also circulating these resources through its campaigns and website. With the collaboration of the *Ordem dos Farmacêuticos – Secção Regional de Coimbra* (Portuguese Pharmaceutical Society – Center Regional Section; SRC-OF), the handouts were also distributed to pharmacies all over the country, in order to reach the population in a larger scale. More recently, the LPCC has also published the decision aids that are being distributed to oncologists and other cancer care clinicians, reproductive medicine specialists and fertility preservation centers, with the cooperation of SPMR and SPO.

Furthermore, all the produced information content is available, in Portuguese, on the website of the *Centre for Fertility Preservation of CHUC, EPE* (www.centropreservacaofertilidade.pt).

4. Discussion

We believe that our systematic method for the provision of patient information—assessing information needs, providing information to meet those needs, pre-testing the information resources with the target population and assessing their quality with validated instruments—is a sound approach to facilitate decision-making among cancer patients in the context of infertility risks and FP options. Moreover, the use of quality decision aids that describe the path of patient decision-making and the consequences of each separate decision will lead patients to more informed clinical judgments.[43-44]

Our results indicate that the developed resources are relevant, reliable, and useful and have the ability to support shared decisions in the context of FP. They were positively evaluated by cancer patients, cancer survivors and health professionals working in the cancer and reproduction settings and achieved high quality scores according to the instruments EQIP and DISCERN. Concerning readability, the general handouts and the decision aids were scored as *fairly difficult* and *difficult* to read, respectively, which means they are suitable for readers with at least high-school grade levels. These low levels of readability are potential barriers for their ability to inform patients and support shared decisions so it is important to further assess the resources in real contexts of decision. Nevertheless, readability scores must always be interpreted with caution. They assume that longer words and sentences are harder to read and do not measure comprehension or indicate if the words are familiar to the reader. For example, some recurrently used Portuguese words in the setting of reproduction and fertility preservation, such as "*espermatozoide*", "*fertilidade*" and "*congelação*", are common and easily understandable words, yet they negatively influence the readability scores because of their many syllables. In the specific case of decision aids (scored as *difficult to read*), it will be important to use direct measures of comprehension, such as their ability to promote shared decision-making and to reduce decisional conflict.

The developed resources were designed to target adult patients in reproductive age faced with a diagnosis of any type of cancer. Accordingly, no cancer-specific information about infertility risks or fertility preservation options was included. Furthermore, they may not be suitable for children or adolescents with cancer, since younger patients may have distinct needs and preferences regarding the provision of information.[45-46] It is also a fact that some of the identified information needs remained unmet. Some topics were beyond the scope of our resources (for instance, the effects of cancer in sexual function, contraception in cancer patients or information about infertility treatments), while for others, the information would

be influenced by the specific type of cancer (such as the risk of genetic transmission of cancer to offspring and the risk of cancer recurrence due to pregnancy). Clearly, these are subjects to include in upcoming information resources. Other limitations of our study are related to the methods for the assessment of information needs. Due to time constraints, qualitative studies were not included in the literature search, and the locally applied questionnaire had a small number of participants.

We wish to highlight the multidisciplinary context in which this project has been carried out, involving cancer patients and survivors, a cancer patients' organization (LPCC), oncologists and other cancer care professionals and professional and scientific societies in the fields of oncology and reproductive medicine. We hope this intense cooperation will contribute to a wider dissemination of the developed information materials to the various stakeholders in the process of cancer care and to a more effective clinical implementation. Additionally, it is important to note that information resources directed to oncologists were also developed in the context of this program, including a main booklet with comprehensive contents, tailored to the needs of clinicians working with cancer patients, and a brochure with summarized contents intended for other cancer care professionals and primary care professionals.[47]

The developed resources are already available to the Portuguese population and to cancer patients in several institutions all over the country. Our next step will be to evaluate the resources with cancer patients using relevant measures such as acceptability, knowledge, decision conflict or self-efficacy.

5. Conclusion

The need to inform cancer patients in an effective and timely manner of their infertility risks and the possibility and options of FP is a recognized relevant issue in the context of quality of life in cancer survival. Through a systematic approach and establishing a multidisciplinary collaboration, information resources directed to cancer patients' needs were successfully developed and disseminated and will contribute to timely, shared and informed clinical decisions in the context of FP.

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References

1. Knopman JM, Papadopoulos EB, Grifo JA, Fino ME, et al. Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood. Lancet Oncol. 2010;11:490–8.
2. Donnez J, Dolmans MM. Preservation of fertility in females with haematological malignancies. Br J Haematol. 2011;154:175-84.
3. Schover LR. Sexuality and Fertility after Cancer. Hematol 2005;1:523-7.
4. Deshpande NA, Braun IM, Meyer FL. Impact of fertility preservation counseling and treatment on psychological outcomes among women with cancer: A systematic review. Cancer. 2015;121:3938-47.
5. Woodruff TK. The Oncofertility Consortium - addressing fertility in young people with cancer. Nat Rev Clin Oncol. 2010;7:466–75.
6. Lee SJ, Schover LR, Partridge AH, Patrizio P, et al. American Society of Clinical Oncology. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24: 2917-31.
7. ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013;24:vi160-70.
8. Quinn GP, Vadaparampil ST, Lee JH, Jacobsen PB, et al. Physician referral for fertility preservation in oncology patients: a national study of practice behaviors. J Clin Oncol. 2009; 27:5952-7.
9. Quinn GP, Vadaparampil ST, Gwede CK, Miree C, et al. Discussion of fertility preservation with newly diagnosed patients: oncologists' views. J Cancer Surviv. 2007;1:146–55.
10. Shimizu C, Bando H, Kato T, Mizota Y, et al. Physicians' knowledge, attitude, and behavior regarding fertility issues for young breast cancer patients: a national survey for breast care specialists. Breast Cancer. 2013;20:230-40.
11. King JW, Davies MC, Roche N, Abraham JM, et al. Fertility preservation in women undergoing treatment for breast cancer in the UK: a questionnaire study. Oncologist. 2012;17:910-6.
12. Schover LR, Brey K, Lichtin A, Lipshultz LI, et al. Oncologists' attitudes and practices regarding banking sperm before cancer treatment. J Clin Oncol. 2002;20:1890-7.
13. Forman EJ, Anders CK, Behera MA. A nationwide survey of oncologists regarding treatment-related infertility and fertility preservation in female cancer patients. Fertil Steril. 2010;94:1652-6.
14. Wilkes S, Coulson S, Crosland A, Rubin G, et al. Experience of fertility preservation among younger people diagnosed with cancer. Hum Fertil (Camb). 2010;13:151-8.
15. Partridge AH, Gelber S, Peppercorn J, Sampson E, et al. Web-Based Survey of Fertility Issues in Young Women With Breast Cancer. J Clin Oncol. 22:4174-83.
16. Peate M, Meiser B, Hickey M, Friedlander M. The fertility-related concerns, needs and preferences of younger women with breast cancer: a systematic review. Breast Cancer Res Treat. 2009;116:215-23.
17. Schover LR, Brey K, Lichtin A, Lipshultz LI, et al. Knowledge and Experience Regarding Cancer, Infertility, and Sperm Banking in Younger Male Survivors. J Clin Oncol. 2002;20:1880-9.
18. Balthazar U, Deal AM, Fritz MA, Kondapalli LA, et al. The current fertility preservation consultation model: are we adequately informing cancer patients of their options? Hum Reprod. 2012, 27:2413–9.
19. Michie S, Dormandy E, Marteau TM. The multi-dimensional measure of informed choice: a validation study. Patient Educ Couns. 2002;48:87-91.
20. Woolf SH, Chan EC, Harris R, Sheridan SL, et al. Promoting Informed Choice: transforming health care to dispense knowledge for decision making. Ann Intern Med. 2005;143:293-300.
21. Stacey D, Samant R, Bennett C. Decision making in oncology: a review of patient decision aids to support patient participation. CA Cancer J Clin. 2008;58:293-304.
22. Stacey D, Bennett CL, Barry MJ, Col NF, et al. Decision aids to help people who are facing health treatment or screening decisions. Cochrane Database Syst Rev. 2011;10:CD001431.
23. Hunink MG. In search of tools to aid logical thinking and communicating about medical decision making. Med Decis Making. 2001;21:267-77.
24. Garvelink MM, ter Kuile MM, Stiggelbout AM, de Vries M. Values clarification in a decision aid about fertility preservation: does it add to information provision? BMC Med Inform Decis Mak. 2014;14:68.
25. Peate M, Meiser B, Cheah BC, Saunders C, et al. Making hard choices easier: a prospective, multicentre study to assess the efficacy of a fertility-related decision aid in young women with early-stage breast cancer. Br J Cancer. 2012;106:1053-61.
26. Almeida Santos AT, Sousa G, Teixeira A, Cardoso P, et al. Recommendations for the preservation of reproductive potential in cancer patients. Rev Port Oncol. 2016; 2:5-24.
27. Armuand GM, Rodriguez-Wallberg KA, Wettergren L, Ahlgren J, et al. Sex Differences in Fertility-Related Information Received by Young Adult Cancer Survivors. J Clin Oncol. 2012;30:2147-53.
28. Jukkala AM, Azuero A, McNees P, Bates GW, et al. Self-assessed knowledge of treatment and fertility preservation in young women with breast cancer. Fertil Steril. 2010;94:2396-8.
29. Scanlon M, Blaes A, Geller M, Majhail NS, et al. Patient Satisfaction with Physician Discussions of Treatment Impact on Fertility, Menopause and Sexual Health among Pre-menopausal Women with Cancer. J Cancer. 2012;3:217-25.

30. Schover LR, Brey K, Lichtin A, Lipshultz LI, et al. Knowledge and Experience Regarding Cancer, Infertility, and Sperm Banking in Younger Male Survivors. J Clin Oncol. 2002;20:1880-9.
31. Zebrack B. Information and service needs for young adult cancer patients. Supp Care Cancer. 2009;17:349-57.
32. Charnock D, Shepperd S, Needham G, Gann R. DISCERN: an instrument for judging the quality of written consumer health information on treatment choices. J Epidemiol Comm Health. 1999;53:105–11.
33. Fernández-Huerta J. Medidas sencillas de lecturabilidad. Consigna. 1959;214:29–33.
34. Flesch R. How to Write Plain English. University of Canterbury Business School. Accessed October 7, 2017 from http://www.mang.canterbury.ac.nz/writing_guide/writing/flesch.shtml.
35. Moult B, Franck LS, Brady H. Ensuring Quality Information for Patients: development and preliminary validation of a new instrument to improve the quality of written health care information. Health Expect. 2004;7:165–75.
36. Balthazar U, Fritz, MA, Mersereau JE. Fertility preservation: a pilot study to assess previsit patient knowledge quantitatively. Fertil Steril. 2011;95:1913-6.
37. Balthazar U, Deal AM, Fritz MA, Kondapalli LM, et al. The current fertility preservation consultation model: are we adequately informing cancer patients of their options? Hum Reprod. 2012;27:2413–19.
38. Meneses K, McNees P, Azuero, A, Jukkala A. Development of the Fertility and Cancer Project: An Internet Approach to Help Young Cancer Survivors. Oncol Nurs Forum. 2010;37:191-7.
39. Peate M, Meiser B, Friedlander M, Zorbas H, et al. It's Now or Never: Fertility-Related Knowledge, Decision-Making Preferences, and Treatment Intentions in Young Women With Breast Cancer—An Australian Fertility Decision Aid Collaborative Group Study. J Clin Oncol. 2011;29:1670-7.
40. Thewes B, Meiser B, Taylor A, Phillips KA, et al. Fertility- and Menopause-Related Information Needs of Younger Women With a Diagnosis of Early Breast Cancer. J Clin Oncol. 2005;23:5155-65.
41. Hoffmann T, Worrall L. Designing effective written health education materials: Considerations for health professionals. Disab Rehabilit. 2004;26:1166–73.
42. General guidance on writing information for patients. NHS Department of Health. In: NHS Toolkit for producing patient information. 2003. Accessed November, 2013 from: https://www.uea.ac.uk/documents/246046/0/Toolkit±for±producing±patient±information.pdf
43. Gardino SL, Jeruss JS, Woodruff TK. Using decision trees to enhance interdisciplinary team work: the case of oncofertility. J Assist Reprod Genet. 2010;27: 227–31.
44. Aleem IS, Jalal H, Aleem IS, Sheikh AA, Bhandari M. Clinical decision analysis: incorporating the evidence with patient preferences. Patient Prefer Adherence. 2009;3:21–4.
45. Murphy D, Sawczyn KK, Quinn GP. Using a patient-centered approach to develop a fertility preservation brochure for pediatric oncology patients: a pilot study. J Pediatr Adolesc Gynecol. 2012;25:114-21.
46. Wyns C, Collienne C, Shenfield F, et al. Fertility preservation in the male pediatric population: factors influencing the decision of parents and children. Hum Reprod. 2015 Sep;30(9):2022-30.
47. Silva C, Almeida-Santos AT, Melo C, Rama ACR. J Adolesc Young Adult Oncol. 2017;6:353-57.

1.1.2 Published information resources for patients

Fertility in Women with Cancer: Know the risks
[*Fertilidade na Mulher com Cancro: Conheça os Riscos*]

Fertility in Men with Cancer: Know the risks
[*Fertilidade no Homem com Cancro: Conheça os Riscos*]

Cristina Silva, Ana Teresa Almeida-Santos, Ana Cristina Ribeiro Rama

LIGA PORTUGUESA
CONTRA O CANCRO

FERTILIDADE NA MULHER COM CANCRO

CONHEÇA OS RISCOS

Como saber se uma mulher é fértil?
- A capacidade de ter filhos depende da presença de óvulos nos ovários e do normal funcionamento do útero.
- Em cada mulher, o número de óvulos é limitado e diminui com a idade, a partir dos 35 anos.
- É possível ter ideia da fertilidade pelos níveis de hormonas e pelo exame dos ovários com ecografia.

Depois do cancro ainda é possível ter filhos?
- É possível. No entanto, as mulheres com cancro têm menos possibilidades de conseguirem ser mães.
- Alguns tratamentos do cancro podem causar alterações no funcionamento dos ovários ou no útero.
- Os problemas de fertilidade podem ser passageiros mas algumas mulheres com cancro entram na menopausa mais cedo.

Quando conversar com o médico sobre os possíveis efeitos do cancro na fertilidade?
- Na altura do diagnóstico e antes de começar os tratamentos.
- Esta discussão é essencial para todas as mulheres com cancro que ainda querem ser mães.

Como saber se a fertilidade pode ser afetada?
- É difícil saber. O médico avalia, em cada caso, o risco de infertilidade.
- O risco depende da idade e também do tipo e dose dos tratamentos a realizar.

Como é que os tratamentos podem afetar a fertilidade?

CIRURGIA
- Quando a cirurgia interfere com a função do útero ou dos ovários.

RADIOTERAPIA / QUIMIOTERAPIA
- Podem diminuir o número de óvulos ou afetar a capacidade de manter uma gravidez.
- O risco é maior quando se administram doses maiores.

TERAPÊUTICA HORMONAL
- Pode afetar a fertilidade mas, em geral, os efeitos são temporários.
- O tratamento com tamoxifeno dura vários anos; a mulher só pode tentar engravidar se suspender ou terminar o tratamento.

TRANSPLANTE
- O transplante de células da medula óssea ou sangue periférico, associado à radioterapia ou quimioterapia em altas doses, provoca muitas vezes infertilidade permanente.

Informe-se com o seu médico, antes de iniciar tratamentos, sobre as instituições que disponibilizam técnicas de preservação da fertilidade.

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LIGA PORTUGUESA
CONTRA O CANCRO

FERTILIDADE NO HOMEM COM CANCRO

CONHEÇA OS RISCOS

Como saber se um homem é fértil?
- A capacidade de ter filhos depende da presença de espermatozóides no esperma e da sua capacidade para fecundar óvulos.
- É possível ter uma ideia da quantidade e qualidade dos espermatozóides com a realização de um exame chamado "espermograma".

Depois do cancro ainda é possível ter filhos?
- É possível. No entanto, os homens com cancro têm menos possibilidades de conseguirem ser pais.
- Alguns tipos de cancro e alguns tratamentos podem afetar, de forma passageira ou permanente, a produção de espermatozóides.
- Alguns tratamentos podem alterar os espermatozóides, que deixam de ser capazes de fecundar óvulos.

Quando conversar com o médico sobre os possíveis efeitos do cancro na fertilidade?
- Na altura do diagnóstico e antes de começar os tratamentos.
- Esta discussão é essencial para todos os homens com cancro que ainda querem ser pais.

Como saber se a fertilidade pode ser afetada?
- É difícil saber. O médico avalia, em cada caso, o risco de infertilidade.
- Em certos casos, o próprio cancro pode afetar a fertilidade (cancro no testículo e alguns linfomas).
- O risco depende também do tipo e dose dos tratamentos.

Como é que os tratamentos podem afetar a fertilidade?

CIRURGIA
- Quando a cirurgia interfere com a função do pénis ou do testículo.

RADIOTERAPIA / QUIMIOTERAPIA
- Podem afetar a produção de espermatozóides ou a sua capacidade de fecundar óvulos.
- O risco é maior quando são administradas doses maiores.

TRANSPLANTE
- O transplante de células da medula óssea ou sangue periférico, associado à radioterapia ou quimioterapia em altas doses, provoca muitas vezes infertilidade permanente.

Informe-se com o seu médico, antes de iniciar tratamentos, sobre as instituições que disponibilizam técnicas de preservação da fertilidade.

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LIGA PORTUGUESA
CONTRA O CANCRO

Fertility in Women with Cancer: Know the Fertility Preservation Options [*Fertilidade na Mulher com Cancro: Conheça as Opções de Preservação de Fertilidade*]

Fertility in Men with Cancer: Know the Fertility Preservation Options [*Fertilidade no Homem com Cancro: Conheça as Opções de Preservação de Fertilidade*]

Cristina Silva, Ana Teresa Almeida-Santos, Ana Cristina Ribeiro Rama

LIGA PORTUGUESA
CONTRA O CANCRO

AO PRESERVAR A FERTILIDADE ESTARÁ A TENTAR PROTEGER A SUA CAPACIDADE PARA SER PAI DEPOIS DO CANCRO.

Esta informação destina-se a homens com cancro e às suas companheiras. Pretende informar sobre as várias opções de preservação da fertilidade no homem com cancro.

PORQUÊ "PRESERVAR A FERTILIDADE"?

Alguns tratamentos do cancro podem diminuir a fertilidade do homem, ou seja, a sua capacidade de ter filhos.

- A capacidade do homem ter filhos depende da existência de espermatozoides (células reprodutoras masculinas) nos testículos e da sua capacidade para fecundarem os óvulos (células reprodutoras femininas);
- O risco de infertilidade é difícil de prever e depende do tipo de cancro e do tipo e/ou dose dos tratamentos que fizer;
- A infertilidade pode ser temporária mas, por vezes, os testículos dos homens com cancro deixam de produzir espermatozoides (azoospermia).

Se gostaria de ter (mais) filhos no futuro:

- Antes de iniciar qualquer tratamento, fale com o seu médico sobre os efeitos dos tratamentos do cancro na fertilidade;
- Faça-o quanto antes, para aumentar as possibilidades de proteger a sua fertilidade;
- O seu médico oncologista vai avaliar o risco de infertilidade associado ao seu tipo de cancro e aos tratamentos que vai realizar.

OPÇÃO I

CONGELAÇÃO DE ESPERMA

Como?

1º São obtidas amostras de esperma, geralmente por masturbação;

2º O esperma é congelado a temperaturas muito baixas (criopreservação);

3º Quando o homem assim desejar, o esperma pode ser descongelado e os espermatozoides introduzidos diretamente no útero da mulher; a este processo dá-se o nome de Inseminação Intrauterina (IIU);

4º Em alternativa, os espermatozoides podem ser usados para fecundar óvulos em laboratório, através de técnicas de Procriação Medicamente Assistida (PMA);

5º Os embriões obtidos são transferidos para o útero da mulher, de forma a tentar iniciar uma gravidez.

Para quem?
Todas os homens e rapazes após a puberdade.

ASPECTOS POSITIVOS

- Na maioria dos homens, esta técnica permite preservar a fertilidade de forma muito fácil e rápida;
- É um procedimento, em geral, eficaz e seguro;
- Há muita experiência na utilização desta técnica para preservar a fertilidade do homem com cancro.

RESULTADOS / TAXAS DE SUCESSO

Em cada 100 ciclos de fertilização *in vitro* utilizando esperma congelado de doentes com cancro, cerca de 40 a 50 resultarão numa gravidez.

ASPECTOS NEGATIVOS

Esta técnica não permite preservar a fertilidade de rapazes antes da puberdade.

RISCOS

- Não existe qualquer risco para o homem que realiza congelação de esperma;
- Até à data, os estudos não revelaram risco aumentado de cancro ou malformações em crianças nascidas utilizando esperma congelado/descongelado.

OPÇÃO II

CONGELAÇÃO DO TECIDO DO TESTÍCULO

Como?

1º É realizada uma pequena cirurgia (biópsia) para se recolherem amostras de tecido do testículo. Geralmente utiliza-se anestesia local;

2º As amostras de tecido são preparadas e congeladas a temperaturas muito baixas (criopreservação);

3º Quando o homem assim desejar, o tecido testicular é descongelado e os espermatozoides usados para fecundar os óvulos da sua companheira, em laboratório, através de técnicas de Procriação Medicamente Assistida (PMA);

4º Os embriões obtidos são transferidos para o útero da mulher, de forma a tentar iniciar uma gravidez.

Esta técnica pode ser usada em duas situações diferentes:

- Nos homens adultos e rapazes após a puberdade, quando o esperma ejaculado não contém espermatozoides; nestas situações, a congelação de tecido do testículo é uma técnica eficaz e segura;
- Em rapazes na pré-puberdade; nestes casos é a única opção disponível mas é uma técnica ainda experimental, ou seja, há pouca informação sobre a sua eficácia e segurança.

ASPECTOS POSITIVOS

- É a única técnica que permite preservar a fertilidade de homens sem espermatozoides no ejaculado;
- É a única técnica que poderá preservar a fertilidade de rapazes na pré-puberdade.

RESULTADOS / TAXAS DE SUCESSO

As taxas de gravidez, embora muito variáveis, são geralmente inferiores às que se obtêm com o uso de esperma congelado.

ASPECTOS NEGATIVOS

Pode não ser possível obter espermatozoides no tecido testicular colhido.

RISCOS

- Após a recolha de tecido por biópsia, podem aparecer hematomas ("sangue pisado"), inchaço ou dor nos testículos. As hemorragias ou infeções são problemas menos frequentes. São raras complicações mais graves, como danos nos nervos ou atrofia do testículo;
- Até à data, os estudos não revelaram risco aumentado de cancro, malformações ou complicações na gravidez ou parto, em crianças nascidas utilizando espermatozoides de tecido testicular congelado/descongelado.

QUAIS SÃO OS CUSTOS ASSOCIADOS ÀS TÉCNICAS DE PRESERVAÇÃO DA FERTILIDADE?

Em geral, nas instituições públicas do Serviço Nacional de Saúde, a recolha e congelação de esperma ou tecido do testículo são procedimentos gratuitos.

POR QUANTO TEMPO PODEM FICAR CONGELADOS O ESPERMA OU O TECIDO DO TESTÍCULO?

O esperma ou o tecido do testículo podem ser congelados por um período máximo de três anos, de acordo com a lei portuguesa. Após este período, terá de ser assinado um consentimento de manutenção da congelação.

O QUE ACONTECE AO ESPERMA OU TECIDO DO TESTÍCULO CONGELADOS QUE NÃO SEJAM UTILIZADOS?

O esperma ou tecido do testículo não utilizados podem ser eliminados ou usados para fins científicos, de acordo com a decisão do homem.

GOSTAVA DE SABER MAIS SOBRE ESTE TEMA?

- Para mais informação sobre os efeitos dos tratamentos do cancro na fertilidade do homem, consulte o folheto **"Fertilidade no Homem com Cancro: Conheça os riscos"**;
- Fale com o seu médico oncologista;
- Contacte a Linha SOS Oncofertilidade: 800 919 940 (2ª a 6ª feira, das 9h às 18h);
- Visite o website do **Centro de Preservação da Fertilidade do CHUC, EPE**, em **www.centropreservacaofertilidade.pt**.

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APOIO

MERCK SERONO

LIGA PORTUGUESA CONTRA O CANCRO

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IL08B

LIGA PORTUGUESA
CONTRA O CANCRO

AO PRESERVAR A FERTILIDADE ESTARÁ A TENTAR PROTEGER A SUA CAPACIDADE PARA ENGRAVIDAR E SER MÃE DEPOIS DO CANCRO.

Esta informação destina-se a mulheres com cancro e aos seus companheiros. Pretende informar sobre as várias opções de preservação da fertilidade e ajudar na escolha da opção mais adequada.

PORQUÊ "PRESERVAR A FERTILIDADE"?

- A capacidade da mulher ter filhos depende da existência de óvulos (células reprodutoras femininas) nos ovários e do normal funcionamento do útero;
- O risco de infertilidade é difícil de prever e depende da sua idade e do tipo e/ou dose dos tratamentos que fizer;
- A infertilidade pode ser temporária ou permanente;
- Por vezes, as mulheres com cancro entram na menopausa mais cedo.

QUAIS SÃO AS OPÇÕES DISPONÍVEIS PARA PRESERVAR A FERTILIDADE?

É muito importante que participe na escolha da opção mais indicada para si. Para isso tem que estar informada! Se gostaria de ter (mais) filhos no futuro:

- Antes de iniciar qualquer tratamento, fale com o seu médico sobre os efeitos dos tratamentos do cancro na fertilidade;
- Faça-o quanto antes para aumentar as possibilidades de proteger a sua fertilidade;
- O seu médico oncologista vai avaliar o risco de infertilidade associado aos tratamentos que vai realizar;
- Todas as decisões devem ser tomadas pela equipa médica e pela doente;
- Se quiser discutir as opções disponíveis com um médico especialista em reprodução, peça para ser encaminhada para um centro de preservação da fertilidade.

1ª DECISÃO

Depois de receber informação sobre o seu risco de infertilidade, deve participar na **decisão de recorrer ou não às técnicas de preservação da fertilidade**.

OPÇÃO I

NÃO QUERO RECORRER A NENHUMA TÉCNICA DE PRESERVAÇÃO DA FERTILIDADE

Esta opção pode ser a melhor para si quando...

- O seu médico considera que não é apropriado, no seu caso, preservar a fertilidade;
- O risco de infertilidade associado aos tratamentos que vai fazer é baixo;
- Não considera importante ser mãe, de forma biológica, depois do cancro;
- Não pretende submeter-se aos procedimentos médicos necessários para preservar a fertilidade.

✚ ASPECTOS POSITIVOS

- Não necessita de se submeter aos procedimentos médicos para preservação da fertilidade.

▬ ASPECTOS NEGATIVOS

- Mesmo que venha a querer engravidar, pode não conseguir depois dos tratamentos do cancro;
- Se não preservar a fertilidade, poderá ter menos hipóteses de vir a ser mãe depois do cancro.

OPÇÃO II

QUERO RECORRER A UMA TÉCNICA DE PRESERVAÇÃO DA FERTILIDADE

✚ ASPECTOS POSITIVOS

- Preservar a fertilidade pode ser a única forma de conseguir ser mãe depois do cancro;

▬ ASPECTOS NEGATIVOS

- As técnicas de preservação da fertilidade não garantem uma gravidez no futuro;
- Para preservar a fertilidade pode ser necessário dispor de 2 a 3 semanas, antes de iniciar tratamentos.

2ª DECISÃO

Se decidir preservar a fertilidade, deve participar na escolha da técnica mais indicada para si. A escolha da técnica deve ter em atenção a sua idade, o seu tipo de cancro, o tipo de tratamentos que vai realizar e as suas preferências.

Existem **duas opções para preservar a fertilidade da mulher** com cancro, antes de iniciar tratamentos:

OPÇÃO I - Congelação de óvulos
OPÇÃO II - Congelação de tecido do ovário

Ambas têm aspetos positivos e negativos. Para que possa participar na escolha, é importante que **leia e entenda as informações sobre cada uma das técnicas**.

OPÇÃO I
CONGELAÇÃO DE ÓVULOS

Como?

1º São administradas hormonas à mulher, para estimular a produção de óvulos;
2º Os óvulos são aspirados por via vaginal com a ajuda de uma agulha (punção folicular). Normalmente utiliza-se anestesia geral;
3º Os óvulos obtidos são congelados a temperaturas muito baixas (criopreservação);
4º Quando a mulher desejar, os óvulos podem ser descongelados e fecundados com espermatozóides, em laboratório, utilizando técnicas de fertilização *in vitro* (FIV);
5º Os embriões obtidos podem ser transferidos para o útero da mulher, de forma a tentar iniciar uma gravidez.

Para quem?
Todas as mulheres e raparigas após a puberdade.

✚ ASPECTOS POSITIVOS

- É geralmente a melhor opção, por ser aquela que tem maior taxa de sucesso;
- É uma técnica eficaz e segura: em todo o mundo, já existem mais de 2000 crianças nascidas após congelação de óvulos.

RESULTADOS / TAXAS DE SUCESSO

- Em teoria, a mulher precisaria de dispor de 20 óvulos congelados para garantir uma gravidez;
- Por cada 100 óvulos congelados, cerca de 5 conduzirão a uma gravidez de sucesso;
- À medida que a idade da mulher aumenta, as hipóteses de sucesso diminuem.

ASPECTOS NEGATIVOS

- Para a recolha de óvulos, é necessário que a mulher fique exposta a níveis elevados de hormonas;
- Pode ser necessário dispor de 2 a 3 semanas para realizar esta técnica;
- É necessário recorrer a fertilização *in vitro* para se produzirem embriões.

RISCOS

- A estimulação do ovário com hormonas pode provocar algum desconforto e inchaço abdominal que desaparecem em poucos dias; alguns casos mais graves podem levar a hospitalização, mas são muito raros;
- A exposição aos níveis elevados de hormonas pode estimular o crescimento de alguns tipos de cancro da mama. Para reduzir este risco pode ser incluído no tratamento um medicamento chamado Letrozol;
- A punção folicular para recolha dos óvulos pode causar dor abdominal passageira, sendo raras as infeções ou hemorragias.

OPÇÃO II

CONGELAÇÃO DE TECIDO DO OVÁRIO

Como?

1º Um dos ovários da mulher é retirado por meio de uma cirurgia pouco invasiva (laparoscopia), sob anestesia geral;

2º Preparam-se pequenos fragmentos, muito finos, de tecido do ovário, que são congelados a temperaturas muito baixas (criopreservação);

3º Quando a mulher desejar, o tecido do ovário pode ser descongelado e reintroduzido na zona do ovário ou noutro local do organismo (transplante);

4º Se o transplante for bem-sucedido, o ovário pode voltar a produzir hormonas e óvulos, e a mulher pode tentar engravidar naturalmente.

Para quem?
Mulheres e raparigas após a puberdade; crianças antes da puberdade.

ASPECTOS POSITIVOS

- É a única técnica que permite preservar a fertilidade em raparigas pré-púberes;
- Não exige tratamento com hormonas;
- Não impede que os tratamentos do cancro sejam iniciados de imediato, se necessário;
- Após o transplante, o ovário pode voltar a funcionar, evitando-se uma menopausa precoce;
- Se esta técnica for bem-sucedida, a mulher pode tentar engravidar de forma natural.

RESULTADOS / TAXAS DE SUCESSO

- As taxas de sucesso são difíceis de calcular porque não se sabe ao certo quantas mulheres já usaram esta técnica;
- De qualquer forma, a congelação de tecido do ovário já permitiu o nascimento de cerca de 40 bebés, em todo o mundo.

ASPECTOS NEGATIVOS

- Esta técnica é considerada experimental, ou seja, ainda não há muita informação sobre a sua eficácia e segurança;
- Exige a realização de uma pequena cirurgia (colheita do ovário por laparoscopia).

RISCOS

- Efeitos adversos, pouco frequentes, em resultado da anestesia geral;
- Após a cirurgia pode surgir dor no local da incisão; são raros efeitos mais graves, como infeções e hemorragias;
- Em certos casos, o transplante de tecido do ovário pode voltar a introduzir células cancerígenas no organismo. No entanto, existem técnicas para avaliar se o tecido está contaminado, antes de ser transplantado.

*NOTA CONGELAÇÃO DE EMBRIÕES

A legislação portuguesa só permite a aplicação de técnicas de Procriação Medicamente Assistida (PMA), ou seja, a produção de embriões em laboratório, em **casais com diagnóstico de infertilidade ou para evitar a transmissão de doença grave**. É ainda importante perceber que os embriões produzidos são propriedade do casal e que esta técnica não protege verdadeiramente a autonomia reprodutiva da mulher. Assim, a congelação de embriões não deverá ser oferecida, às mulheres com cancro, como técnica de preservação da fertilidade.

QUAIS SÃO OS CUSTOS ASSOCIADOS ÀS TÉCNICAS DE PRESERVAÇÃO DA FERTILIDADE?

CONGELAÇÃO DE ÓVULOS

Nas instituições do Serviço Nacional de Saúde, a doente apenas terá que pagar os medicamentos para estimulação do ovário. **O custo, para a doente, pode variar entre 200 a 500 euros**, dependendo do tipo de medicamentos utilizados. A Liga Portuguesa Contra o Cancro, no âmbito do apoio social, poderá minimizar estes custos.

CONGELAÇÃO DE TECIDO DO OVÁRIO

Nas instituições do Serviço Nacional de Saúde, a recolha e congelação de tecido do ovário não implicam, em geral, quaisquer custos para as doentes oncológicas.

POR QUANTO TEMPO PODEM FICAR CONGELADOS OS ÓVULOS E O TECIDO DO OVÁRIO?

Os óvulos ou tecido do ovário congelados podem ser conservados por um prazo máximo de 3 anos, de acordo com a lei portuguesa. Após este período, terá de ser assinado um consentimento para a manutenção da congelação.

O QUE ACONTECE AOS ÓVULOS OU TECIDO DO OVÁRIO CONGELADOS QUE NÃO SEJAM UTILIZADOS?

Os óvulos ou tecido do ovário que não sejam utilizados poderão ser eliminados ou usados em investigação, de acordo com a decisão da mulher.

GOSTAVA DE SABER MAIS SOBRE ESTE TEMA?

- Para mais informação sobre os efeitos dos tratamentos do cancro na fertilidade da mulher, consulte o folheto **"Fertilidade na Mulher com Cancro: Conheça os riscos"**;
- Fale com o seu médico oncologista;
- Contacte a Linha SOS Oncofertilidade: 800 919 940 (2ª a 6ª feira, das 9h às 18h);
- Visite o website do **Centro de Preservação da Fertilidade do CHUC, EPE**, em **www.centropreservacaofertilidade.pt**.

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OUTUBRO 2015

APOIO

MERCK SERONO

LIGA PORTUGUESA CONTRA O CANCRO

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1.2 Development of information resources for healthcare professionals

1.2.1 Decision on Fertility Preservation in Cancer Patients: Development of Information Materials for Healthcare Professionals

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Abstract

Infertility is a potential side effect of cancer chemotherapy. As the number of AYA-aged survivors increases, future fertility becomes an important issue. However, many patients are not adequately informed and oncologists point the lack of information as a barrier to discussion. Our aim was to produce information materials, tailored to oncologists' needs to promote and support discussion on infertility risk and fertility preservation with AYA-aged patients.

After literature review, information materials were successfully developed and are currently being distributed to healthcare professionals, in Portugal, with the collaboration of several national organizations. These information materials will contribute to shared, informed decisions regarding fertility preservation in AYA-aged patients.

1. Introduction

Infertility is a recognized potential adverse effect of several cancer treatments. In relation to chemotherapy, the degree of gonadal toxicity is influenced by several factors such as the nature of antineoplastic agents, total dose administered and patients' age.[1,2] Additionally, the number of AYA-aged survivors is increasing, as a consequence of earlier diagnosis and significant progresses in cancer treatment.[1,3] Besides the repercussions of the disease, these patients will have additional concerns related with the effects on their future fertility. Several studies document that future fertility is an important issue for cancer patients and survivors[4-6] and, therefore, shared decision concerning fertility preservation (FP) must take place at the time of diagnosis. In this context, oncofertility, a term created in 2006 by Professor Teresa Woodruff, has emerged as a multidisciplinary field with the purpose to fulfil the needs of AYA-aged patients regarding their reproductive potential.[7]

According to recommendations of international organizations on cancer care, namely the European Society of Medical Oncology (ESMO)[8] and the American Society of Clinical Oncology (ASCO),[9] healthcare providers should address infertility risks with all cancer patients treated during their reproductive years. Moreover, they must be prepared to discuss FP options or to refer potential patients to reproductive medicine specialists. Despite the above recommendations, several international studies indicate that professionals caring for cancer patients do not address these issues and a considerable proportion of AYA-aged patients is not informed on the possibilities regarding FP.[10–13] The main reasons reported by health professionals were the lack of knowledge, access to reproduction specialists, and information on FP options, especially those remaining experimental. Patient-related factors such as bad

prognosis, terminal disease, or the need to postpone treatments were also pointed to contribute.[11]

In Portugal, the oncofertility area is taking its first steps. Nevertheless, a variety of techniques for male and female FP are available at a few specialized institutions of the National Healthcare System, including the *Centre for Fertility Preservation* (Centro para a Preservação da Fertilidade, CPF) of CHUC, EPE, in Coimbra. Recently, the *Sociedade Portuguesa de Medicina Reprodutiva* (SPMR; Portuguese Society of Reproductive Medicine) endorsed the organization of the first and second Portuguese Oncofertility Meetings, with the purpose of implementing an integrated national practice concerning FP for cancer patients. In this process, the CFP has been at the front line, actively promoting awareness of this new field and disseminating information regarding infertility risks and FP options both to AYA-aged patients and healthcare professionals. One specific objective of this information program was the production of information materials for Portuguese cancer care professionals, tailored to the respective reported information needs, to promote and support discussion with AYA-aged patients on the topics of infertility risks and FP.

2. Methods

2.1 Assessment of information needs

To identify worldwide reported information needs, a literature search was conducted on Medline, through PubMed, combining the following MeSH terms: Neoplasms, Antineoplastic Agents/adverse effects, Fertility/drug effects, Fertility Preservation, Sperm banks, Health Knowledge, Practice, and Attitude of Health Personnel. Quantitative studies reporting oncologists' information needs or gaps in knowledge concerning infertility risks and FP or barriers to FP implementation were selected and critically evaluated.

2.2 Production of information

Information contents were selected to accomplish two main objectives: 1) to alert for the need to discuss infertility risks with patients and to help healthcare professionals estimating those risks; 2) to promote knowledge on the available male and female FP options. The latest published evidence on infertility risks associated with cancer treatments was identified through literature search, namely regarding mechanisms and adverse effects of cancer treatments on fertility, factors associated with infertility risk and tools available for risk calculation. Regarding FP techniques, current evidence-based information on clinical indications, time requisites, success rates, risks and advantages/disadvantages of each FP

technique was gathered, also by literature search. Published clinical guidelines on FP in cancer patients were also identified.

A main booklet directed to clinicians working with cancer patients with comprehensive contents was prepared. This professional group presents the greatest information needs as they have the responsibility to initiate FP discussion with patients and referencing them to FP specialists. A booklet with summarized contents was also produced and intended to inform other healthcare professionals working in the cancer setting. This resumed booklet is also intended to primary care professionals, which many times make the first contact with AYA cancer patients, so that they can promote awareness of the FP subject.

3. Results

3.1 Information needs

Twelve (12) published articles were selected and analyzed.[13-24] Data on methods and relevant results (reported information needs, gaps in knowledge or barriers to FP discussion) was collected from each individual article (Table 1.8).

Table 1.8 Studies concerning information needs, gaps in knowledge and barriers to FP implementation reported by oncologists.

Title	Methods (sample)	Information needs/gaps in knowledge/barriers to FP
Oncologists' Attitudes and Practices Regarding Banking Sperm Before Cancer Treatment[13]	A postal survey was sent to 718 oncology staff physicians and fellows (n=162).	FP options costs; FP facilities; risk of infertility in male versus female patients; treatment delay needed for FP
Fertility preservation in cancer survivors: a national survey of oncologists' current knowledge, practice and attitudes[14]	National online survey of oncologists (n=100).	FP options, specially testicular cryopreservation and ovarian cryopreservation; FP techniques success rates; FP in patients with hormonally sensitive malignancy
Do doctors discuss fertility issues before they treat young patients with cancer?[15]	Paediatric oncologists prospectively completed a data form for each new patient registered over a 12 month period (n=1030).	FP options in pre-pubertal patients; Experimental/established FP techniques; facilities available for FP

Title	Methods (sample)	Information needs/gaps in knowledge/barriers to FP
Strategies for Fertility Preservation after chemotherapy: Awareness among Irish cancer specialists[16]	Online questionnaire to cancer specialists (n=50).	Success rates; low awareness of published guidelines; available facilities for FP; treatment delay needed for FP; FP in patients with estrogen receptor (ER) positive disease
Oncologists' confidence in knowledge of fertility issues for young women with cancer[17]	National sample of medical oncologists, hematology/oncologists, radiation oncologists and gynecologic oncologists (n=344).	Infertility risk estimation; risks of pregnancy for the woman and the fetus; surgical techniques to protect the ovaries from radiation; new IVF stimulation protocols with less delay of cancer treatment or less estrogen exposure; cryopreservation of ovarian tissue and oocytes
A nationwide survey of oncologists regarding treatment-related infertility and fertility preservation in female cancer patients[18]	Email survey to a database of oncologists at the top 25 cancer hospitals as ranked by U.S. News & World Report (n=249).	Risk of gonadotoxicity from specific regimens
Who should be offered sperm banking for fertility preservation? A survey of UK oncologists and haematologists[19]	Post questionnaire to all members of the Royal College of Radiotherapists' Faculty of Oncology and the British Society for Haematology (n = 499).	Need to offer sperm banking to patients before they go through chemo- or radiotherapy; treatment delay needed for FP
Attitudes and Practices of Pediatric Oncology Providers Regarding Fertility Issues[20]	Survey to healthcare providers in a pediatric hematology/oncology clinic (n=30).	Risks of infertility in boys versus girls; risks of ovarian failure in pre-pubertal versus post-pubertal girls; FP techniques in pre-pubertal girls; risk of cancer or birth defects in the offspring of cancer survivors

Title	Methods (sample)	Information needs/gaps in knowledge/barriers to FP
Fertility Preservation in Women Undergoing Treatment for Breast Cancer in the U.K.: A Questionnaire Study[21]	Online questionnaire to surgeons, oncologists, and clinical nurse specialists who manage patients with breast cancer in the United Kingdom (n=306).	Treatment delay needed for FP; FP in patients with estrogen receptor (ER) positive disease; FP options available; interference of FP with the success of cancer treatment
Results from the survey for preservation of adolescent reproduction (SPARE) study: gender disparity in delivery of fertility preservation message to adolescents with cancer[22]	Survey by email to all members of a nationwide pediatric oncology subspecialty group (n=180).	FP in pre-pubertal patients; low awareness of published guidelines
Fertility preservation among patients with cancer: report of a French regional practical experience[23]	Prospective survey amongst oncologists working in Provence Alpes Côte d'Azur region (n=225).	FP options available and indications
Fertility Preservation Practices Among Ontario Oncologists[24]	Questionnaire to Ontario physicians with specialties in medical oncology, radiation oncology, gynecologic oncology, and urology (n=152)	FP specialists for referral; FP costs

3.2 Information contents

All the identified information topics were included in the main booklet, named “Oncofertility - Fertility Preservation in Cancer Patients”. In this booklet, contents were organized in 4 main sections:

Section 1. Why the need for fertility preservation in cancer patients?

In this first section the relevance of oncofertility in the present context is discussed. Moreover, information on the topics of fertility outcomes of cancer survivors, evaluation of reproductive potential and (in)fertility markers, risk factors for infertility in cancer patients and infertility risks associated both with cancer and cancer treatments is also provided.

Section 2. How can cancer patients' fertility be preserved?

This section includes the following sub-sections: Preserving fertility: which patients and when?; Male fertility preservation techniques; Female fertility preservation techniques (organized according to their classification as established and experimental); Other FP procedures (ovarian transposition, GnRH agonists administration). For each FP technique information is provided regarding procedure, classification as established/experimental, indications (for whom and when), time requisites, success rates, risks for man/woman and offspring, ideal time for conception/pregnancy, using the cryopreserved cells/tissue and costs.

Section 3. Questions & Answers

For the most frequently reported topics, information was reinforced in a series of Questions & Answers (total of 15). Some examples are FP in estrogen-positive breast cancer (Which FP techniques are available for hormone-sensitive tumors?), FP in pre-pubertal patients (Which FP techniques are available for pre-pubertal patients?), time requisites for FP (Is there a need to postpone cancer treatments to allow for FP procedures in a cancer patient?), available guidelines (Are there national or international guidelines on FP in cancer patients?), patient referral (What is the procedure for referencing patients to a FP consultation?) or established *versus* experimental techniques (Which FP techniques are acknowledged as established medical practice?),

Section 4. Information Tools

A variety of practical tools were developed and included in this last section, including a compilation of electronic tools to estimate infertility risks, the infertility risk tables published by ASCO in 2013 (translated and adapted to Portuguese), a list of published international guidelines regarding FP in oncology and a comparative table of the female FP techniques. In addition, a list of recommended e-books and review articles was prepared.

3.3 Information dissemination

The materials produced are being distributed with the collaboration of the *Liga Portuguesa contra o Cancro* (LPCC; Portuguese League Against Cancer), a nonprofit cancer patients organization, the *Sociedade Portuguesa de Medicina Reprodutiva* (SPMR; Portuguese Society of Reproductive Medicine), the *Sociedade Portuguesa de Oncologia* (SPO; Portuguese Society of Oncology) and the *Ordem dos Farmacêuticos* (OF; Portuguese Pharmaceutical Society). The LPCC published the summarized booklet and is disseminating both materials to primary care

and cancer care health professionals, through its website and promotion campaigns. This smaller booklet is also being distributed to the Portuguese hospital and community pharmacists through the efforts of the OF. The comprehensive information booklet was printed with the support of the SPMR and is being distributed in cancer care institutions and to oncologists with the collaboration of the SPO. Moreover, all information contents of the produced materials are available through the website of the Centre for Fertility Preservation of CHUC, EPE (www.centropreservacaofertilidade.pt), in Portuguese.

4. Discussion

It is important to note that information materials directed to AYA-aged cancer patients were also developed in the context of this program, including decision aids to support the decision of preserving fertility (or not) and the choice of the FP technique (results to publish). Moreover, we would like to highlight the multidisciplinary context in which it this project has been out, involving oncologists, reproductive medicine physicians, pharmacists, psychologists and the professional societies from the mentioned areas. This cooperation will certainly contribute to a wider dissemination of the developed information materials to the various intervenients in the process of cancer care and to a more effective clinical implementation.

Although the present information materials have been developed based on the internationally reported needs, the identified information topics are in accordance with the results from a locally applied questionnaire to a sample of 37 oncologists (response rate of 50%) from two hospitals in the center region of Portugal (unpublished results). Answers came mainly from clinicians working in CHUC EPE, although a few more were filled by oncologists in the Coimbra Regional Institute of Oncology (IPO Coimbra). The participants were mainly female clinicians n=25) with a mean age of 43 years. Several medical specialties were represented, with preponderance of clinicians from the clinical haematology (n=13), gynaecology (n=13) and oncology (n=4) specialties. The results of this investigation point towards the need for more information about infertility risks and FP in cancer patients, in order to improve clinical practice. In a scale of *Not important* (0) to *Extremely important* (4), most topics (9/12) had a mean score above 3 (Very important) and the topics *Types of cancer treatments associated to a greater risk* (mean score 3.32 ± 0.63), *Interference of FP techniques with cancer were those with higher rates* (mean score 3.32 ± 0.85), *Available FP techniques* (mean score 3.22 ± 0.79) and *Factors influencing the risk of infertility* (mean score 3.16 ± 0.76) were the ones with higher mean scores.

The developed information materials will support the role of cancer care professionals as patients' educators, increasing their participation in clinical decisions. Additionally, health professionals working in primary care settings can significantly raise awareness of this relevant subject, as they are in a privileged position to disseminate information to the general population.

The next step will be to disseminate these materials to other Portuguese language countries and the translation to English and French. Moreover, it is our intention to perform, in cooperation with the SPO, an evaluation study of cancer care clinicians' perceptions on the relevance, reliability and completeness of contents and on the usefulness of this information for their clinical practice.

5. Conclusions

The opportune information of AYA-aged cancer patients on their risk of infertility and the possibilities concerning FP is recognized as a highly relevant issue, in the context of cancer survival quality of life. Our work confirms the significant information needs of oncologists on these subjects and, by fulfilling those needs, contributes to timely, shared and informed clinical decisions on FP.

Acknowledgments

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Author Disclosure Statement

The authors state that there is no competing financial interest.

References

1. Knopman JM, Papadopoulos EB, Grifo JA, et al. Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood. Lancet Oncol. 2010;11:490–98.
2. Donnez J, Dolmans MM. Preservation of fertility in females with haematological malignancies. Br J Haematol. 2011;154:175-184.
3. Siegel R, DeSantis C, Virgo K, et al. Cancer Treatment and Survivorship Statistics, 2012. CA Cancer J Clin. 2012;62:220-41.
4. Burns KC, Boudreau C, Panepinto JA, et al. Attitudes regarding fertility preservation in female adolescent cancer patients. J Pediatr Hematol Oncol. 2006;28:350-354.
5. Schover LR, Rybicki LA, Martin BA, Bringelsen KA. Having children after cancer: a pilot survey of survivors' attitudes and experiences. Cancer. 1999; 86:697-709.
6. Huyghe E, Sui D, Odensky E, Schover LR. Needs assessment survey to justify establishing a reproductive health clinic at a comprehensive cancer center. J Sex Med. 2009 Jan;6:149-63.
7. Woodruff TK. The Oncofertility Consortium—addressing fertility in young people with cancer. Nat Rev Clin Oncol. 2010;7:466–475.
8. Peccatori FA, Azim HA, Orecchia R, et al. ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013;24:vi160-vi170.
9. Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24:2917-3.
10. Quinn GP, Vadaparampil ST, Lee JH, et al. Physician referral for fertility preservation in oncology patients: a national study of practice behaviors. J Clin Oncol. 2009;27:5952-7.
11. Quinn GP, Vadaparampil ST, Gwede CK, et al. Discussion of fertility preservation with newly diagnosed patients: oncologists' views. J Cancer Surviv. 2007;1:146–155.
12. Shimizu C, Bando H, Kato T, et al. Physicians' knowledge, attitude, and behavior regarding fertility issues for young breast cancer patients: a national survey for breast care specialists. Breast Cancer. 2013;20:230-40.
13. Schover LR, Brey K, Lichtin A, et al. Oncologists' attitudes and practices regarding banking sperm before cancer treatment. J Clin Oncol. 2002;20:1890-7.
14. Adams E, Hill E, Watson E. Fertility preservation in cancer survivors: a national survey of oncologists' current knowledge, practice and attitudes. Br J Cancer. 2013 30;108:1602-15.
15. Anderson RA, Weddell A, Spoudeas HA, Douglas C, Shalet SM, Levitt G, Wallace WH. Do doctors discuss fertility issues before they treat young patients with cancer? Hum Reprod. 2008;23:2246-51.
16. Collins IM, Fay L, Kennedy MJ. Strategies for fertility preservation after chemotherapy: awareness among Irish cancer specialists. Ir Med J. 2011;104:6-9.
17. Duffy C, Allen SM, Dube C, Dickersin K. Oncologists' confidence in knowledge of fertility issues for young women with cancer. J Cancer Educ. 2012;27:369-76.
18. Forman EJ, Anders CK, Behera MA. A nationwide survey of oncologists regarding treatment-related infertility and fertility preservation in female cancer patients. Fertil Steril. 2010;94:1652-6.
19. Gilbert E, Adams A, Mehanna H, Harrison B, Hartshorne GM. Who should be offered sperm banking for fertility preservation? A survey of UK oncologists and haematologists. Ann Oncol. 201;22:1209-14.
20. Goodwin T, Elizabeth Oosterhuis B, Kiernan M, Hudson MM, Dahl GV. Attitudes and practices of pediatric oncology providers regarding fertility issues. Pediatr Blood Cancer. 2007;48:80-5.
21. King JW, Davies MC, Roche N, Abraham JM, Jones AL. Fertility preservation in women undergoing treatment for breast cancer in the UK: a questionnaire study. Oncologist. 2012;17:910-6.
22. Köhler TS, Kondapalli LA, Shah A, Chan S, Woodruff TK, Brannigan RE. Results from the survey for preservation of adolescent reproduction (SPARE) study: gender disparity in delivery of fertility preservation message to adolescents with cancer. J Assist Reprod Genet. 2011;28:269-77.
23. Préaubert L, Poggi P, Pibarot M, Delotte J, Thibault E, Saias-Magnan J, Courbière B. Fertility preservation among patients with cancer: report of a French regional practical experience. J Gynecol Obstet Biol Reprod (Paris). 2013;42:246-51.
24. Yee S, Fuller-Thomson E, Lau A, Greenblatt EM. Fertility preservation practices among Ontario oncologists. J Cancer Educ. 2012 Jun;27(2):362-8.

1.2.2 Published information resources for healthcare professionals

Fertility Preservation in Cancer Patients: General Information for Healthcare Professionals

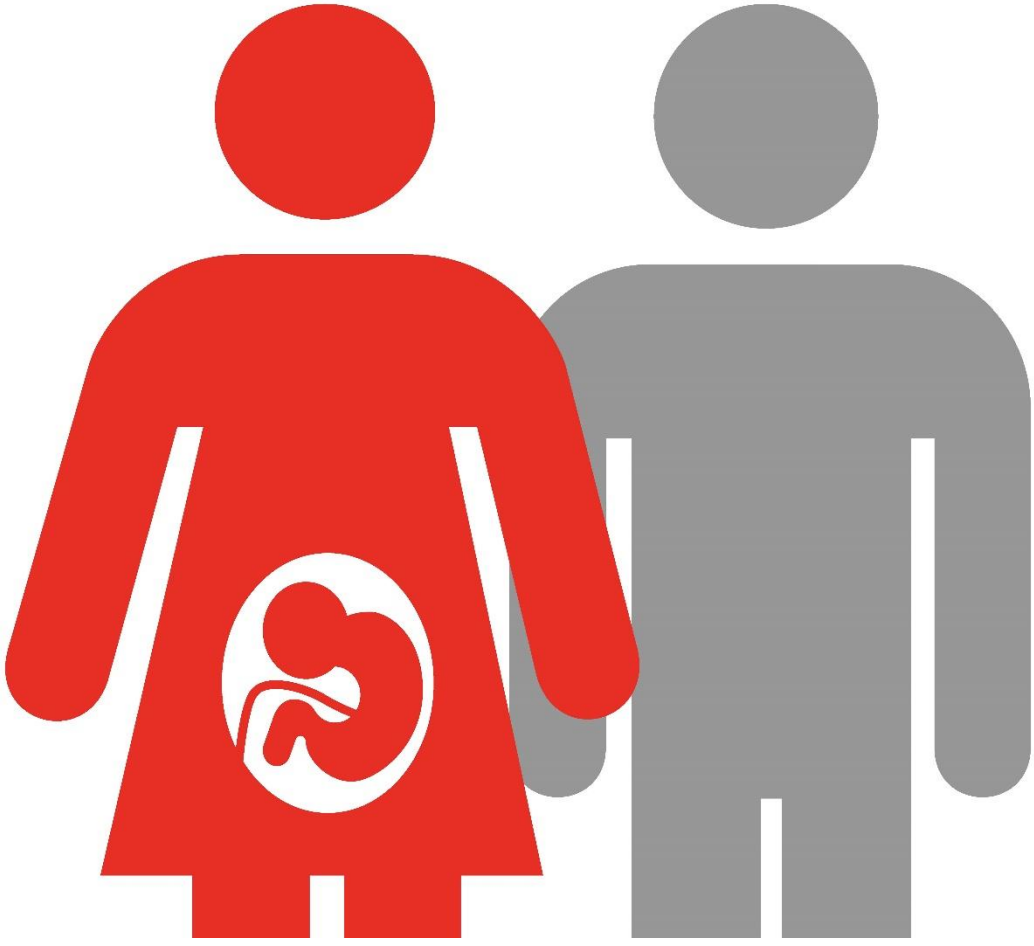
[*Preservação da Fertilidade em Doentes Oncológicos: Informação Geral para Profissionais de Saúde*]

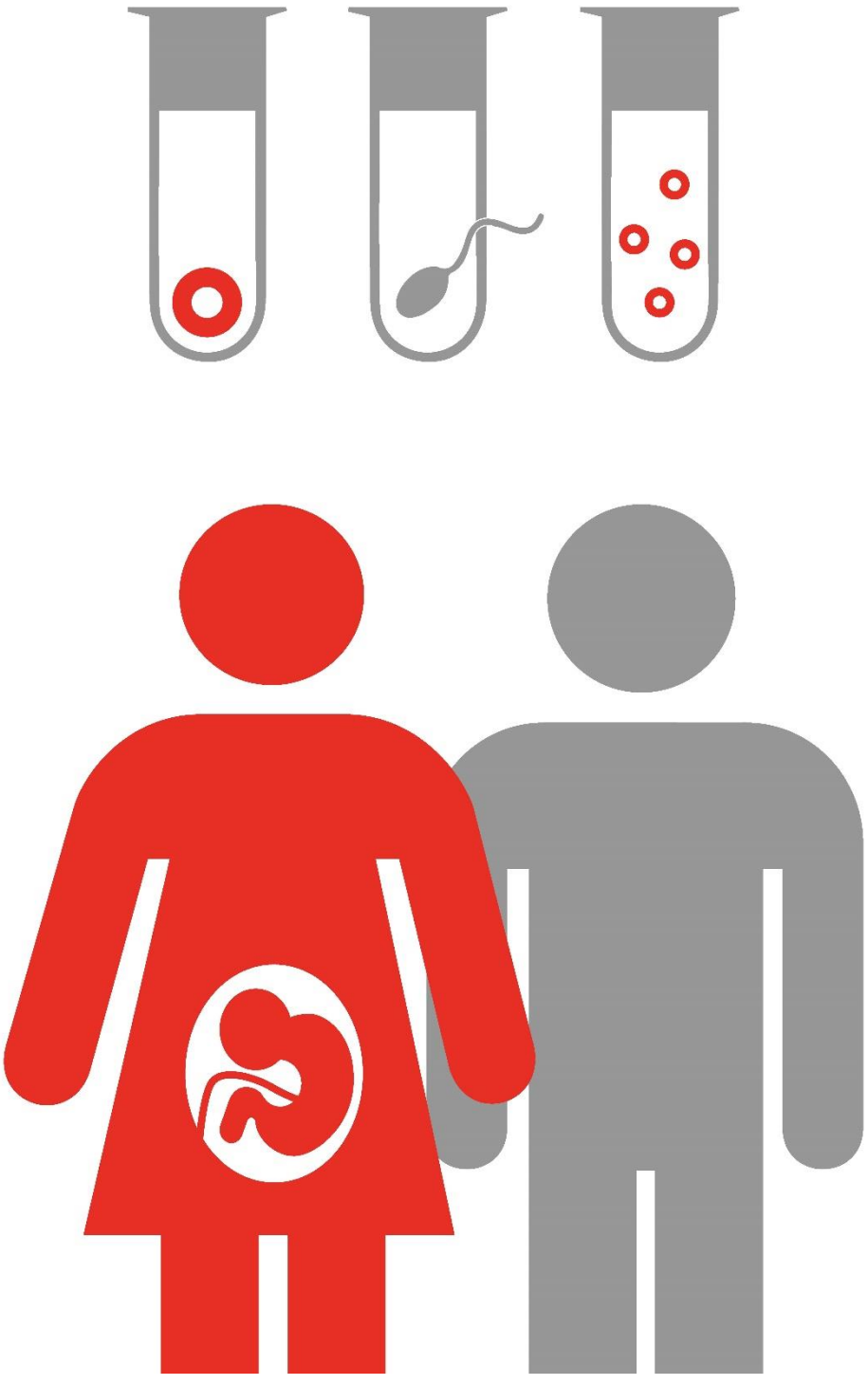
Cristina Silva, Ana Teresa Almeida-Santos, Ana Cristina Ribeiro Rama

LIGA PORTUGUESA
CONTRA O CANCRO
NÚCLEO REGIONAL DO CENTRO

PRESERVAÇÃO DA FERTILIDADE EM DOENTES ONCOLÓGICOS

INFORMAÇÃO GERAL PARA PROFISSIONAIS DE SAÚDE





PORQUÊ PRESERVAR A FERTILIDADE EM DOENTES ONCOLÓGICOS?

- As **alterações na fertilidade** são um potencial efeito adverso associado à doença oncológica e/ou aos tratamentos ministrados.
- Diversos agentes antineoplásicos, como os alquilantes, estão associados a um **risco de toxicidade ao nível da função reprodutora**, quer na mulher quer no homem.
- Há diversos factores, relacionados com o doente, com os tratamentos e até com a doença oncológica, que influenciam o risco de infertilidade em cada doente (Tabela 1).
- Observa-se um número crescente de **sobreviventes de doença oncológica em idade reprodutiva** devido, por um lado, aos avanços consideráveis no diagnóstico e tratamento e, por outro, ao aumento da incidência de doença oncológica em adolescentes e jovens adultos (Knopman, 2010).
- Torna-se imperativo focar as atenções em aspetos relacionados com a **qualidade de vida na sobrevivência**, nos quais se inclui a **fertilidade futura**. Diversos estudos indicam que as discussões sobre fertilidade e preservação da fertilidade são da maior importância para os doentes oncológicos (Burns e Boudreau e Panepinto, 2006; Schover et al., 1999).
- A **Oncofertilidade** surge, neste contexto, como uma nova **área clínica de intervenção multidisciplinar** que pretende ir ao encontro das necessidades dos doentes oncológicos, no que diz respeito ao seu potencial reprodutivo (Woodruff, 2010).

Burns KC, Boudreau C, Panepinto JA. Attitudes regarding fertility preservation in female adolescent cancer patients. J Pediatr Hematol Oncol. 2006; 28(6):350-354.

Knopman JM, Papadopoulos EB, Grifo JA, Fino ME, Noyes N. Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood. Lancet Oncol 2010; 11: 490–98.

Schover LR, Rybicki LA, Martin BA, Bringelsen KA. Having children after cancer: a pilot survey of survivors' attitudes and experiences. Cancer. 1999; 86(4):697-709.

Woodruff TK. The Oncofertility Consortium—addressing fertility in young people with cancer. Nat Rev Clin Oncol. 2010 August ; 7(8): 466–475.

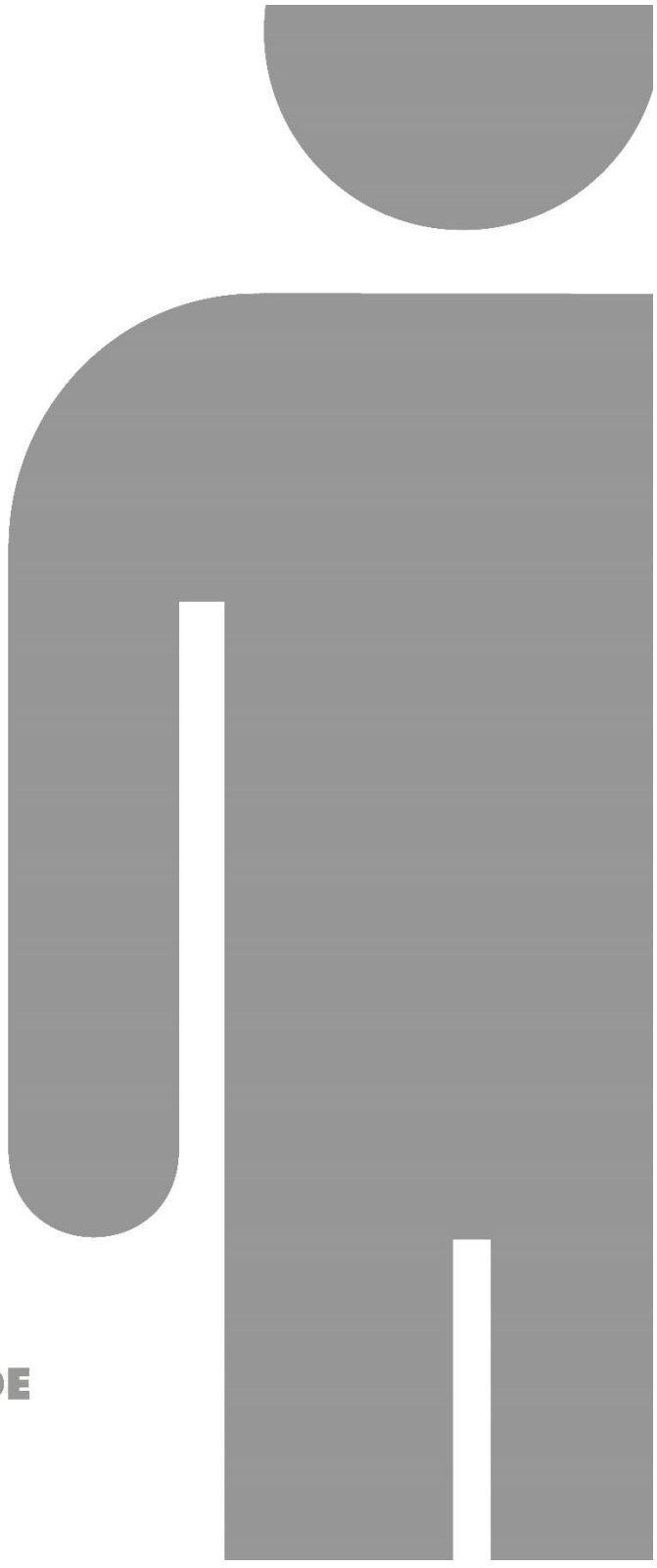
1

TABELA 1. FATORES INFLUENCIADORES DO RISCO DE INFERTILIDADE EM DOENTES ONCOLÓGICOS.

GRUPO DE FATORES	MULHER	HOMEM
FATORES RELACIONADOS COM O DOENTE	Idade (+)	Idade (–)
	Fertilidade inicial	Fertilidade inicial
	Fatores genéticos (?)	
FATORES RELACIONADOS COM OS TRATAMENTOS DA DOENÇA ONCOLÓGICA	Tipo e dose cumulativa dos fármacos antineoplásicos	Tipo e dose cumulativa dos fármacos antineoplásicos
	Localização e dose da radioterapia	Localização e dose da radioterapia
	Área de cirurgia	Área de cirurgia
FATORES RELACIONADOS COM A DOENÇA ONCOLÓGICA		Tipo de doença oncológica

COMO PRESERVAR A FERTILIDADE EM DOENTES ONCOLÓGICOS?

- Orientações internacionais recentes recomendam que os profissionais de saúde devem referir a possibilidade de infertilidade a **todos os doentes oncológicos em idade reprodutiva** (e aos seus pais, no caso de crianças ou adolescentes menores) (Loren et al., 2013; Peccatori et al., 2013; The Practice Committee of the American Society for Reproductive Medicine, 2012).
- Os profissionais de saúde deverão ainda estar preparados para **discutir as opções de preservação da fertilidade e/ou referenciar os doentes a especialistas em medicina da reprodução**. Estas informações devem ser parte integrante do processo de esclarecimento e consentimento informado, antes de iniciar tratamento.
- Estão disponíveis várias **técnicas para preservação da fertilidade**, sendo que a criopreservação de esperma, no homem, e a criopreservação de embriões ou ovócitos, na mulher, são consideradas técnicas estabelecidas (Tabelas 2 e 3); na mulher, estão ainda disponíveis as práticas de ooforopexia e de administração de agonistas GnRH (Tabela 4).



PRESERVAÇÃO DA FERTILIDADE NO HOMEM

PRESERVAÇÃO DA FERTILIDADE NO HOMEM

TABELA 2. TÉCNICAS DE PRESERVAÇÃO DA FERTILIDADE NO HOMEM.

	CRIOPRESERVAÇÃO DE ESPERMA	CRIOPRESERVAÇÃO DE TECIDO TESTICULAR
DESCRIÇÃO	Criopreservação de amostras de esperma, obtidas por masturbação, biópsia testicular ou eletroejaculação.	As amostras de tecido testicular são obtidas através de biópsia, preparadas e criopreservadas.
POPULAÇÃO-ALVO	Todos os homens e rapazes na pós-puberdade.	• Homens e rapazes na pós-puberdade nos quais a recolha de espermatozoides no ejaculado não seja possível (p. ex. em casos de azoospermia). • Crianças e jovens na pré-puberdade: única opção disponível, embora seja uma técnica ainda experimental, sem prova de sucesso clínico.
TAXA DE SUCESSO	Taxa de gravidez: 40 a 50%.	Geralmente inferiores às que se obtêm com o uso de esperma congelado.
RISCOS	Não existe qualquer risco associado a esta técnica.	• Biópsia testicular: risco de hematoma, edema ou dor na zona escrotal e, menos frequentemente, hemorragia ou infecção; lesão nervosa ou atrofia testicular são complicações muito raras. • Pode não ser possível obter espermatozoides no tecido testicular colhido.
CUSTOS	No âmbito do Serviço Nacional de Saúde (SNS), a criopreservação de esperma ou de tecido testicular de doentes oncológicos e a sua manutenção são, em geral, procedimentos gratuitos.	

4


PRESERVAÇÃO
DA FERTILIDADE
NA MULHER

PRESERVAÇÃO DA FERTILIDADE NA MULHER

TABELA 3. TÉCNICAS DE PRESERVAÇÃO DA FERTILIDADE NA MULHER.

	CRIOPRESERVAÇÃO DE EMBRIÕES	CRIOPRESERVAÇÃO DE OVÓCITOS	CRIOPRESERVAÇÃO DE TECIDO OVÁRICO
DESCRIÇÃO	Estimulação hormonal, seguida de punção folicular para recolha de ovócitos e posterior inseminação in vitro. Os embriões obtidos são então criopreservados. Técnica estabelecida.	Estimulação hormonal, seguida de punção folicular e criopreservação dos ovócitos obtidos por vitrificação. Técnica estabelecida.	Colheita, por cirurgia laparoscópica, de fragmentos de tecido ovárico, que são preparados e criopreservados. Após transplante, o tecido ovárico pode repor a função endócrina e a fertilidade, possibilitando uma conceção natural. Técnica experimental.
POPULAÇÃO-ALVO	Mulheres em idade reprodutiva, com parceiro, desde que não existam objeções morais e/ou éticas à criopreservação de embriões.	Todas as raparigas na pós-puberdade e mulheres em idade reprodutiva, mesmo na ausência de parceiro, ou quando existem objeções morais/éticas à criopreservação de embriões.	• Mulheres e raparigas na pós-puberdade, quando é indesejável a estimulação hormonal e/ou há necessidade de iniciar tratamento com urgência. • Crianças e raparigas na pré-puberdade (única técnica de preservação da fertilidade que pode ser utilizada).

	CRIOPRESERVAÇÃO DE EMBRIÕES	CRIOPRESERVAÇÃO DE OVÓCITOS	CRIOPRESERVAÇÃO DE TECIDO OVÁRICO
TAXAS DE SUCESSO	Está descrita uma taxa de gravidez de 35,6%, em mulheres até aos 35 anos, por ciclo (Westphal & Massie, 2012). À medida que a idade da mulher aumenta, a probabilidade de conseguir iniciar uma gravidez diminui de forma significativa.	A taxa de nascimentos por ovócito criopreservado, nos centros com maior experiência, é de 5,7% pelo que, teoricamente, seria necessário criopreservar 20 ovócitos para assegurar uma probabilidade de sucesso de 100%.	Não é possível calcular a taxa de sucesso uma vez que não é conhecido o nº total de transplantes realizados. Até à data, estão relatados em todo o mundo mais de 40 nascimentos em resultado da utilização de tecido ovárico criopreservado e transplantado.
RISCOS	Estimulação hormonal: risco de Síndroma de Hiperestimulação Ovárica; em doentes com tumores hormono-dependentes, a exposição a níveis elevados de estrogénios pode ser minimizada com o uso de inibidores da aromatase. Punção folicular: riscos menores, como infeções ou hemorragias, que ocorrem em menos de 1% dos casos.	Estimulação hormonal: risco de Síndroma de Hiperestimulação Ovárica; em doentes com tumores hormono-dependentes, a exposição a níveis elevados de estrogénios pode ser minimizada com o uso de inibidores da aromatase. Punção folicular: riscos menores, como infeções ou hemorragias, que ocorrem em menos de 1% dos casos.	Riscos associados à anestesia e cirurgia laparoscópica. Risco de transplantar tecido ovárico com células neoplásicas: estão em desenvolvimento técnicas histológicas, de PCR, de citometria de fluxo e de xenotransplantação, que ajudam a excluir estes casos.
CUSTOS	No âmbito do Serviço Nacional de Saúde, a doente oncológica apenas terá que suportar o custo dos medicamentos para estimulação da ovulação, que pode variar entre 200 a 500 euros, de acordo com o protocolo de estimulação utilizado.		No âmbito do Serviço Nacional de Saúde, estes procedimentos são, em geral, gratuitos.

7

TABELA 4. OUTRAS PRÁTICAS PARA PRESERVAÇÃO DA FERTILIDADE NA MULHER.

TRANSPOSIÇÃO OVÁRICA OU OOFOROPEXIA	A transposição ovárica ou ooforopexia pode ser oferecida quando a doente vai ser sujeita a radioterapia pélvica, de forma a minimizar a exposição dos ovários à radiação. No entanto, devido à dispersão de radiação, os ovários nem sempre ficam protegidos e as doentes devem ser informadas de que esta técnica nem sempre é totalmente eficaz. Este procedimento deve ser realizado o mais próximo possível do momento em que é administrada a radioterapia, devido ao risco de migração dos ovários (Loren et al., 2013).
ADMINISTRAÇÃO DE AGONISTAS DA GNRH (HORMONA LIBERTADORA DE GONADOTROFINAS)	Atualmente, os resultados da efetividade dos agonistas GnRH para preservação da fertilidade feminina não são ainda conclusivos (Roness et al, 2014) e a sua utilização não é recomendada como método de preservação da fertilidade (Loren et al., 2013; Peccatori et al., 2013). Outros benefícios possivelmente associados à sua utilização, como a redução de hemorragias vaginais, devem ser ponderados face a riscos como a perda de massa óssea, os afrontamentos e a potencial interferência com a resposta ao tratamento em tumores hormono-dependentes (Loren et al., 2013).

Roness H, Kalich-Philosoph L, Meirow D. Prevention of chemotherapy-induced ovarian damage: possible roles for hormonal and non-hormonal attenuating agents. Hum Reprod Update 2014.20(5): 759–774.

Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K; American Society of Clinical Oncology. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013 Jul 1;31(19):2500-10.

Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G; ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013 Oct;24 Suppl 6:vi160-70.

TAMBÉM DISPONÍVEL (MONOFOLHAS):

FERTILIDADE NO HOMEM COM CANCRO: CONHEÇA OS RISCOS

FERTILIDADE NA MULHER COM CANCRO: CONHEÇA OS RISCOS

FICHA TÉCNICA
EDITOR: NÚCLEO REGIONAL DO CENTRO DA LIGA PORTUGUESA CONTRA O CANCRO
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TIRAGEM: 8.000 EXEMPLARES [2015]

O PRESENTE TRABALHO FOI PATROCINADO POR UMA BOLSA DE INVESTIGAÇÃO
ATRIBUÍDA PELA LIGA PORTUGUESA CONTRA O CANCRO

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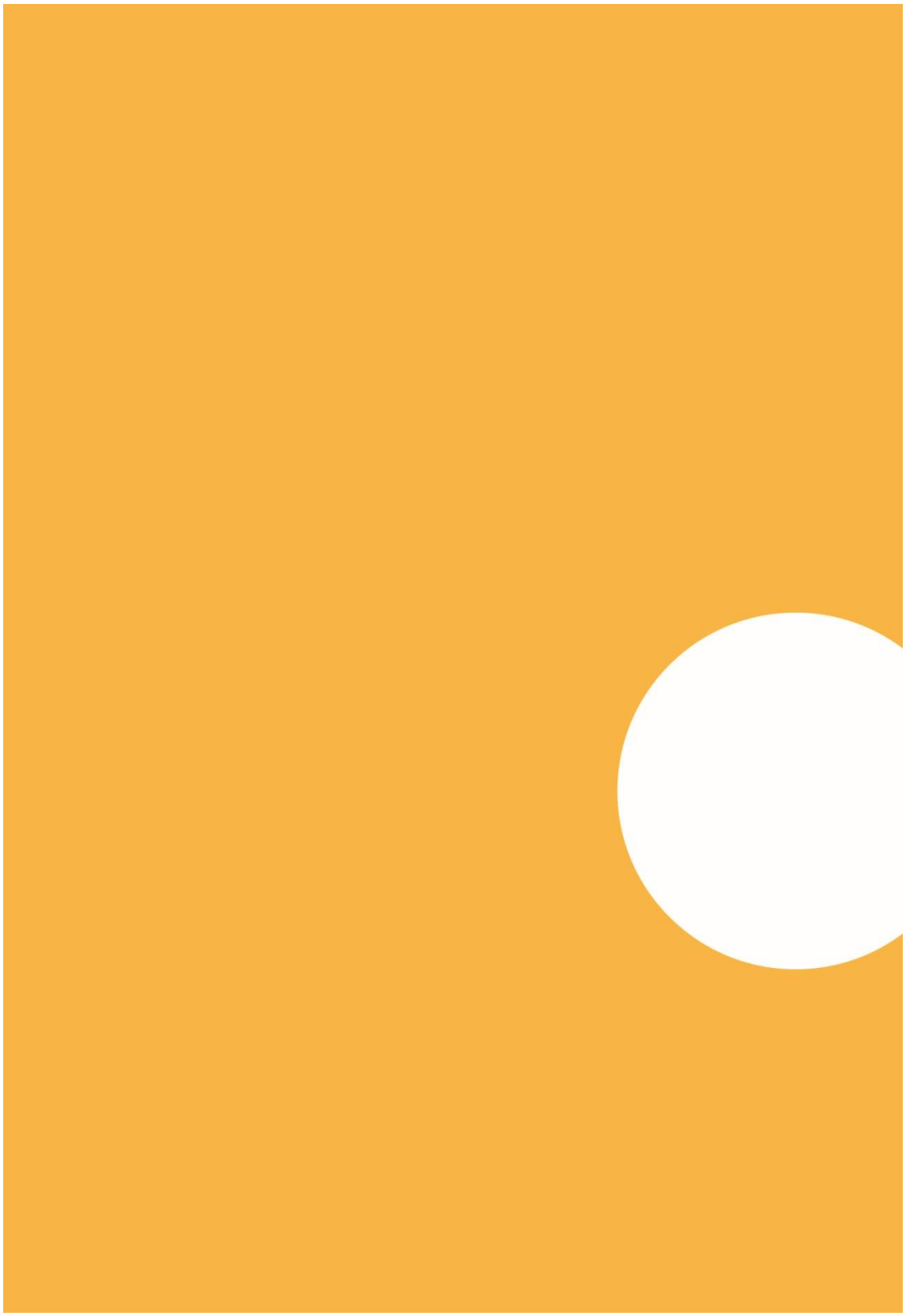
Administração Regional de Saúde do Centro

Fertility Preservation in Cancer Patients

[*Preservação da Fertilidade em Doentes Oncológicos*]

Cristina Silva, Ana Teresa Almeida-Santos, Ana Cristina Ribeiro Rama


PRESERVAÇÃO
DA FERTILIDADE
EM DOENTES
ONCOLÓGICOS



As alterações na fertilidade são um potencial efeito adverso associado à doença oncológica e sobretudo aos tratamentos para a combater. No que concerne à terapêutica antineoplásica, diversos agentes estão associados a um risco moderado ou elevado de toxicidade ao nível da função reprodutora, tanto na mulher como no homem. Os avanços consideráveis ao nível do diagnóstico e tratamento da doença oncológica e o aumento da incidência desta em adolescentes e jovens adultos contribuem para um número crescente de sobreviventes de cancro em idade reprodutiva. Esta nova realidade implica focar a atenção dos médicos, em particular dos oncologistas, para aspectos relacionados com a qualidade de vida na sobrevivência, nos quais se inclui a fertilidade futura.

Em Portugal, a criopreservação de gâmetas masculinos vem sendo praticada há largos anos. A preservação da fertilidade feminina, bastante mais complexa, iniciou-se em 2010, no Serviço de Reprodução Humana do Centro Hospitalar e Universitário de Coimbra (CHUC) tendo em 2013 sido criado o Centro de Preservação da Fertilidade desta instituição hospitalar, dotado de instalações especificamente dedicadas a esta atividade multidisciplinar.

ÍNDICE

3. PERGUNTAS E RESPOSTAS

1. Qual o momento ideal para discutir com os doentes oncológicos a possibilidade de preservarem a sua fertilidade?
2. Se referenciar um doente para o Centro de Preservação da Fertilidade, que técnicas estão disponíveis?
3. Em que consiste a consulta de preservação da fertilidade?
4. Como devo proceder para referenciar um doente oncológico para uma consulta de preservação da fertilidade?
5. Existem orientações, nacionais ou internacionais, sobre a preservação da fertilidade em doentes oncológicos?
6. Quais as técnicas de preservação da fertilidade atualmente consideradas como prática médica estabelecida?
7. Quais as técnicas de preservação da fertilidade que são ainda consideradas experimentais?
8. As técnicas de preservação da fertilidade podem, de alguma forma, interferir no tratamento da doença oncológica?
9. Para que um doente possa preservar a sua fertilidade é necessário adiar o início dos tratamentos da doença oncológica?
10. Quais os custos associados às várias técnicas de preservação da fertilidade?
11. Que técnicas de preservação da fertilidade estão disponíveis para doentes oncológicos na pré-puberdade?
12. Que técnicas de preservação da fertilidade estão disponíveis para doentes com tumores hormono-dependentes?
13. Em doentes oncológicos nos quais é urgente iniciar tratamento, quais as opções de preservação da fertilidade disponíveis?
14. Existe um risco aumentado de doença oncológica e/ou malformações congénitas na descendência de doentes oncológicos?
15. Quanto tempo se deve aguardar para tentar iniciar uma gravidez/a conceção, depois de terminarem os tratamentos da doença oncológica?

BIBLIOGRAFIA RECOMENDADA

Orientações clínicas
Livros online
Artigos de revisão

ANEXOS

ANEXO I • Compilação de ferramentas para apoio à identificação do risco de infertilidade.
ANEXO II • Compilação de orientações clínicas internacionais sobre a preservação da fertilidade em doentes oncológicos.
ANEXO III A • Categorização do risco de amenorreia permanente em mulheres tratadas com regimes modernos de quimioterapia e radioterapia (ASCO, 2013).
ANEXO III B • Categorização do risco de azoospermia em homens tratados com regimes modernos de quimioterapia e radioterapia (ASCO, 2013).
ANEXO IV • Tabela comparativa das várias técnicas de preservação da fertilidade na mulher.

PORQUÊ PRESERVAR A FERTILIDADE EM DOENTES ONCOLÓGICOS?

1.1 Relevância da oncofertilidade no contexto atual

As alterações na fertilidade são um potencial efeito adverso associado à doença oncológica e/ou aos tratamentos ministrados. No que diz respeito à terapêutica antineoplásica, diversos agentes, como os alquilantes, estão associados a um risco moderado ou elevado de toxicidade ao nível da função reprodutora, quer na mulher quer no homem. Paralelamente, os avanços consideráveis ao nível do diagnóstico e tratamento da doença oncológica e o aumento da sua incidência em adolescentes e jovens adultos, contribuem para um número crescente de sobreviventes de cancro em idade reprodutiva. Os diagnósticos mais comuns em indivíduos jovens incluem melanoma, linfomas, leucemias e carcinomas da mama, do colo do útero e do testículo. De entre as mulheres diagnosticadas com cancro da mama e ginecológico, até 15% e 43%, respetivamente, têm idade inferior a 45 anos. Os homens em idade reprodutiva são frequentemente confrontados com linfoma Hodgkin e cancro testicular e, neste último, representam 90% dos novos casos. Doenças hemato-oncológicas, como as leucemias e linfomas, são frequentes em crianças e adultos jovens (Knopman et al., 2010). Desta forma, torna-se imperativo focar as atenções em aspetos relacionados com a qualidade de vida na sobrevivência, nos quais se inclui a fertilidade futura. Diversos estudos indicam que as discussões sobre fertilidade e preservação da fertilidade são da maior importância para os doentes oncológicos (Burns e Boudreau e Panepinto, 2006; Schover et al., 1999). A oncofertilidade surge, neste contexto, como uma nova área clínica de intervenção multidisciplinar que pretende ir ao encontro das necessidades dos doentes oncológicos, relativas ao seu potencial reprodutivo (Woodruff, 2010).

Burns KC, Boudreau C, Panepinto JA. Attitudes regarding fertility preservation in female adolescent cancer patients. J Pediatr Hematol Oncol. 2006; 28(6):350-354.
Knopman JM, Papadopoulos EB, Grifo JA, Fino ME, Noyes N. Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood. Lancet Oncol 2010; 11: 490-98.
Schover LR, Rybicki LA, Martin BA, Bringelsen KA. Having children after cancer: a pilot survey of survivors' attitudes and experiences. Cancer. 1999; 86(4):697-709.
Woodruff TK. The Oncofertility Consortium - addressing fertility in young people with cancer. Nat Rev Clin Oncol. 2010 August; 7(8): 466-475.

1.2 Risco de infertilidade em doentes oncológicos

1.2.1 FERTILIDADE EM SOBREVIVENTES DE DOENÇA ONCOLÓGICA

Diversos estudos epidemiológicos internacionais (Green et al., 2010; Green et al., 2009; Madanat et al., 2008; Schover, 2008) investigaram a fertilidade de sobreviventes de doença oncológica, face a controlos saudáveis, e demonstraram que a probabilidade destes doentes produzirem descendência é significativamente inferior, quer em homens quer em mulheres. No caso concreto da mulher sobrevivente de doença oncológica, observa-se ainda a ocorrência de falência ovárica aguda e uma incidência aumentada de menopausa precoce (Green et al., 2009).

Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, Donaldson SS, Byrne J, Robison LL. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol. 2009 Jun 1;27(16):2677-85.
Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, Donaldson SS, Byrne J, Robison LL. Fertility of male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2010 Jan 10;28(2):332-9
Madanat LM, Malila N, Dyba T, Hakulinen T, Sankila R, Boice JD Jr, Lahteenmaki PM. Probability of parenthood after early onset cancer: a population-based study. Int J Cancer. 2008; 123: 2891–2898.
Schover LR. Premature ovarian failure and its consequences: vasomotor symptoms, sexuality, and fertility. J Clin Oncol. 2008; 26: 753–758.

1.2.2 POTENCIAL REPRODUTIVO E MARCADORES DE (IN)FERTILIDADE

No homem, o potencial reprodutivo está diretamente relacionado com a espermatogénese, pelo que os principais indicadores para avaliação da fertilidade masculina são a quantidade e qualidade do esperma produzido, avaliadas por meio da realização de um espermograma.

Na mulher, a fertilidade depende do normal funcionamento do eixo hipotálamo-hipófise e ainda de um útero funcionante e de uma função ovárica normal. Esta função ovárica está diretamente relacionada com a denominada reserva ovárica (RO), i.e. o *pool* de folículos primordiais remanescente no ovário num determinado momento (Partridge et al., 2010). Este *pool* é limitado e diminui fisiologicamente com a idade, atingindo um mínimo na altura da menopausa, sendo ainda afetado por diversos fatores externos como a terapêutica antineoplásica ou a radioterapia. Tradicionalmente, a existência e regularidade de períodos menstruais têm sido usadas como indicadores da fertilidade feminina e, de forma inversa, a amenorreia como identificadora de infertilidade. No entanto, a ausência de períodos menstruais revela-se um marcador pouco fiável, uma vez que só deteta o declínio da função ovárica numa fase terminal e porque a presença de ciclos menstruais, mesmo regulares, não exclui a existência de danos sub-clínicos no ovário (Donnez e Kim, 2011). Neste sentido, preconiza-se que, na avaliação da (in)fertilidade feminina, sejam utilizados marcadores da RO pois identificam, com maior sensibilidade, falência ovárica fisiológica ou induzida (Domingues e Rocha e Serafini, 2010). Os testes de RO incluem marcadores hormonais e ecográficos. De entre os vários marcadores, destacam-se a Hormona Anti-Mülleriana (HAM), hormona produzida pelas células da granulosa dos folículos em desenvolvimento, e a Contagem de Folículos Antrais (CFA), marcador ecográfico da RO, pois ambos se correlacionam fortemente com a RO e depleção folicular precoce, sendo apontados como úteis na deteção da RO diminuída após terapêutica antineoplásica (Partridge et al., 2010; Donnez e Kim, 2011; Hansen et al., 2011).

Partridge AH, Ruddy KJ, Gelber S, Schapira L, Abusief M, Meyer M, Ginsburg E. Ovarian reserve in women who remain premenopausal after chemotherapy for early stage breast cancer. Fertil Steril. 2010; 94(2):638-44.
Donnez J, Kim S. Principles and practice of fertility preservation. Cambridge University Press. 2011. ISBN: 9780521196956.
Domingues TS, Rocha AM, Serafini PC. Tests for ovarian reserve: reliability and utility. Curr Opinion Obstetr Gynecol. 2010; 22:271-76.
Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril. 2011 Jan;95(1):170-5.

1.2.3 FATORES DE RISCO PARA INFERTILIDADE EM DOENTES ONCOLÓGICOS

No doente oncológico, a fertilidade futura é influenciada por um conjunto de fatores que poderemos organizar em três grandes grupos: fatores relacionados com o próprio doente, fatores relacionados com os tratamentos realizados ou a realizar e ainda, no caso do homem, o tipo de doença oncológica (tabela 1).

TABELA 1. **FATORES INFLUENCIADORES DA FERTILIDADE EM DOENTES ONCOLÓGICOS.**

GRUPO DE FATORES	MULHER	HOMEM
Fatores relacionados com o doente	Idade Fertilidade inicial	Idade (-) Fertilidade inicial
Fatores relacionados com os tratamentos da doença oncológica	Fatores genéticos (?) Tipo, dose e duração do tratamento antineoplásico Localização e dose da radioterapia Área de cirurgia	Tipo e dose cumulativa dos fármacos antineoplásicos Localização e dose da radioterapia Área de cirurgia
Fatores relacionados com a doença oncológica		Tipo de doença oncológica

Na mulher, o fator idade é muito relevante uma vez que o seu potencial reprodutivo diminui de forma fisiológica com o envelhecimento, como já referido. Também a fertilidade à altura do diagnóstico é condicionadora da fertilidade futura, quer na mulher quer no homem. Alguns estudos indicam que a toxicidade gonadal associada à terapêutica antineoplásica, em mulheres com doença oncológica, pode ainda ser influenciada pela suscetibilidade genética (Su et al., 2010). No que diz respeito aos fatores relacionados com os tratamentos, sabe-se que determinados grupos de fármacos antineoplásicos, como os agentes alquilantes, estão associados a um maior risco de infertilidade futura, ao passo que para outros o risco é muito baixo ou mesmo inexistente. O risco está ainda dependente, para o mesmo fármaco, da dose total administrada. Relativamente ao risco de infertilidade associada à radioterapia, verifica-se maior risco quando há irradiação hipofisária ou pélvica. Mais uma vez os efeitos são dose-dependentes, sendo que doses fracionadas têm geralmente menor toxicidade.
Finalmente, o local de cirurgia é, obviamente, um fator relevante, na medida em que algumas cirurgias dos aparelhos reprodutores masculino e feminino influenciam a fertilidade, podendo mesmo ser esterilizantes. No homem será ainda necessário considerar a influência do tipo de doença oncológica.

Su HI, Sammel MD, Velders L, Horn M, Stankiewicz C, Matro J, Gracia CR, Green J, DeMichele A. Association of cyclophosphamide drug-metabolizing enzyme polymorphisms and chemotherapy-related ovarian failure in breast cancer survivors. Fertil Steril. 2010 Jul;94(2):645-54.

1.2.4. RISCO DE INFERTILIDADE ASSOCIADA À DOENÇA ONCOLÓGICA

No que diz respeito à fertilidade feminina, o impacto da doença oncológica parece ser mínimo ou mesmo inexistente (Levin e Almog, 2013). No homem, no entanto, alguns tipos de doença oncológica, como o cancro do testículo e o linfoma Hodgkin, implicam, por si só, contagens mais baixas de espermatozoides, mesmo antes de iniciar tratamentos (van Casteren, 2010). Este efeito é mediado por mecanismos imunológicos ou citotóxicos, ainda não completamente esclarecidos (Knopman, 2010).

Knopman JM, Papadopoulos EB, Grifo JA, Fino ME, Noyes N. Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood. Lancet Oncol 2010; 11: 490-98.
Levin I, Almog B. Effect of cancer on ovarian function in patients undergoing in vitro fertilization for fertility preservation: a reappraisal. Curr Oncol. 2013 Feb;20(1):e1-3. doi: 10.3747/co.20.1193.
van Casteren NJ, Boellaard WP, Romijn JC, Dohle GR. Gonadal dysfunction in male cancer patients before cytotoxic treatment. Int J Androl. 2010;33:73-9.

1.2.5. RISCO DE INFERTILIDADE ASSOCIADA AOS TRATAMENTOS DA DOENÇA ONCOLÓGICA

MECANISMOS

Relativamente ao impacto dos vários tratamentos da doença oncológica, sabe-se que poderão influenciar a fertilidade por meio de um ou mais dos seguintes mecanismos (Bahadur, 2000; Fleischer, Vollenhoven e Weston, 2011, Meistrich, 2009):

Gonadotoxicidade direta, quando ocorre lesão direta do ovário ou do epitélio seminífero no testículo; este mecanismo intervém no efeito dos agentes antineoplásicos alquilantes ou da radioterapia pélvica;

Gonadotoxicidade indireta, quando as alterações são ao nível do funcionamento do eixo hipotálamo-hipófise-gónadas, como poderá acontecer após radioterapia craniana;

Alterações ao nível da função uterina, na mulher, e das **funções erétil ou ejaculatória**, no homem, que podem ser causadas por irradiação pélvica ou por alguns tipos de cirurgia do aparelho reprodutor, feminino ou masculino.

Bahadur G. Fertility issues for cancer patients. Mol Cell End 169 : 117-122. 2010
Fleischer RT, Vollenhoven BJ, Weston GC. The effects of chemotherapy and radiotherapy on fertility in premenopausal women. Obstet Gynecol Surv. 2011 Apr;66(4):248-54. doi: 10.1097/OGX.0b013e318224e97b.
Meistrich M.L. Male Gonadal Toxicity. Pediatr Blood Cancer. 2009 August ; 53(2): 261-266.

No caso específico da terapêutica antineoplásica, e na mulher, a toxicidade direta no ovário pode acontecer por depleção direta do *pool* folicular, i.e. da RO (ex. agentes alquilantes), mas também por efeitos a nível celular, mediados por stress oxidativo (ex. ciclofosfamida e antraciclinas) ou ainda por toxicidade vascular (ex. doxorubicina) (Ben-Aharon, 2012). No homem, os antineoplásicos causam, predominantemente, lesões no epitélio seminífero e, consequentemente, alterações da espermatogénese, embora possam também danificar as células de Leydig, responsáveis pela produção de testosterona. Uma vez que a sensibilidade das várias células produzidas ao longo do processo de espermatogénese é variável, o impacto está dependente do tipo de fármacos administrados e, por consequência, do tipo de células afetadas. As espermatogónias em diferenciação são extremamente sensíveis à maioria dos agentes antineoplásicos, e daí resulta uma redução transitória das contagens de esperma, ao passo que as células das fases mais terminais do processo são relativamente resistentes e, portanto, o impacto de uma determinada terapêutica pode não ser visível de imediato. Adicionalmente, os tratamentos da doença oncológica são potencialmente causadores de mutações nas células germinais do epitélio seminífero (Meistrich, 2009). A radioterapia, de uma forma geral, só é condicionadora da fertilidade quando são irradiadas as gónadas (ovário ou testículo), o eixo hipotálamo-hipófise ou o útero. Salienta-se que o epitélio germinal seminífero é extremamente sensível à radiação pelo que, mesmo em doses baixas, a radiação provoca danos funcionais significativos.

Ben-Aharon I. Shalgi R. What lies behind chemotherapy-induced ovarian toxicity? Reproduction. 2012; 144: 153-163.
Meistrich M. L. Male Gonadal Toxicity. Pediatr Blood Cancer. 2009 August ; 53(2): 261-266.
Howell S. J. , Shalet S. M. Spermatogenesis After Cancer Treatment: Damage and Recovery. J Natl Cancer Monogr Inst 2005;34:12-7.

CATEGORIZAÇÃO DE RISCO DA TERAPÊUTICA ANTINEOPLÁSICA E RADIOTERAPIA NA MULHER E NO HOMEM

Face ao exposto, a identificação do risco de infertilidade, em cada doente oncológico, não se revela tarefa fácil. A crescente complexidade dos tratamentos oncológicos, a utilização frequente de associações terapêuticas e o surgimento de novos fármacos antineoplásicos, para os quais não há qualquer informação sobre os efeitos na fertilidade humana, aliam-se à multiplicidade de fatores já referidos (idade, doses administradas, entre outros) e tornam a quantificação do risco, em cada doente, uma tarefa complexa e delicada.

No sentido de facilitar esta categorização de risco, em cada doente individual, diversas instituições e organismos internacionais, da área médica e não só, disponibilizam ferramentas que permitem aos clínicos recolher dados para, posteriormente, informar o doente oncológico sobre o seu risco individual. O **Anexo I** apresenta algumas dessas ferramentas, bem como as suas principais características, potencialidades, vantagens e desvantagens. A informação recolhida permitirá, ao doente e equipa clínica, fazer uma estimativa do risco de infertilidade associado aos tratamentos que vai realizar e ponderar a decisão de se submeter a alguma das técnicas de preservação da fertilidade disponíveis e que se revelem adequadas a cada caso.

Na utilização destas ferramentas, cabe ao clínico e/ou equipa analisar criticamente a informação disponibilizada, tendo em atenção aspetos como a origem da informação, o seu grau de evidência e atualidade, os indicadores de (in)fertilidade utilizados e as características dos doentes incluídos nos estudos. Só desta forma se conseguirá avaliar a sua aplicabilidade a um doente em concreto.

Nas orientações da American Society of Clinical Oncology (ASCO), atualizadas em 2013, é apresentada uma categorização do risco de infertilidade associado a diversos tratamentos da doença oncológica. Estas tabelas informam sobre o risco de efeitos na fertilidade, masculina e feminina, decorrentes de diversos tratamentos antineoplásicos e radioterapia, compilando informação de estudos que utilizaram como marcadores a produção de esperma, no homem, e a presença de amenorreia, na mulher. Esta categorização tem ainda em atenção alguns dos principais fatores influenciadores da fertilidade, nomeadamente a idade (fator muito relevante na mulher, como já referido), a dose e a duração da terapêutica antineoplásica ou a dose cumulativa de radiação. Para cada categoria de risco, a ASCO emite ainda recomendações relativas ao aconselhamento reprodutivo. Tendo em conta que as tabelas disponibilizadas pela ASCO são as de publicação mais recente e que a informação resultou de uma revisão sistemática da evidência publicada, considerou-se que seria pertinente incluir nesta brochura uma tradução/adaptação dessas tabelas (cf. **Anexo III A** e **Anexo III B**).

Novamente se salienta que todas as ferramentas apresentam limitações (cf. **Anexo I**) e, portanto, a informação que disponibilizam deverá ser cuidadosamente avaliada e interpretada.

2.

COMO PRESERVAR A FERTILIDADE EM DOENTES ONCOLÓGICOS?

2.1 Preservar a fertilidade: em que doentes e quando

Diversas orientações internacionais recentes (Loren et al., 2013; Peccatori et al., 2013; The Practice Committee of the American Society for Reproductive Medicine, 2012) recomendam que os profissionais de saúde devem referir a possibilidade de infertilidade a todos os doentes oncológicos em idade reprodutiva (e aos seus pais, no caso de crianças ou adolescentes menores). Deverão ainda estar preparados para discutir as opções de preservação da fertilidade e ou referenciar os doentes a especialistas em medicina da reprodução. Estas informações devem ser parte integrante do processo de esclarecimento e consentimento informado, antes de iniciar tratamento (Loren et al., 2013).
No **Anexo II** apresenta-se uma compilação das diversas orientações clínicas publicadas, no âmbito da preservação da fertilidade em doentes oncológicos.

As orientações da ASCO (Loren et al., 2013) sugerem que, na discussão deste tema com os doentes, sejam abordados os seguintes tópicos:

1. INFORMAÇÃO SOBRE O RISCO INDIVIDUAL

a. Alguns tratamentos da doença oncológica podem causar infertilidade ou menopausa prematura.
b. Na determinação do seu risco específico considerámos os seus fatores individuais (tipo de cancro, idade e tratamentos planeados).
c. De acordo com essa informação, acreditamos que o risco específico, no seu caso, é (elevado, médio, baixo, não existe).
d. A sua fertilidade antes do cancro poderá também influenciar o seu risco individual (discutir se relevante).

2. DISCUSSÃO DE PREOCUPAÇÕES FREQUENTES

a. Opções

I. Existem várias opções disponíveis para preservar a fertilidade e a parentalidade após o cancro.
II. Para o homem, a opção mais utilizada e bem-sucedida é a congelação de esperma; existem outras opções, se a congelação de esperma não for uma opção viável no seu caso.
III. Para a mulher, as opções mais estabelecidas são a congelação de embriões e de óvulos; de notar que a lei portuguesa não permite a utilização de técnicas de PMA (Procriação Medicamente Assistida) e, portanto, a produção de embriões, fora do contexto da infertilidade. Existem outras opções, experimentais, se estas não forem opções viáveis no seu caso.
IV. Se pretender ter mais informação poderá ser feita referenciação para consulta com um especialista em medicina reprodutiva.

b. Tempo

I. A questão tempo é essencial; os tratamentos para preservação da fertilidade devem ser realizados antes de iniciar a quimioterapia e/ou radioterapia.
II. No caso do homem, a colheita e congelação de esperma podem ser realizadas de forma rápida e repetidas a cada 24h, enquanto seja necessário.
III. Na mulher, a preservação da fertilidade poderá demorar 2 a 3 semanas, no caso das técnicas estabelecidas; no entanto, algumas opções experimentais poderão ser realizadas mais rapidamente e, por isso, a referenciação atempada ao especialista em reprodução é importante.

C. Riscos associados à gravidez e filhos após o cancro

I. Muitas doentes preocupam-se com a segurança de uma gravidez após o cancro. Os dados são limitados, mas não parece haver um risco aumentado de recorrência associado às técnicas de preservação da fertilidade ou à gravidez, mesmo em tumores hormonodependentes.
II. De igual modo, muitos doentes têm preocupações relacionadas com o risco de transmitirem o cancro à descendência. À exceção das síndromas genéticas hereditárias e da exposição *in utero* a alguns tratamentos de quimioterapia, não há qualquer evidência de que a história de cancro, tratamento do cancro ou intervenções na área da fertilidade, aumentem o risco de cancro ou anomalias congénitas na descendência.

Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K; American Society of Clinical Oncology. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013 Jul 1;31(19):2500-10.
Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G; ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013 Oct;24 Suppl 6:vi160-70.
The Practice Committee of the American Society for Reproductive Medicine. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril. 2013 Nov;100(5):1214-23.

2.2 Técnicas de preservação da fertilidade no homem

Criopreservação de esperma

As amostras de esperma, obtidas por masturbação, biópsia testicular ou eletroejaculação, são criopreservadas. Posteriormente, quando o doente assim o pretender, o esperma poderá ser usado para a criação de embriões, por inseminação intra-uterina (IIU), fertilização in-vitro (FIV) ou injeção intra-citoplasmática de espermatozoides (ICSI).

Classificação
Técnica Estabelecida.

Indicações
PARA QUEM: Homens e jovens na pós-puberdade.
QUANDO: Antes de iniciar os tratamentos potencialmente gonadotóxicos.

Taxas de sucesso
A taxa de gravidez é variável e depende, entre outros fatores, da técnica de fecundação utilizada. Há centros que relatam taxas de gravidez, por ciclo, de 40 a 50%, com a utilização de esperma criopreservado de sobreviventes de doença oncológica e recorrendo a técnicas de FIV/ICSI (Selk et al., 2009; van Casteren et al., 2008).

Selk A, Belej-Rak T, Shapiro H, Greenblatt E. Use of an oncology sperm bank: a Canadian experience. Can Urol Assoc J. 2009 Jun;3(3):219-222.
van Casteren NJ, van Santbrink EJ, van Inzen W, Romijn JC, Dohle GR. Use rate and assisted reproduction technologies outcome of cryopreserved semen from 629 cancer patients. Fertil Steril. 2008 Dec;90(6):2245-50. doi: 10.1016/j.fertnstert.2007.10.055. Epub 2008 Jan 14.

Riscos para o homem

Não existe qualquer risco associado a esta técnica.

Riscos para a descendência

Os tratamentos da doença oncológica são potencialmente causadores de mutações nas células germinais do epitélio seminífero. No entanto, os dados atualmente disponíveis indicam que não há um risco aumentado de malformações congénitas ou de doença oncológica nas crianças nascidas com utilização de esperma criopreservado de sobreviventes de doença oncológica. De igual forma, a descendência de sobreviventes de doença oncológica do sexo masculino não apresenta risco aumentado de complicações obstétricas e perinatais ou de baixo peso à nascença (Dohle, 2010).

Dohle GR. Male infertility in cancer patients: Review of the literature. Int J Urol. 2010 Apr;17(4):327-31.

Conservação do esperma criopreservado

De acordo com a lei vigente em Portugal, o esperma é criopreservado por um período de três anos. Findo esse período, o proprietário do material biológico tem de se deslocar ao centro onde foi realizada a criopreservação para assinar um consentimento de manutenção desta. Na ausência deste consentimento, o esperma deverá ser descongelado e eliminado. O esperma poderá ser utilizado para fins científicos desde que tenha ficado expressa, no consentimento informado, autorização para essa utilização.

Momento ideal para conceção

Tendo em conta o tempo necessário para reparar eventuais alterações genéticas, recomenda-se que o doente oncológico aguarde pelo menos 2 anos, após terminarem os tratamentos, antes de tentar ser pai (Fertile Hope, 2013).
Salienta-se que o esperma criopreservado antes de iniciar qualquer tratamento oncológico não foi exposto às substâncias potencialmente mutagénicas e, teoricamente, pode ser utilizado de imediato.

Fertile Hope 2013. Pregnancy & Children After Cancer. Acessível em www.fertilehope.org/learn-more/cancer-and-fertility-info/pregnancy-and-children-after-cancer.cfm

Custos

No âmbito do Serviço Nacional de Saúde (SNS), a criopreservação de esperma de doentes oncológicos e a sua manutenção são, em geral, procedimentos que não envolvem custos para o doente.

Criopreservação de tecido testicular

Em homens adultos, esta técnica é uma alternativa à criopreservação de esperma, quando não é possível a ejaculação ou quando o esperma ejaculado não contém espermatozóides. As amostras de tecido testicular são obtidas através de biópsia, preparadas e criopreservadas. Após terminar os tratamentos, e quando o doente assim pretender, as amostras podem ser descongeladas e os espermatozoides utilizados para fecundação por ICSI.

Em crianças ou jovens pré-púberes, a criopreservação de tecido testicular é a única opção de preservação da fertilidade, embora, nesta população, seja uma técnica experimental, sem registo de experiências clínicas de sucesso. Prevê-se que, num futuro próximo, venha a ser possível transplantar o tecido criopreservado/descongelado ou realizar maturação *in vitro* das células espermáticas (Dohle, 2010).

Dohle GR. Male infertility in cancer patients: Review of the literature. Int J Urol. 2010 Apr;17(4):327-31.

Classificação

Em homens e jovens na pós-puberdade, a recolha de tecido testicular através de biópsia e subsequente criopreservação é uma técnica estabelecida.

Em indivíduos pré-púberes, a recolha e criopreservação de tecido testicular imaturo é uma técnica ainda experimental. Tendo em conta os avanços promissores da investigação nesta área, espera-se que, num futuro próximo, possa ser utilizada com sucesso.

Indicações

PARA QUEM: única opção disponível para crianças e jovens na pré-puberdade; homens e rapazes na pós-puberdade nos quais a recolha de espermatozoides no ejaculado não seja possível (p. ex. em casos de azoospermia).

QUANDO: antes de iniciar os tratamentos potencialmente gonadotóxicos.

Taxas de sucesso

As taxas de sucesso, embora muito variáveis, são geralmente inferiores às que se obtêm com o uso de esperma criopreservado. Não obstante, há relatos de taxas de gravidez de 40% e 55,8%, por ciclo, utilizando esperma testicular criopreservado/descongelado (Ulug et al.,2005; Kalsi et al., 2011).

No que se refere à criopreservação de tecido testicular imaturo, até à data a investigação está limitada a estudos pré-clínicos.

Ulug U, Bener F, Karagenc L, Ciray N, Bahceci M. Outcomes in couples undergoing ICSI: comparison between fresh and frozen thawed surgically retrieved spermatozoa. Int J Androl. 2005;28(6):343–9.

Kalsi J, Thum MY, Muneer A, Pryor J, Abdullah H, Minhas S. Analysis of the outcome of intracytoplasmic sperm injection using fresh or frozen sperm. BJU Int. 2011 Apr;107(7):1124-8.

Riscos para o homem

Alguns riscos associados à biópsia testicular incluem o desenvolvimento de hematoma, edema ou dor na zona escrotal e, menos frequentemente, o risco de hemorragia ou infeção. Algumas complicações mais graves, que são muito raras, incluem lesão nervosa ou atrofia testicular. Por outro lado, pode não ser possível obter espermatozoides no tecido testicular colhido (Urology Care Foundation, 2011).

Urology Care Foundation. Sperm retrieval. Consultado em 04/12/2013. Acessível em http://www.urologyhealth.org/urology/index.cfm?article=133. Last updated April 2013.

Riscos para a descendência

A descendência de sobreviventes de doença oncológica do sexo masculino não apresenta risco aumentado de complicações obstétricas e perinatais ou de baixo peso à nascença. Por outro lado, embora os tratamentos da doença oncológica sejam potencialmente causadores de mu-

tações nas células germinais do epitélio seminífero, não se verificou um risco aumentado de malformações congénitas ou de doença oncológica nas crianças nascidas com utilização de esperma criopreservado de sobreviventes de doença oncológica (Dohle, 2010).

Dohle GR. Male infertility in cancer patients: Review of the literature. Int J Urol. 2010 Apr;17(4):327-31.

Conservação do tecido testicular

De acordo com a lei vigente em Portugal, o tecido testicular é criopreservado por um período de três anos. Findo esse período, o proprietário do material biológico tem de se deslocar ao centro onde foi realizada a criopreservação para assinar um consentimento de manutenção desta. Na ausência deste consentimento, o tecido testicular deverá ser descongelado e eliminado. O tecido pode ser utilizado para fins científicos desde que tenha ficado expressa, no consentimento informado, autorização para essa utilização.

Momento ideal para conceção

Tendo em conta o tempo necessário para reparar eventuais alterações genéticas, recomenda-se que o doente oncológico aguarde pelo menos 2 anos, após terminarem os tratamentos, antes de tentar ser pai (Fertile Hope, 2013).

Se o tecido testicular a utilizar tiver sido criopreservado antes de iniciar tratamentos, não foi exposto às substâncias potencialmente mutagénicas e, teoricamente, pode ser utilizado de imediato.

Fertile Hope 2013. Pregnancy & Children After Cancer. Acessível em http://www.fertilehope.org/learn-more/cancer-and-fertility-info/pregnancy-and-children-after-cancer.cfm

Custos

No âmbito do Serviço Nacional de Saúde (SNS), a criopreservação de tecido testicular de doentes oncológicos e a sua manutenção são, em geral, procedimentos que não envolvem custos para o doente.

2.3 Técnicas de preservação da fertilidade na mulher

2.3.1 TÉCNICAS ESTABELECIDAS

Criopreservação de embriões

A preservação da fertilidade através da criopreservação de embriões compreende uma fase inicial de estimulação hormonal, seguida de punção folicular para recolha de ovócitos e posterior inseminação por fertilização in vitro (FIV) ou por microinjeção de espermatozoides (ICSI). Os embriões obtidos são então criopreservados. Quando o casal assim o pretender, os embriões são descongelados e transferidos para o útero da mulher.

Classificação

Técnica Estabelecida.

De notar que a legislação portuguesa restringe a aplicação de técnicas de Procriação Medicamente Assistida (PMA) a casais com diagnóstico de infertilidade, não contemplando estas situações específicas em que se pretende uma alternativa para uma situação de infertilidade induzida pela terapêutica.

Indicações
PARA QUEM: mulheres na pós-puberdade, com parceiro, desde que não existam objeções morais e/ou éticas à criopreservação de embriões.
QUANDO: antes de iniciar os tratamentos potencialmente gonadotóxicos.

Requisitos em termos de tempo
A utilização de protocolos de estimulação após administração de antagonistas da GnRH (Hormona libertadora de Gonadotrofinas) permite iniciar a estimulação na fase lútea e é, portanto, uma estratégia útil quando a mulher se encontra nessa fase do ciclo menstrual e há necessidade de iniciar rapidamente os tratamentos. Neste caso, o processo fica concluído em 2 a 3 semanas (Nayak & Wakim, 2011; von Wolff et al., 2009). Se a estimulação hormonal for iniciada na fase folicular o processo demora apenas 2 semanas.

Nayak SR, Wakim AN. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles with GnRH agonist trigger for fertility preservation. Fertil Steril. 2011 Jul;96(1):e51-4.
von Wolff M, Thaler CJ, Frambach T, Zeeb C, Lawrenz B, Popovici RM, Strowitzki T. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. Fertil Steril 2009; 92: 1360-1365.

Taxas de sucesso
Está descrita uma taxa de gravidez de 35,6%, em mulheres até aos 35 anos, por ciclo (Westphal & Massie, 2012). À medida que a idade da mulher aumenta, a probabilidade de conseguir iniciar uma gravidez diminui de forma significativa.

Westphal LM, Massie JAM. Oncofertility Medical Practice: Clinical Issues and Implementation; Chapter 4: Embryo and Oocyte Banking. C. Gracia and T.K. Woodruff (eds.). Springer 2012. Acessível em http://oncofertility.northwestern.edu/sites/default/files/uploadedfilecontent/embryo_and_oocyte_banking_-_lynn_m._westphal_and_jamie_a.m._massie.pdf

Riscos para a mulher
A administração de indutores da ovulação poderá resultar numa resposta exagerada - Síndroma de Hiperestimulação Ovárica - cujas formas ligeiras são relativamente frequentes (1 em cada 3 mulheres apresenta sintomas ligeiros). O risco de situações mais graves é baixo com o recurso a protocolos que incluem antagonistas da GnRH (De Sutter, Gherris & Dhont, 2008). A exposição a níveis elevados de estrogénios pode ser preocupante em doentes com tumores dependentes de estrogénios (nomeadamente cancro da mama); para obviar este problema têm vindo a ser propostos protocolos de estimulação que utilizam inibidores da aromatase. Estes protocolos permitem evitar os níveis séricos elevados de estrogénios, resultantes da estimulação ovárica (Rodriguez-Wallberg & Oktay, 2012). Relativamente à punção folicular, existem alguns riscos menores, como infeções ou hemorragias, que ocorrem em menos de 1% dos casos (De Sutter, Gherris & Dhont, 2008).
No que diz respeito ao eventual risco aumentado de cancro associado às técnicas de FIV, uma meta-análise recente, que incluiu um total de 109.969 mulheres expostas, não demonstrou associação significativa com a ocorrência de cancro do ovário, endométrio ou colo do útero, quando se utilizou como grupo de controlo mulheres inférteis (Siristatidis et al., 2013).

Riscos para a descendência
Os dados disponíveis indicam que não existe um risco aumentado de parto prematuro, baixo peso à nascença ou malformações nas crianças nascidas de embriões criopreservados, descongelados e transferidos, face à transferência de embriões frescos (Wennerholm et al., 2009).

De Sutter P, Gerris J, Dhont M. Assisted reproductive technologies: how to minimize the risks and complications in developing countries? ESHRE Monogr (2008) 2008 (1): 73-76.
Rodriguez-Wallberg KA, Oktay K. Fertility Preservation and Pregnancy in Women With and Without BRCA Mutation–Positive Breast Cancer. The Oncologist 2012; 17: 1409–1417
Siristatidis C, Sergentanis TN, Kanavidis P, Trivella M, Sotiraki M, Mavromatis I, Psaltopoulou T, Skalkidou A,Petridou ET. Controlled ovarian hyperstimulation for IVF: impact on ovarian, endometrial and cervical cancer—a systematic review and meta-analysis. Hum Reprod Update 2013; Vol.19, No.2 pp. 105–123.
Wennerholm UB, Söderström-Anttila V, Bergh C, Aittomäki K, Hazekamp J, Nygren KG, Selbing A, Loft A. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. Hum Reprod. 2009 Sep;24(9):2158-72.

Momento ideal para gravidez

A determinação da altura ideal para iniciar uma gravidez deve ser feita caso a caso, considerando vários fatores como a data em que terminam os tratamentos, o risco de recidiva, a idade e a função ovárica de cada doente (Peccatori, 2013).

Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G; ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013 Oct;24 Suppl 6:vi160-70.

Utilização dos embriões criopreservados

De acordo com a lei vigente em Portugal, se os embriões criopreservados não forem utilizados no prazo de três anos, o casal terá de se deslocar ao centro onde foi realizada a criopreservação para assinar um consentimento de manutenção desta, por um período adicional de 3 anos. Findo este prazo, se os embriões não tiverem sido utilizados pelo casal, deverão ser descongelados e eliminados. Mediante autorização do casal, na altura do consentimento, os embriões não utilizados podem ser doados a outros casais inférteis e/ou utilizados em projetos de investigação científica.

De notar que só poderão ser produzidos embriões para efeito de preservação da fertilidade se a doença oncológica surgir num casal com uma situação de infertilidade documentada, uma vez que a lei portuguesa não permite a utilização de técnicas de PMA fora do contexto da infertilidade.

Custos

No âmbito do Serviço Nacional de Saúde, a doente oncológica apenas terá que suportar os custos dos medicamentos utilizados para estimulação da ovulação, que pode variar entre 200 e 500 euros, aproximadamente. Estes custos dependem do protocolo de estimulação utilizado.

Criopreservação de ovócitos

A preservação da fertilidade através da criopreservação de ovócitos compreende uma fase inicial de estimulação hormonal, seguida de punção folicular e criopreservação dos ovócitos obtidos por vitrificação. A técnica de congelação por vitrificação veio melhorar significativamente a sobrevivência dos ovócitos, as taxas de fertilização e a proporção de embriões de elevada qualidade, face à congelação lenta, permitindo, inclusive, obter resultados comparáveis aos da utilização de ovócitos frescos (Cobo & Diaz, 2011).

Quando a mulher assim o pretender, os gâmetas preservados são descongelados e fecundados com recurso a FIV/ICSI. Os embriões obtidos são então transferidos para o útero da mulher.

Cobo & Diaz, 2011
Nayak SR, Wakim AN. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles with GnRH agonist trigger for fertility preservation. Fertil Steril. 2011 Jul;96(1):e51-4.
von Wolff M, Thaler CJ, Frambach T, Zeeb C, Lawrenz B, Popovici RM, Strowitzki T. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. Fertil Steril 2009; 92: 1360-1365.

Classificação
Técnica Estabelecida.

Indicações
PARA QUEM: jovens na pós-puberdade; mulheres em idade reprodutiva, especialmente na ausência de parceiro ou quando existem objeções morais/éticas à criopreservação de embriões.
QUANDO: antes de iniciar os tratamentos potencialmente gonadotóxicos.

Requisitos em termos de tempo
A utilização de protocolos de estimulação após administração de antagonistas da GnRH (Hormona libertadora de Gonadotrofinas) permite iniciar a estimulação na fase lútea e é, portanto, uma estratégia útil quando a mulher se encontra nessa fase do ciclo menstrual e há necessidade de iniciar rapidamente os tratamentos. Neste caso, o processo fica concluído em 2 a 3 semanas (Nayak & Wakim, 2011; von Wolff et al., 2009). Se a estimulação hormonal for iniciada na fase folicular o processo demora apenas 2 semanas.

Taxas de sucesso
A evidência mais recente indica que as taxas de fertilização e gravidez resultantes da FIV/ICSI são similares quando se utilizam ovócitos frescos ou quando se utilizam ovócitos vitrificados/desvitrificados (The Practice Committee of the ASRM, 2012; Cobo & Diaz, 2011).
A taxa de nascimentos por ovócito criopreservado, nos centros com maior experiência, é de 5,7% (Donnez, 2013) pelo que, teoricamente, será necessário criopreservar 20 ovócitos para assegurar uma probabilidade de cerca de 100% de sucesso.

Cobo A, Diaz C. Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril 2011; 96: 277-85.
Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol. 2013 Dec;9(12):735-49.
The Practice Committee of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. 2012. Mature oocyte cryopreservation: a guideline. Fertil Steril 2012.

Momento ideal para gravidez
A determinação da altura ideal para iniciar uma gravidez deve ser feita caso a caso, considerando vários fatores como a data em que terminam os tratamentos, o risco de recidiva, a idade e a função ovárica de cada doente (Peccatori, 2013). Para reduzir a probabilidade de fecundação de ovócitos com eventuais alterações genéticas, recomenda-se aguardar um mínimo de 6 meses, após terminar o tratamento (FertileHope, 2013).

Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G; ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013 Oct;24 Suppl 6:vi160-70.
Fertile Hope 2013. Pregnancy & Children After Cancer. Acessível em http://www.fertilehope.org/learn-more/cancer-and-fertility-info/pregnancy-and-children-after-cancer.cfm

Utilização dos gâmetas criopreservados
De acordo com a lei vigente em Portugal, se os ovócitos criopreservados não forem utilizados no prazo de três anos, a mulher terá de se deslocar ao centro onde foi realizada a criopreservação para assinar um consentimento de manutenção desta, por um período adicional de 3 anos. Na ausência de uma declaração assinada a solicitar a manutenção da criopreservação, e decorrido este prazo, os ovócitos deverão ser descongelados e eliminados, a menos que tenha ficado expresso, no consentimento, autorização para o seu uso em projetos de investigação científica.

Riscos para a mulher

A administração de indutores da ovulação poderá resultar numa resposta exagerada - Síndroma de Hiperestimulação Ovárica - cujas formas ligeiras são relativamente frequentes (1 em cada 3 mulheres apresenta sintomas ligeiros). O risco de situações mais graves é baixo devido à utilização de protocolos com antagonistas da GnRH (De Sutter, Gherris & Dhont, 2008). A exposição a níveis elevados de estrogénios pode ser preocupante em doentes com tumores dependentes de estrogénios (nomeadamente cancro da mama); para obviar este problema têm vindo a ser propostos protocolos de estimulação que utilizam inibidores da aromatase. Estes protocolos permitem evitar os níveis séricos elevados de estrogénios, resultantes da estimulação ovárica (Rodriguez-Wallberg & Oktay, 2012). Relativamente à punção folicular, existem alguns riscos menores, como infeções ou hemorragias, que ocorrem em menos de 1% dos casos (De Sutter, Gherris & Dhont, 2008).

No que diz respeito ao eventual risco aumentado de cancro associado às técnicas de FIV, uma meta-análise recente, que incluiu um total de 109.969 mulheres expostas, não demonstrou associação significativa com a ocorrência de cancro do ovário, endométrio ou colo do útero, quando se utilizou como grupo de controlo mulheres inférteis (Siristatidis et al., 2013).

De Sutter P, Gerris J, Dhont M. Assisted reproductive technologies: how to minimize the risks and complications in developing countries? ESHRE Monogr (2008) 2008 (1): 73-76.
Rodriguez-Wallberg KA, Oktay K. Fertility Preservation and Pregnancy in Women With and Without BRCA Mutation–Positive Breast Cancer. The Oncologist 2012; 17: 1409-1417
Siristatidis C, Sergentanis TN, Kanavidis P, Trivella M, Sotiraki M, Mavromatis I, Psaltopoulou T, Skalkidou A,Petridou ET. Controlled ovarian hyperstimulation for IVF: impact on ovarian, endometrial and cervical cancer - a systematic review and meta-analysis. Hum Reprod Update 2013; Vol.19, No.2 pp. 105-123.

Riscos para a descendência

Uma revisão de estudos publicados não identificou risco aumentado de abortos ou malformações em mais de 900 bebés nascidos de ovócitos criopreservados (por congelação lenta ou vitrificação), comparativamente a outros concebidos naturalmente (Noyes, 2009).

Noyes N, Porcu E, Borini A. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. Reprod Biomed Online. 2009 Jun;18(6):769-76.

Custos

No âmbito do Serviço Nacional de Saúde, a doente oncológica apenas terá que suportar os custos dos medicamentos utilizados para estimulação da ovulação, que pode variar entre 200 e 500 euros, aproximadamente. Estes custos dependem do protocolo de estimulação utilizado.

2.3.2 TÉCNICAS EXPERIMENTAIS

Criopreservação de tecido ovárico

Procede-se a colheita, por cirurgia laparoscópica, de um fragmento ou da totalidade do ovário. O tecido ovárico é devidamente preparado, isolando-se fragmentos do córtex que são, então, criopreservados. Quando a mulher assim o pretender, os fragmentos são descongelados e poderão ser enxertados no ovário remanescente - transplante ortotópico - ou noutra localização – transplante heterotópico. Há vantagem em remover o ovário completo pois permite obter maior quantidade de tecido para criopreservação e ainda repetir o transplante, caso o primeiro enxerto não seja bem-sucedido (Rosendhal et al., 2011). Após transplante, o tecido ovárico pode repor

a função endócrina e a fertilidade, possibilitando uma conceção natural (Dolmans et al., 2013; Schmidt et al., 2013).

Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, Donnez J, Van Langendonckt A. A review of 15 years of ovarian tissue bank activities. J Assist Reprod Genet. 2013 Mar;30(3):305-14.
Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, Andersen AN, Andersen CY. Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique. Reprod BioMed Online (2011) 22, 162– 171.
Schmidt KT, Ernst E, Greve T, Andersen CY.Transplantation of Frozen Thawed Ovarian Tissue – State of the Art. J. Reproduktionsmed. Endokrinol 2013; 10 (Sonderheft 1), 55-58.

Classificação
Técnica Experimental.

Indicações
PARA QUEM: única técnica de preservação da fertilidade que pode ser utilizada em crianças e raparigas na pré-puberdade; raparigas e mulheres na pós-puberdade, nas situações em que é indesejável a estimulação hormonal e/ou há necessidade de iniciar tratamento com urgência.
QUANDO: antes de iniciar os tratamentos potencialmente gonadotóxicos.

Requisitos em termos de tempo
Uma vez que não exige estimulação hormonal, esta técnica não implica qualquer adiamento do tratamento da doença oncológica.

Taxas de sucesso
Não é possível calcular a taxa de sucesso do transplante de tecido ovárico uma vez que não é conhecido o número total de transplantes já efetuados. No entanto, diversos centros a nível mundial têm reportado experiências de criopreservação e posterior transplantação ovárica, com resultados promissores. Num total de 60 doentes em que foi reimplantado tecido ovárico, de forma ortotópica, 11 conseguiram engravidaram e a maioria (93%) recuperou a função ovárica (Donnez et al., 2013). Só na Bélgica, em 15 anos de experiência realizaram-se 582 criopreservações de tecido ovárico, das quais resultaram 11 transplantes ortotópicos e 5 nascimentos, 4 deles decorrentes de conceção natural (Dolmans et al., 2013).
Até à data, estão relatados em todo o mundo 40 nascimentos em resultado da utilização de tecido ovárico, transplantado de forma ortotópica (Donnez & Dolmans, 2013). Recentemente foi relatado o primeiro caso de gravidez após transplante heterotópico de tecido ovárico (Stern et al., 2013).
A duração da manutenção da função ovárica, após transplante, está dependente da quantidade de tecido transplantado e da idade da mulher aquando do procedimento. Em média, a função ovárica permanece durante 4 a 5 anos (Donnez & Dolmans, 2013) mas há casos em que persistiu durante mais de 7 anos (Andersen et al., 2012).

Andersen CY, Silber SJ, Bergholdt SH, Jorgensen JS, Ernst E. Long-term duration of function of ovarian tissue transplants: case reports. Reprod Biomed Online. 2012 Aug;25(2):128-32.
Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, Donnez J, Van Langendonckt A. A review of 15 years of ovarian tissue bank activities. J Assist Reprod Genet. 2013 Mar;30(3):305-14.
Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Sanchez Serrano M, Schmidt KT, Ernst E, Luyckx V, Andersen CY. Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. Fertil Steril. 2013 May;99(6):1503-13.
Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol. 2013 Dec;9(12):735-49.
Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, Jobling T. First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. Hum Reprod. 2013 Nov;28(11):2996-9.

Utilização do tecido ovárico criopreservado

De acordo com a lei vigente em Portugal, o tecido ovárico permanece criopreservado por um período de três anos, findo o qual a mulher deverá deslocar-se ao centro onde foi realizada a criopreservação para assinar um consentimento de manutenção desta. Na ausência de uma declaração assinada a solicitar a manutenção da criopreservação por um período adicional de 3 anos, e decorrido este prazo, o tecido ovárico deverá ser eliminado, a menos que tenha ficado expresso, no consentimento, autorização para o seu uso em projetos de investigação científica.

Momento ideal para gravidez

A determinação da altura ideal para iniciar uma gravidez deve ser feita caso a caso, considerando vários fatores como a data em que terminam os tratamentos, o risco de recidiva, a idade e a função ovárica de cada doente (Peccatori, 2013).

Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G; ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013 Oct;24 Suppl 6:vi160-70.

Riscos

Existem riscos associados à anestesia e cirurgia laparoscópica, embora complicações graves sejam raras e este procedimento seja, geralmente, bem tolerado pelas doentes (Dolmans et al., 2013; Rosendhal et al., 2008).

Relativamente ao risco teórico de criopreservar, e posteriormente transplantar, tecido ovárico com células neoplásicas, estão em desenvolvimento diversas técnicas histológicas, de PCR (*Polymerase Chain Reaction*), de citometria de fluxo e de xenotransplantação que ajudam a excluir estes casos. Por outro lado, o transplante de folículos de forma isolada, após maturação *in vitro* ou através do "ovário artificial", poderão permitir este risco, no futuro (Donnez & Dolmans, 2013).

Muito recentemente, foi publicado o primeiro relato de nascimento com sucesso, resultante da maturação *in vitro* de ovócitos obtidos após ooforectomia numa doente com cancro do ovário (Prasath et al., 2013).

Uma revisão recente categorizou o risco de metástases ováricas de acordo com o tipo de cancro, concluindo que é elevado para as situações de leucemia, neuroblastoma e linfoma de Burkitt; moderado para cancro da mama em estadio IV ou do subtipo lobular, cancro do cólon, adenocarcinoma do colo uterino, linfoma não-Hodgkin e sarcoma de Ewing; e baixo para cancro da mama em estadios I-II ou do subtipo ductal, carcinoma de células escamosas do colo uterino, linfoma Hodgkin, carcinoma osteogénico, rabdomiossarcoma não-genital e tumor de Wilms (Dolmans et al., 2013).

Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. Fertil Steril. 2013 May;99(6):1514-22.
Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, Donnez J, Van Langendonckt A. A review of 15 years of ovarian tissue bank activities. J Assist Reprod Genet. 2013 Mar;30(3):305-14.
Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol. 2013 Dec;9(12):735-49.
Prasath EB, Chan MLH,Wong WHW, Lim CJW, Tharmalingam MD, Hendricks M, Loh, Chia WN. First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient. Hum. Reprod. Advance Access published December 9, 2013.
Rosendahl, M., Andersen, C.Y., Ernst, E., et al. Ovarian function after removal of an entire ovary for cryopreservation of pieces of cortex prior to gonadotoxic treatment: a follow-up study. Hum Reprod 2008. 23, 2475–2483.

Custos

No âmbito do Serviço Nacional de Saúde (SNS), a criopreservação de tecido ovárico de doen-

tes oncológicas e a sua manutenção são, em geral, procedimentos que não envolvem custos para as doentes.

2.3.3 COMPARAÇÃO DAS TÉCNICAS DE PRESERVAÇÃO DA FERTILIDADE FEMININA

Está disponível, no **Anexo IV**, uma tabela que sumaria as características das várias técnicas de preservação da fertilidade feminina, comparando-as em termos de indicações, requisitos de tempo, taxas de sucesso, riscos, vantagens e desvantagens.

2.3.4 OUTRAS PRÁTICAS

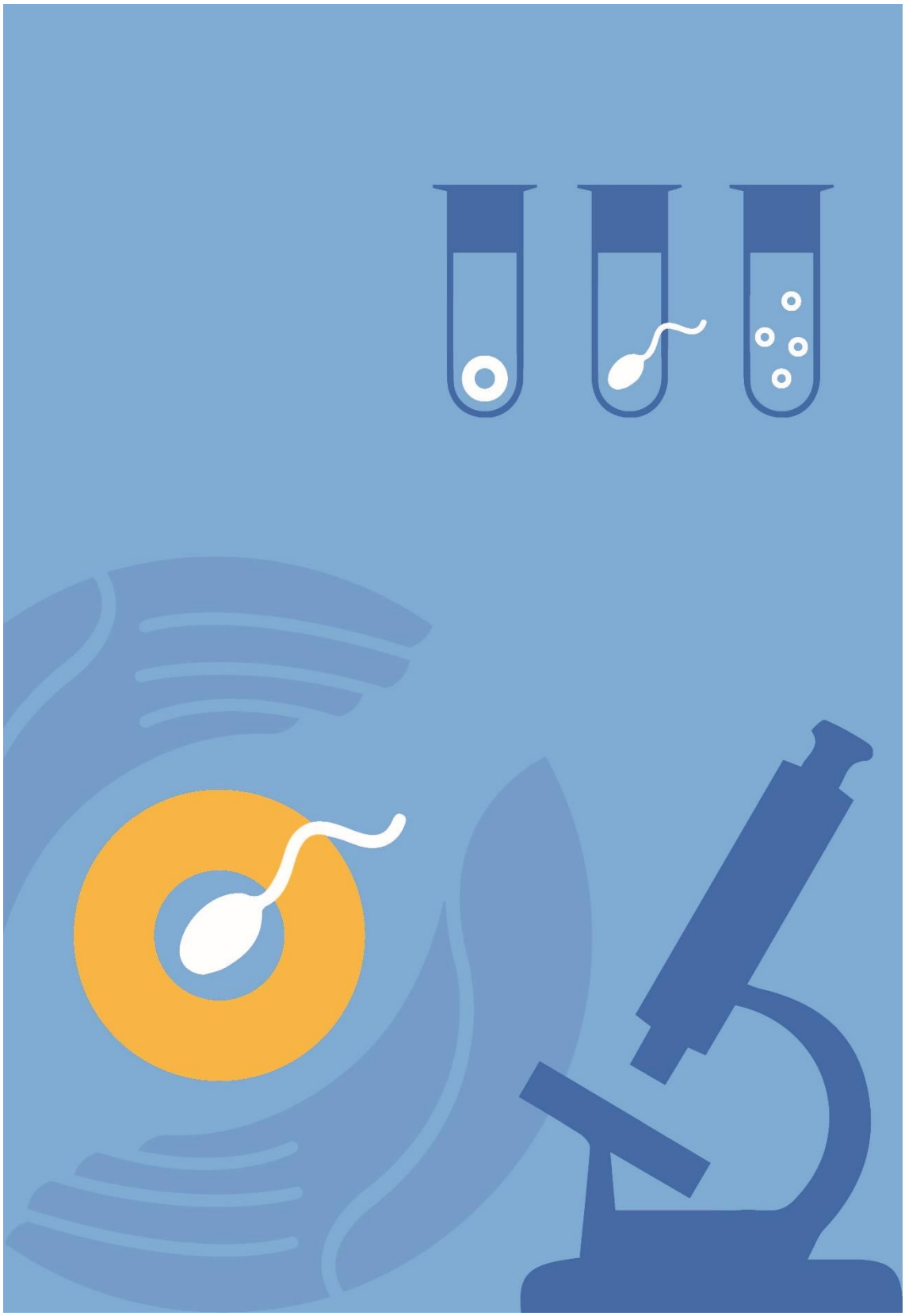
Transposição ovárica ou ooforopexia

A transposição ovárica ou ooforopexia pode ser oferecida quando a doente vai ser sujeita a radioterapia pélvica, de forma a minimizar a exposição dos ovários à radiação. No entanto, devido à dispersão de radiação, os ovários nem sempre ficam protegidos e as doentes devem ser informadas de que esta técnica nem sempre é totalmente eficaz. Este procedimento deve ser realizado o mais próximo possível do momento em que é administrada a radioterapia, devido ao risco de migração dos ovários (Loren et al., 2013).

Administração de agonistas da GnRH

Atualmente, os resultados de efetividade dos agonistas da GnRH (Hormona Libertadora de Gonadotrofinas), para preservação da fertilidade feminina, não são ainda conclusivos (Roness et al, 2014) e a sua utilização não é recomendada como método de preservação da fertilidade (Loren et al., 2013; Peccatori et al., 2013). Outros benefícios possivelmente associados à sua utilização, como a redução de hemorragias vaginais, devem ser ponderados face a riscos como a perda de massa óssea, os afrontamentos e a potencial interferência com a resposta ao tratamento em tumores hormono-dependentes (Loren et al., 2013).

Roness H, Kalich-Philosoph L, Meirow D. Prevention of chemotherapy-induced ovarian damage: possible roles for hormonal and non-hormonal attenuating agents. Hum Reprod Update 2014.20(5): 759–774.
Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K; American Society of Clinical Oncology. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013 Jul 1;31(19):2500-10.
Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G; ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013 Oct;24 Suppl 6:vi160-70.



3.

PERGUNTAS E RESPOSTAS

1. Qual o momento ideal para discutir com os doentes oncológicos a possibilidade de preservarem a sua fertilidade?

A discussão sobre os potenciais efeitos dos tratamentos oncológicos na fertilidade e sobre a possibilidade de preservar a fertilidade deve acontecer, idealmente, na altura do diagnóstico da doença oncológica. As mais recentes orientações internacionais indicam que estas informações fazem parte do processo de educação e consentimento informado junto de todos os doentes oncológicos em idade reprodutiva, antes de iniciarem a terapêutica oncológica.

Quanto mais precoce for a discussão destes temas, maior será o leque de opções disponíveis, na medida em que algumas técnicas necessitam de 2 a 3 semanas para poderem ser realizadas. Por outro lado, a discussão atempada permitirá dispor de mais tempo para um processo de decisão informada e partilhada.

2. Se referenciar um doente para o Centro de Preservação da Fertilidade, que técnicas estão disponíveis?

O Centro de Preservação da Fertilidade do CHUC, EPE, é um centro altamente especializado nas técnicas de preservação da fertilidade feminina, que se revelam tecnicamente mais complexas e exigem um trabalho de equipa multidisciplinar. Neste âmbito, está disponível a criopreservação de ovócitos, técnica estabelecida, e a criopreservação de tecido ovárico, técnica considerada ainda experimental.

No âmbito da preservação da fertilidade masculina, o Centro de Preservação da Fertilidade do CHUC, EPE realiza criopreservação de esperma e de fragmentos de biópsia testicular, quando indicado.

3. Em que consiste a consulta de preservação da fertilidade?

No Centro de Preservação da Fertilidade, a consulta de preservação da fertilidade é realizada por uma equipa clínica multidisciplinar, constituída por médico especialista em reprodução humana e psicólogo clínico. Fazem ainda parte da equipa do centro outros técnicos, como embriologista e farmacêutico. A decisão relativa à preservação (ou não) da fertilidade e à seleção da(s) técnica(s) a utilizar é feita de forma partilhada, depois do(a) doente ser convenientemente informado(a) dos riscos e benefícios de cada uma das opções disponíveis. Na tomada de decisão são tidos em conta fatores clínicos, pessoais, éticos e legais e, sempre que possível, discutidas as opções com o médico oncologista.

4. Como devo proceder para referenciar um doente oncológico para uma consulta de Preservação da Fertilidade?

Poderá referenciar um doente ao Centro de Preservação da Fertilidade do CHUC, EPE através dos

seguintes contactos:
Centro de Preservação da Fertilidade do CHUC, EPE
Tel. 239 400 698
preservacaofertilidade@huc.min-saude.pt

O Centro de Preservação da Fertilidade do CHUC, EPE compromete-se a marcar uma consulta num prazo máximo de uma semana sendo que, em geral, as doentes são consultadas em 1 a 3 dias, de acordo com a sua disponibilidade para se deslocarem ao centro.

5. Existem orientações, nacionais ou internacionais, sobre a preservação da fertilidade em doentes oncológicos?

A nível internacional, diversas organizações e sociedades científicas, europeias e norte-americanas, publicaram orientações nesta área, que se apresentam no **Anexo II**. À data da elaboração desta brochura não existiam quaisquer orientações nacionais sobre este tema.

6. Quais as técnicas de preservação da fertilidade atualmente consideradas como prática médica estabelecida?

No âmbito da preservação da fertilidade feminina, quer a criopreservação de embriões quer a criopreservação de ovócitos são atualmente consideradas como prática médica estabelecida, tendo em conta a vasta informação disponível relativamente à sua eficácia e segurança. No entanto, a legislação portuguesa restringe a aplicação de técnicas de PMA a casais com diagnóstico de infertilidade, não contemplando estas situações específicas em que se pretende uma alternativa para uma situação de infertilidade induzida pela terapêutica.
A criopreservação de esperma e de tecido testicular para preservação da fertilidade masculina em indivíduos pós-púberes, é também considerada estabelecida.

7. Quais as técnicas de preservação da fertilidade que são ainda consideradas experimentais?

Quer a criopreservação de tecido ovárico quer a criopreservação de tecido testicular em crianças e rapazes pré-púberes são classificadas como técnicas experimentais, na medida em que é considerada insuficiente a evidência relativa à sua eficácia e segurança. Não obstante, estas técnicas estão disponíveis no Centro de Preservação da Fertilidade do CHUC, EPE e a sua utilização poderá revelar-se adequada em determinados doentes oncológicos (cf. Indicações da criopreservação de tecido ovárico e da criopreservação de tecido testicular).

8. As técnicas de preservação da fertilidade podem, de alguma forma, interferir com o tratamento da doença oncológica?

O único tipo de interferência está relacionado com o início dos tratamentos potencialmente causadores de infertilidade, na medida em que a criopreservação de embriões e/ou ovócitos pode requerer o adiamento do início dos tratamentos em cerca de 2 a 3 semanas (para mais informação cf. resposta à questão nº 9). Salientamos, no entanto, que uma referenciação atempada, na altura do diagnóstico, pode permitir facilmente ultrapassar esta limitação. Nas doentes em que é urgente iniciar tratamento e que, ainda assim, queiram preservar a fertilidade, pode ser ponderada a realização de criopreservação de tecido ovárico.

9. Para que um doente possa preservar a sua fertilidade é necessário adiar o início dos tratamentos da doença oncológica?

Graças a evoluções significativas no que diz respeito aos protocolos de estimulação hormonal, as técnicas estabelecidas de criopreservação de embriões e de criopreservação de ovócitos requerem um período máximo de 2 a 3 semanas para poderem ser executadas, incluindo já os processos de estimulação hormonal, punção folicular, fecundação *in vitro* (no caso dos embriões) e criopreservação dos embriões ou gâmetas.

Se a opção for a criopreservação de esperma (no homem) ou de tecido ovárico (na mulher) os tratamentos oncológicos poderão ser iniciados quase de imediato. A criopreservação de tecido ovárico é a única técnica de preservação da fertilidade feminina que pode ser executada quando se revela urgente iniciar os tratamentos oncológicos potencialmente causadores de infertilidade.

10. Quais os custos associados às várias técnicas de preservação da fertilidade?

No caso das técnicas de criopreservação de embriões e criopreservação de ovócitos a doente terá que suportar os custos com os medicamentos para estimulação ovárica. Estes custos variam entre 200 e 500 euros, aproximadamente, de acordo com o protocolo de estimulação utilizado. As técnicas de criopreservação de tecido ovárico, esperma e tecido testicular, não implicam, em geral quaisquer custos para os doentes oncológicos.

11. Que técnicas de preservação da fertilidade estão disponíveis para doentes oncológicos na pré-puberdade?

No caso da criança ou rapariga pré-púbere, a única opção disponível é a criopreservação de tecido ovárico. Embora ainda seja considerada uma técnica experimental, há relatos publicados (Ernst et al., 2013; Poirot et al., 2012) que comprovam a capacidade de um posterior transplante permitir a estas jovens iniciar a puberdade e conseguir um normal funcionamento do sistema reprodutivo.

Relativamente aos doentes pré-púberes do sexo masculino, não existem ainda técnicas que tenham permitido, com sucesso, preservar a fertilidade futura. No entanto, as investigações prosseguem e prevê-se que a cultura e maturação *in vitro* de espermatogónias, ou mesmo de células testiculares estaminais, e posterior transplante, venham a ser uma opção num futuro próximo.

Ernst E, Kjærsgaard M, Birkebæk NH, Clausen N, Andersen CY. Case report: stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. Eur J Cancer. 2013 Mar;49(4):911-4.

Poirot C, Abirached F, Prades M, Coussieu C, Bernaudin F, Piver P. Induction of puberty by autograft of cryopreserved ovarian tissue. Lancet 2012; 379: 588.

12. Que técnicas de preservação da fertilidade estão disponíveis para doentes com tumores hormono-dependentes?

Quando o tumor diagnosticado apresenta positividade para recetores hormonais, como acontece em muitas situações de cancro da mama, coloca-se a dúvida sobre a segurança da exposição a níveis elevados de estrogénios, em consequência da estimulação ovárica necessária às técnicas de criopreservação de embriões ou de ovócitos. No entanto, existe a possibilidade de incluir

inibidores da aromatase (normalmente letrozol), no protocolo de estimulação que, comprovadamente, impedem que se atinjam níveis elevados de estrogénios. Caso se pretenda evitar completamente a exposição aos estrogénios, existe sempre a opção de criopreservar tecido ovárico, na medida em que esta técnica não exige qualquer estimulação hormonal.

13. Em doentes oncológicos nos quais é urgente iniciar tratamento, quais as opções de preservação da fertilidade disponíveis?

A criopreservação de tecido ovárico, embora considerada ainda experimental, é a única técnica de preservação da fertilidade feminina que pode ser executada num tempo muito curto, quando se revela urgente iniciar os tratamentos oncológicos potencialmente causadores de infertilidade. As restantes técnicas, criopreservação de embriões ou criopreservação de ovócitos exigem um período mínimo de 2 semanas para serem executadas, dada a necessidade de estimulação hormonal.

No homem, a criopreservação de esperma e a criopreservação de tecido testicular não exigem qualquer adiamento do início dos tratamentos.

14. Existe um risco aumentado de doença oncológica e/ou malformações congénitas na descendência de doentes oncológicos?

Diversos estudos prospetivos publicados não detetaram risco aumentado de doença oncológica e/ou malformações congénitas em descendentes de sobreviventes de doença oncológica (UptoDate, 2013). Não há evidência de que a história de doença oncológica, respetivos tratamentos ou intervenções na área da fertilidade aumentem o risco de doença oncológica ou malformações na descendência, exceto nas situações genéticas hereditárias ou no caso de exposição *in utero* a determinados tratamentos (Loren et al., 2013).

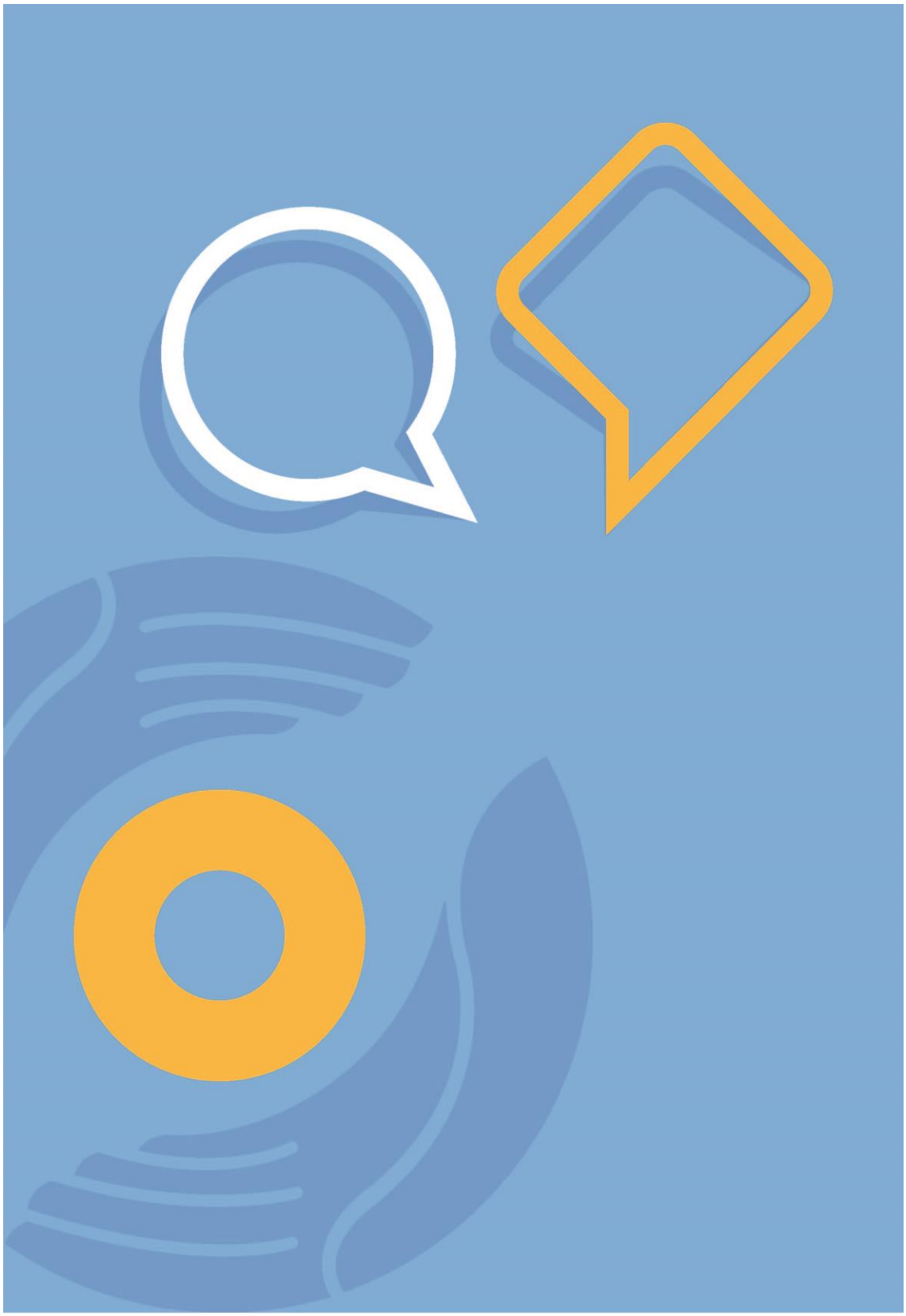
Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K; American Society of Clinical Oncology. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013 Jul 1;31(19):2500-10.

UpToDate®. Overview of fertility and pregnancy in cancer survivors. Literature review current through: Oct 2013. Topic last updated: Jul 29, 2013. Acessível em http://www.uptodate.com/contents/overview-of-fertility-and-pregnancy-in-cancer-survivors.

15. Quanto tempo se deve aguardar para tentar iniciar uma gravidez, após terminarem os tratamentos da doença oncológica?

Recomenda-se que o homem com doença oncológica aguarde, pelo menos, 2 anos após terminarem os tratamentos, antes de tentar ser pai. No entanto, se o esperma foi criopreservado antes de iniciar tratamentos, não foi exposto às substâncias potencialmente mutagénicas e, teoricamente, pode ser utilizado de imediato.

Na mulher, a determinação da altura ideal para iniciar uma gravidez deve ser feita caso a caso, considerando vários fatores como a data em que terminam os tratamentos, o risco de recidiva, a idade e a função ovárica de cada doente. Para reduzir a probabilidade de fecundação de ovócitos com eventuais alterações genéticas, recomenda-se aguardar um mínimo de 6 meses, após terminar o tratamento.



BIBLIOGRAFIA RECOMENDADA

Orientações clínicas

AMERICAN SOCIETY OF CLINICAL ONCOLOGY
Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K; American Society of Clinical Oncology. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013 Jul 1;31(19):2500-10.
Acessíveis em http://www.asco.org/quality-guidelines/fertility-preservation-patients-cancer-american-society-clinical-oncology

AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE
The Practice Committee of the American Society for Reproductive Medicine. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril 2013; 100:1214–23.

EUROPEAN SOCIETY OF MEDICAL ONCOLOGY
Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G; ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013 Oct;24 Suppl 6:vi160-70.
Acessíveis em http://annonc.oxfordjournals.org/content/24/suppl_6/vi160.full.pdf+html

ROYAL COLLEGE OF PHYSICIANS
Royal College of Physicians, The Royal College of Radiologists, Royal College of Obstetricians and Gynaecologists. The effects of cancer treatment on reproductive functions: Guidance on management. Report of a Working Party. London: RCP, 2007.
Acessível em http://www.rcplondon.ac.uk/publications/effects-cancer-treatment-reproductive-functions

Livros

Oncofertility: Fertility Preservation for Cancer Survivors.
Editors: Director Teresa K. Woodruff PhD, Research Assistant Professor Karrie Ann Snyder PhD
ISBN: 978-0-387-72292-4 (Print) 978-0-387-72293-1 (Online)
Acessível em http://oncofertility.northwestern.edu/files/oncofertility-and-fertility-preservation/

oncofertility-fertility-preservation-cancer-survivors
Oncofertility: Ethical, Legal, Social, and Medical Perspectives.
Co-editors Teresa K. Woodruff, Laurie Zoloth, Lisa Campo-Engelstein, and Sarah Rodriguez.
http://oncofertility.northwestern.edu/files/health-care-professionals/oncofertility-reflections-humanities-and-social-sciences-table-conten
Oncofertility Medical Practice: Clinical Issues and Implementation.
Co-editors Clarisa Gracia and Teresa K. Woodruff
http://oncofertility.northwestern.edu/files/health-care-professionals/oncofertility-clinical-issues-and-implementation
Oncofertility Communication. Sharing Information and Building Relationships across Disciplines
Editors: Woodruff, Teresa K; Clayman, Marla L; Waimey, Kate E
2014, Springer
ISBN 978-1-4614-8235-2

Artigos de revisão

Knopman JM, Papadopoulos EB, Grifo JA, Fino ME, Noyes N.Surviving childhood and reproductive-age malignancy:
effects on fertility and future parenthood. Lancet Oncol 2010; 11: 490–98.
Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol 2005; 6: 209–18.
Blumenfeld Z. Chemotherapy and fertility. Best Pract Res Clin Obst Gynaecol 2012. 26: 379–390.
Ben-Aharon I, Shalgi R. What lies behind chemotherapy-induced ovarian toxicity? Reprod 2012; 144: 153–163.
Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? Hum Reprod Upd 2012; 18(5): 525–535.
Meistrich ML. Male Gonadal Toxicity. Pediatr Blood Cancer 2009 Aug; 53(2): 261–266.
Dohle GR. Male infertility in cancer patients: Review of the literature. Int J Urol 2010; 17: 327–331.
Jeruss JS, Woodruff TK. Preservation of Fertility in Patients with Cancer. N Engl J Med. 2009 Feb 26; 360(9): 902–911.
Woodruff TK. The Oncofertility Consortium—addressing fertility in young people with cancer. Nat Rev Clin Oncol. 2010 August ; 7(8): 466–475.
Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol. 2013 Dec;9(12):735-49.

ANEXOS

ANEXO I

COMPILAÇÃO E CARACTERIZAÇÃOA DE FERRAMENTAS PARA APOIAR A IDENTIFICAÇÃO DO RISCO DE INFERTILIDADE.

FERRAMENTA E ACESSO	TIPO DE INFORMAÇÃO DISPONIBILIZADA	VANTAGENS	DESVANTAGENS
Fertile Hope **www.fertilehope.org**	Tabelas que sumariam a informação disponível sobre os riscos de infertilidade associados a tratamentos oncológicos específicos. Ferramentas de cálculo de risco, para homens e mulheres, pesquisável por tipo de doença oncológica ou tratamento.	Ferramenta de cálculo on-line, interativa, o que permite uma pesquisa muito fácil, rápida e intuitiva. Inclui ainda ferramenta para identificação das opções de preservação da fertilidade disponíveis, de acordo com características pessoais e do tratamento.	Última atualização em 2007.
Fertility preservation for young patients with cancer: who is at risk and what can be offered? The Lancet Oncology, Volume 6, Issue 4, Pages 209 - 218, April 2005.	Tabela que classifica o risco de infertilidade em baixo, médio ou elevado, de acordo com o tipo de doença oncológica na infância ou adolescência.	Permite estimar o risco de infertilidade num determinado doente mesmo antes de planear os tratamentos oncológicos.	Tabela publicada em 2005. Considera certos tratamentos como standard para cada tipo de doença oncológica, pelo que pode não ser aplicável a todos os doentes.
Fertility Preservation for Patients with Cancer: ASCO Clinical Practice Guideline Update (2013). Data supplement: #5 Effects of Different Antitumor Agents on Sperm Production in Men. #6 Risks of Permanent Amenorrhea in Women Treated With Modern Chemotherapy and Radiotherapy. **www.asco.org/sites/www.asco.org/files/fp_data_supplements_final_052813_0.pdf**	Tabelas que classificam o risco de diversos tratamentos oncológicos (antineoplásicos e radioterapia) causarem azoospermia (no homem) ou amenorreia (na mulher).	Última atualização em 2013. Para cada tratamento, são considerados fatores como a idade do doente e as doses administradas. Para cada uma das categorias de risco são feitas recomendações relevantes para o aconselhamento reprodutivo.	No que diz respeito aos efeitos na fertilidade feminina, não é incluída informação de estudos que tenham utilizado marcadores de (in)fertilidade feminina mais fiáveis, como os marcadores de reserva ovárica.

ANEXO II

COMPILAÇÃO DE ORIENTAÇÕES CLÍNICAS INTERNACIONAIS SOBRE A PRESERVAÇÃO DA FERTILIDADE EM DOENTES ONCOLÓGICOS.

INSTITUIÇÃO/ ORGANIZAÇÃO	ANO	TITULO	ENDEREÇO ELETRÓNICO
American Society of Clinical Oncology	2006	**ASCO recommendations on fertility preservation in cancer patients**	jop.ascopubs.org/content/2/3/143/suppl/DC1
	2013	**Fertility preservation in patients with cancer: ASCO guideline update**	www.asco.org/quality-guidelines/fertility-preservation-patients-cancer-american-society-clinical-oncology
The Practice Committee of the American Society for Reproductive Medicine	2013	**Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion**	www.asrm.org/uploadedFiles/ASRM_Content/News_and_Publications/Ethics_Committee_Reports_and_Statements/FertilityPreservation.pdf
The Ethics Committee of the American Society for Reproductive Medicine	2013	**Fertility preservation and reproduction in patients facing gonadotoxic therapies: a committee opinion**	www.fertstert.org/article/S0015-0282(13)02957-9/pdf
International Society for Fertility Preservation Practice Committee	2012	**Recommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer**	www.ncbi.nlm.nih.gov/pmc/articles/PMC3370045/
European Society for Medical Oncology	2013	**Cancer, fertility and pregnancy: ESMO clinical practice guidelines for diagnosis, treatment and follow-up**	annonc.oxfordjournals.org/content/24/suppl_6/vi160.full.pdf+html
FertiPROTEKT network	2011	**Fertility preservation in women-a practical guide to preservation techniques and therapeutic strategies in breast cancer, Hodgkin's lymphoma and borderline ovarian tumours**	link.springer.com/article/10.1007%-2Fs00404-011-1874-1
Royal College of Physicians, The Royal College of Radiologists, Royal College of Obstetricians and Gynaecologists	2008	**Effects of cancer treatment on reproductive functions - guidance on management.**	www.rcplondon.ac.uk/publications/effects-cancer-treatment-reproductive-functions
American Academy of Pediatrics	2008	**Preservation of fertility in pediatric and adolescent patients with cancer**	pediatrics.aappublications.org/content/121/5/e1461.full.pdf+html

ANEXO III A

CATEGORIZAÇÃO DO RISCO DE AZOOSPERMIA EM HOMENS TRATADOS COM REGIMES MODERNOS DE QUIMIOTERAPIA E RADIOTERAPIA (ASCO, 2013).

Fertility Preservation for Patients with Cancer: ASCO Clinical Practice Guideline Update (2013).
Data supplement #5 - Effects of Different Antitumor Agents on Sperm Production in Men. Documentos originais acessíveis em www.asco.org/sites/www.asco.org/files/fp_data_supplements_final_052813_0.pdf

Esta tabela foi produzida de acordo com a literatura disponível relativa aos efeitos de tratamentos oncológicos de uso frequente na produção de espermatozoides em homens na pós-puberdade (exceto quando outras faixas etárias são indicadas) e inclui as seguintes categorias de risco:

RISCO ELEVADO

É FREQUENTE A OCORRÊNCIA DE AZOOSPERMIA PROLONGADA OU PERMANENTE APÓS TRATAMENTO

Considerações no aconselhamento reprodutivo: quaisquer tratamentos que incluam doses elevadas de agentes alquilantes e/ou radiação testicular, pélvica ou do eixo hipotálamo-hipófise representam o nível mais elevado de risco de impacto gonadal. Os doentes devem ser aconselhados sobre a preservação da fertilidade antes de iniciar tratamento.

RISCO MODERADO

EMBORA NÃO SEJA FREQUENTE, PODE OCORRER AZOOSPERMIA PROLONGADA OU PERMANENTE APÓS TRATAMENTO

Considerações no aconselhamento reprodutivo: níveis mais reduzidos de agentes alquilantes e/ou irradiação testicular, pélvica ou do eixo hipotálamo-hipófise diminuem mas não eliminam o risco de esterilidade. Os doentes devem ser aconselhados sobre a preservação da fertilidade antes de iniciar tratamento.

RISCO BAIXO

TRATAMENTOS QUE CARACTERISTICAMENTE CAUSAM APENAS DANO TEMPORÁRIO NA PRODUÇÃO DE ESPERMATOZOIDES

Considerações no aconselhamento reprodutivo: apesar de ser pouco provável que o tratamento provoque esterilidade, os doentes poderão querer considerar preservar a fertilidade devido à possibilidade de recidiva e/ou necessidade de tratamento adicional.

RISCO MUITO BAIXO/SEM RISCO

TRATAMENTOS QUE NÃO AFETAM A PRODUÇÃO DE ESPERMA

Considerações no aconselhamento reprodutivo: apesar de ser pouco provável que o tratamento provoque esterilidade, os doentes poderão querer considerar preservar a fertilidade devido à possibilidade de recidiva e/ou necessidade de tratamento adicional.

RISCO DESCONHECIDO

Considerações no aconselhamento reprodutivo: os doentes devem ser informados sobre a inexistência de dados conclusivos sobre os efeitos destes fármacos na produção de espermatozoides; devem ser discutidas as opções de preservação da fertilidade.

NÍVEL DE RISCO	PROTOCOLO DE TRATAMENTO	FATORES RELACIONADOS COM O DOENTE E A DOSE	INDICAÇÕES TERAPÊUTICAS HABITUAIS	CONSIDERAÇÕES NO ACONSELHAMENTO REPRODUTIVO
RISCO ELEVADO É frequente a ocorrência de azoospermia prolongada ou permanente após tratamento.	Qualquer agente alquilante (ex. Bussulfano, Carmustina, Ciclofosfamida, Ifosfamida, Melfalano, Procarbazina) + irradiação corporal total		Preparação para transplante de células estaminais hematopoiéticas (TCEH) em leucemias, linfomas, mielomas, sarcoma de Ewing, neuroblastoma	Quaisquer tratamentos que incluam doses elevadas de agentes alquilantes e/ou irradiação testicular, pélvica ou do eixo hipotálamo-hipófise representam o nível mais elevado de risco de impacto gonadal. Os doentes devem ser aconselhados sobre a preservação da fertilidade antes de iniciar tratamento.
	Qualquer agente alquilante + Radiação pélvica ou testicular		Sarcomas, tumores testiculares	
	Ciclofosfamida	< 7,5 g/m^2	Vários tipos de cancro e preparação para transplante de células estaminais hematopoiéticas	
	Protocolos que incluam Procarbazina: MOPP e BEACOPP	> 3 ciclos > 6 ciclos	Linfoma Hodgkin	
	Protocolos que incluam Temozolomida ou Carmustina + irradiação craniana		Tumores do SNC	
	Irradiação testicular	> 2,5 Gy em homens > 6 Gy em rapazes pré-púberes	Leucemia linfoblástica aguda, linfoma não-Hodgkin, sarcoma,tumores de células germinais	
	Doses de irradiação corporal total		Transplante de células estaminais hematopoiéticas	
	Irradiação craniana	> 40 Gy	Tumores do SNC	
RISCO MODERADO Pode ocorrer azoospermia prolongada ou permanente após tratamento, embora não seja frequente.	Protocolos que incluam metais pesados: BEP Cisplatina Carboplatina	2 - 4 ciclos > 400 mg/m^2 > 2 g/m2	Tumores testiculares	Níveis mais reduzidos de agentes alquilantes e/ou irradiação testicular, pélvica ou do eixo hipotálamo-hipófise diminuem mas não eliminam o risco de esterilidade. Os doentes devem ser aconselhados sobre a preservação da fertilidade antes de iniciar tratamento.
	Irradiação testicular (devido a dispersão)	1 - 6 Gy	Tumor de Wilms, neuroblastoma	
RISCO BAIXO Tratamentos que normalmente causam apenas dano temporário na produção de espermatozoides.	Protocolos que incluam agentes não-alquilantes (ex. ABVD, CHOP, COP, protocolos multi-agente para Leucemia)		Linfoma Hodgkin, linfoma não-Hodgkin, leucemia	Apesar de ser pouco provável que o tratamento provoque esterilidade, os doentes poderão querer considerar preservar a fertilidade devido à possibilidade de recidiva e/ou necessidade de tratamento adicional.
	Irradiação testicular	< 0,2 - 0,7 Gy	Tumores testiculares	
	Antraciclina + Citarabina		Leucemia mieloblástica aguda	
RISCO MUITO BAIXO/ SEM RISCO Tratamentos que não afetam a produção de espermatozoides.	Protocolos multi-fármaco que incluam Vincristina		Leucemia, linfoma e cancro do pulmão	
	Iodo radioativo		Tumores da tiróide	
	Irradiação testicular (devido a dispersão)	< 0,2 Gy	Vários tipos de tumores	
RISCO DESCONHECIDO	Anticorpos monoclonais (ex. Bevacizumab (Avastin), Cetuximab (Erbitux))		Tumores do cólon, células não-pequenas do pulmão, cabeça e pescoço	Os doentes devem ser informados sobre a inexistência de dados conclusivos sobre os efeitos destes fármacos na produção de espermatozoides; devem ser discutidas as opções de preservação da fertilidade.
	Inibidores das Tirosinacinases (ex. Erlotinib (Tarceva), Imatinib (Gleevec))		Tumores de células não-pequenas do pulmão e pancreático, leucemia mielóide crónica, GIST	

Loren et al: J Clin Oncol Vol. 31(19), 2013: 2500-2510

Anexo III B

CATEGORIZAÇÃO DO RISCO DE AMENORREIA PERMANENTE EM MULHERES TRATADAS COM REGIMES MODERNOS DE QUIMIOTERAPIA E RADIOTERAPIA (ASCO, 2013).

Fertility Preservation for Patients with Cancer: ASCO Clinical Practice Guideline Update (2013).
Data supplement #6 - Risks of Permanent Amenorrhea in Women Treated With Modern Chemotherapy and Radiotherapy.

Esta tabela representa uma compilação da experiência clínica e de dados publicados sobre o efeito de tratamentos oncológicos de uso frequente na função menstrual. Outras medidas de potencial reprodutivo como níveis hormonais, contagem de folículos e resultados clínicos de gravidez não estão refletidas nesta tabela. Os riscos são categorizados para mulheres na pós-puberdade, de acordo com a literatura disponível (exceto quando outras faixas etárias são indicadas). As categorias de risco são as seguintes:

RISCO ELEVADO

>70% DAS MULHERES DESENVOLVE AMENORREIA APÓS TRATAMENTO

Considerações no aconselhamento reprodutivo: quaisquer tratamentos que incluam doses elevadas de agentes alquilantes e/ou radiação do abdómen, pélvis ou eixo hipotálamo-hipófise apresentam o nível mais elevado de risco de impacto gonadal e amenorreia imediata. As doentes devem ser aconselhadas sobre a preservação da fertilidade antes de iniciar tratamento.

RISCO MODERADO

APROXIMADAMENTE 30 A 70% DAS MULHERES DESENVOLVE AMENORREIA APÓS TRATAMENTO

Considerações no aconselhamento reprodutivo: níveis mais reduzidos de agentes alquilantes e/ou radiação abdominal, pélvica ou do eixo hipotálamo-hipófise diminuem o risco de amenorreia imediata mas não eliminam o risco de lesão gonadal. As doentes devem ser aconselhadas sobre a preservação da fertilidade antes de iniciar tratamento.

RISCO BAIXO

<30% DAS MULHERES DESENVOLVE AMENORREIA APÓS TRATAMENTO

Considerações no aconselhamento reprodutivo: é pouco provável que estes tratamentos provoquem amenorreia imediata nas doses normalmente utilizadas; no entanto, as doentes devem ser aconselhadas relativamente ao risco de menopausa prematura. As doentes poderão pretender considerar preservação da fertilidade antes ou depois do tratamento.

RISCO MUITO BAIXO/SEM RISCO

RISCO NEGLIGENCIÁVEL; TRATAMENTOS SEM EFEITO NO PERÍODO MENSTRUAL

Considerações no aconselhamento reprodutivo: é pouco provável que estes tratamentos provoquem amenorreia imediata nas doses normalmente utilizadas; no entanto, as doentes devem ser aconselhadas relativamente ao risco de menopausa prematura. As doentes poderão pretender considerar preservação da fertilidade antes ou depois do tratamento.

RISCO DESCONHECIDO

Considerações no aconselhamento reprodutivo: as doentes devem ser informadas sobre a inexistência de dados conclusivos sobre os efeitos reprodutivos destes fármacos; devem ser discutidas as opções de preservação da fertilidade.

NÍVEL DE RISCO	PROTOCOLO DE TRATAMENTO	FATORES RELACIONADOS COM O DOENTE E A DOSE	INDICAÇÕES TERAPÊUTICAS HABITUAIS	CONSIDERAÇÕES NO ACONSELHAMENTO REPRODUTIVO
RISCO ELEVADO Mais de 70% das mulheres desenvolve amenorreia após tratamento.	Qualquer agente alquilante (ex. Bussulfano, Carmustina, Ciclofosfamida, Ifosfamida, Lomustina, Melfalano, Procarbazina) + irradiação corporal total		Preparação para transplante de células estaminais hematopoiéticas (TCEH) em leucemias, linfomas, mielomas, sarcoma de Ewing, neuroblastoma, coriocarcinoma	Quaisquer tratamentos que incluam doses elevadas de agentes alquilantes e/ou radiação do abdómen, pélvis ou eixo hipotálamo-hipófise apresentam o nível mais elevado de risco de impacto gonadal e amenorreia imediata. As doentes devem ser aconselhadas sobre a preservação da fertilidade antes de iniciar tratamento.
	Qualquer agente alquilante + irradiação pélvica		Sarcomas, tumores do ovário	
	Ciclofosfamida	5 g/m^2 em mulheres com idade > 40 anos 7,5 g/m^2 em mulheres e raparigas de idade < 20 anos	Vários tipos: cancro da mama, linfoma não-Hodgkin, preparação para transplante de células estaminais hematopoiéticas	
	Protocolos que incluam procarbazina: MOPP BEACOPP	 > 3 ciclos > 6 ciclos	Linfoma Hodgkin	
	Protocolos que incluam Temozolomida ou Carmustina + irradiação craniana		Tumores do SNC	
	Doses totais de irradiação abdominal ou pélvica	> 6 Gy em mulheres adultas > 10 Gy em raparigas pós-púberes > 15 Gy em raparigas pré-púberes	Tumor de Wilms, neuroblastoma, sarcomas, linfoma Hodgkin, tumores do ovário	
	Doses de irradiação corporal total		Transplante de células estaminais hematopoiéticas	
	Irradiação craniana	> 40 Gy	Tumores do SNC	
RISCO MODERADO Cerca de 30 a 70% das mulheres desenvolve amenorreia após tratamento.	Ciclofosfamida	5 g/m^2 em mulheres de idade entre 30 e 40 anos	Vários tipos de cancro, cancro da mama	Níveis mais reduzidos de agentes alquilantes e/ou irradiação abdominal, pélvica ou do eixo hipotálamo-hipófise diminuem o risco de amenorreia imediata mas não eliminam o risco de lesão gonadal. As doentes devem ser aconselhadas sobre a preservação da fertilidade antes de iniciar tratamento.
	Protocolo AC (Doxorrubicina e Ciclofosfamida) para cancro da mama	4 ciclos + Paclitaxel ou Docetaxel em mulheres com idade <40 anos	Cancro da mama	
	Protocolo FOLFOX4		Cancro do cólon	
	Protocolos que incluam cisplatina		Cancro do colo do útero	
	Irradiação abdominal ou pélvica	10-15 Gy em raparigas pré-púberes 5-10 Gy em raparigas pós-púberes	Tumor de Wilms, neuroblastoma, tumores da espinal medula, tumores cerebrais, recidiva de leucemia linfoblástica aguda ou de linfoma não-Hodgkin	
RISCO BAIXO Menos de 30% das mulheres desenvolve amenorreia após tratamento	Protocolos que incluam agentes não-alquilantes (ex. ABVD, CHOP, COP, protocolos multi-fármaco para Leucemia)		Linfoma Hodgkin, linfoma não-Hodgkin, leucemia	É pouco provável que estes tratamentos provoquem amenorreia imediata nas doses normalmente utilizadas; no entanto, as doentes devem ser aconselhadas relativamente ao risco de menopausa precoce. As doentes poderão pretender considerar preservação da fertilidade antes ou depois do tratamento.
	Protocolos para cancro da mama que incluam Ciclofosfamida (ex. CMF, FEC ou FAC)	Mulheres de idade < 30 anos	Cancro da mama	
	Antraciclina + Citarabina		Leucemia mieloblástica aguda	
RISCO MUITO BAIXO/ SEM RISCO Risco negligenciável; sem efeito na função menstrual	Protocolos multi-fármaco que incluam Vincristina		Leucemia, linfoma, cancro da mama e cancro do pulmão	
	Iodo radioativo		Tumores da tiróide	
RISCO DESCONHECIDO	Anticorpos monoclonais (ex. Bevacizumab (Avastin), Cetuximab (Erbitux), Trastuzumab (Herceptin))		Tumores do cólon, células não-pequenas do pulmão, cabeça e pescoço e mama	As doentes devem ser informadas sobre a inexistência de dados conclusivos sobre os efeitos reprodutivos destes fármacos; devem ser discutidas as opções de preservação da fertilidade.
	Inibidores das Tirosinacinases (ex. Erlotinib (Tarceva), Imatinib (Gleevec))		Tumores de células não-pequenas do pulmão e pancreático, leucemia mielóide crónica, GIST	

Loren et al: J Clin Oncol Vol. 31(19), 2013: 2500-2510.

Anexo IV

TABELA COMPARATIVA DAS VÁRIAS TÉCNICAS DE PRESERVAÇÃO DA FERTILIDADE NA MULHER.

	CRIOPRESERVAÇÃO DE EMBRIÕES	CRIOPRESERVAÇÃO DE OVÓCITOS	CRIOPRESERVAÇÃO DE TECIDO OVÁRICO
CLASSIFICAÇÃO	TÉCNICA ESTABELECIDA	TÉCNICA ESTABELECIDA	TÉCNICA EXPERIMENTAL
DESCRIÇÃO	Estimulação hormonal, seguida de punção folicular para recolha de ovócitos e posterior inseminação por FIV ou ICSI. Os embriões obtidos são criopreservados por congelação lenta ou vitrificação.	Estimulação hormonal, seguida de punção folicular e criopreservação dos ovócitos por vitrificação. Após desvitrificação, os gâmetas preservados são fecundados (FIV), com posterior transferência de embriões.	Colheita por laparoscopia, de tecido do córtex ovárico/totalidade do ovário, que é criopreservado; depois de descongelado, o tecido ovárico pode ser enxertado de forma ortotópica ou heterotópica.
PARA QUEM	Mulheres em idade reprodutiva, com parceiro e desde que não existam objeções morais e/ou éticas à criopreservação de embriões.	Jovens na pós-puberdade. Mulheres em idade reprodutiva, especialmente na ausência de parceiro ou quando existem objeções morais/éticas à CP de embriões.	Crianças e jovens na pré-puberdade (única técnica disponível) Mulheres em idade reprodutiva, se indesejável a estimulação hormonal e/ou necessidade urgente de iniciar tratamento.
QUANDO	Antes de iniciar tratamentos potencialmente gonadotóxicos.	Antes de iniciar tratamentos potencialmente gonadotóxicos.	Antes de iniciar tratamentos potencialmente gonadotóxicos.
REQUISITOS DE TEMPO	2 a 3 semanas	2 a 3 semanas	Não exige adiamento dos tratamentos.
TAXAS DE SUCESSO	Taxa de gravidez por embrião transferido: 35,6%, em mulheres até aos 35 anos.	Taxa de nascimentos por ovócito desvitrificado: 4,5 a 12%.	Só na Dinamarca mais de 600 mulheres já recorreram a esta técnica (35 transplantes em 25 mulheres). Todas recuperaram a função ovárica e, atualmente, há mais de 40 bebés nascidos após enxerto ortotópico de tecido ovárico criopreservado.
RISCOS	Técnicas de FIV: riscos menores como infeções ou hemorragias, que ocorrem raramente; não foi identificado risco aumentado de cancro do ovário, endométrio ou colo do útero.	Técnicas de FIV: raramente podem ocorrer infeções ou hemorragias; não foi identificado risco aumentado de cancro do ovário, endométrio ou colo do útero.	Riscos menores associados à cirurgia laparoscópica. Risco teórico de criopreservar/transplantar tecido ovárico com células neoplásicas: estão em desenvolvimento diversas técnicas que ajudam a excluir estes casos
VANTAGENS	**FACE ÀS RESTANTES TÉCNICAS** Maior evidência de eficácia e segurança. Maior taxa de sucesso. **FACE À CRIOPRESERVAÇÃO DE TECIDO OVÁRICO** Evita o risco de reimplantação de células malignas. Não requer cirurgia.	**FACE À CRIOPRESERVAÇÃO DE EMBRIÕES** Permite manter a autonomia reprodutiva da mulher. Não exige gâmetas masculinos / parceiro. Pode ser usada quando há objeções legais/morais/éticas à CP de embriões. **FACE À CRIOPRESERVAÇÃO DE TECIDO OVÁRICO** Maior evidência de eficácia e segurança e taxas de sucesso. Evita o risco de reimplantação de células malignas. Não requer cirurgia.	**FACE ÀS RESTANTES TÉCNICAS** Permite manter a autonomia reprodutiva da mulher. Permite preservar a fertilidade em raparigas pré-púberes. Não exige estimulação hormonal/adiamento tratamentos. Permite preservar um número elevado de células germinais. Permite preservar a função hormonal ovárica e conceção natural. **FACE À CRIOPRESERVAÇÃO DE EMBRIÕES** Não exige gâmetas masculinos / parceiro.
DESVANTAGENS	**FACE ÀS RESTANTES TÉCNICAS** Não respeita a autonomia reprodutiva da mulher. Problemas éticos/legais (lei portuguesa só contempla PMA para infertilidade; embriões excedentários/órfãos). **FACE À CRIOPRESERVAÇÃO DE TECIDO OVÁRICO** Exige adiamento do início dos tratamentos. Exposição a níveis elevados de estrogénios; pode ser reduzida usando estimulação com inibidores da aromatase. Não permite conceção natural (FIV/ICSI).	**FACE À CRIOPRESERVAÇÃO DE TECIDO OVÁRICO** Exige adiamento do início dos tratamentos. Exposição a níveis elevados de estrogénios, que pode ser reduzida usando estimulação com inibidores da aromatase. Não permite conceção natural (FIV/ICSI).	**FACE ÀS RESTANTES TÉCNICAS** Técnica experimental: a evidência médica publicada relativa aos riscos, benefícios, segurança e eficácia não é suficiente para a considerar como prática médica estabelecida.

FICHA TÉCNICA

Título
Preservação da Fertilidade em Doentes Oncológicos

Autores
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SPMR
SOCIEDADE PORTUGUESA DE MEDICINA DA REPRODUÇÃO
LIGA PORTUGUESA CONTRA O CANCRO
CENTRO DE PRESERVAÇÃO DA FERTILIDADE
www.centropreservacaofertilidade.pt

Chapter II

Participation in the development of clinical guidance and other national education and information initiatives concerning infertility risks and fertility preservation in cancer patients

Preamble

The concept of oncofertility was introduced in 2006 by Professor Teresa Woodruff and describes an integrated network of clinical resources that are focused on developing methods to spare or restore reproductive function in patients diagnosed with cancer. The *Oncofertility Consortium* was subsequently founded to address the questions that exist at the intersection of oncology, reproductive medicine, and the public. This consortium integrates the bench (basic and social research sciences), the bedside (clinicians and clinical researchers), and the community (the humanities, law, and education) in an overarching program, representing a new way to approach a previously intractable problem (Woodruff 2010).

The *Centre for Fertility Preservation* (CFP) is located at the Reproductive Department of the Coimbra Hospital and University Centre (CHUC, EPE). It was created in 2010 to fulfil the reproductive needs of patients undergoing treatments possibly threatening to their reproductive function and it is, currently, the only public dedicated Centre for Fertility Preservation in Portugal. In Portugal, FP in males is done since the 90's in several public institutions. However, the female FP techniques weren't available before in the Portuguese public practice so it was clearly important to do something to try to assure the biological parenthood of the female patient too. Since 2010, the main goal of the CPF is to provide reproductive monitoring and counselling to male and female patients from every part of the country that are about to initiate possibly gonadotoxic treatments. This centre is the only one in the country that provides all FP techniques to both men and women. The team includes six physicians, one embryologist, one psychologist, and one pharmacist. Through a multidisciplinary approach, the main goal is to provide reproductive monitoring and counselling to male and female patients undergoing gonadotoxic treatments. Also, the Centre aims to support patient's decision-making process concerning fertility preservation and, afterwards, their post-treatment reproductive decisions by the means of annual follow-up consultations. Although most cancer patients are referred to the Centre by their oncologists, it is important to note that a significant number of them ask for a consultation on their own initiative. According to research conducted at the Centre, the opportunity to make a decision about FP is stated by all cancer patients as crucial regardless they were or not referred by the oncologist. This possibility gives them some hope and sense of control in a context that is mainly overwhelming. Therefore, another goal of the Centre activities is to better inform patients, health professionals and the general population about the possible impact of cancer

(and cancer treatments) in fertility, the available techniques for FP and how to reach the team in a timely and effective manner (Ataman, Rodrigues et al. 2016).

The CPF has also been at the front line of the oncofertility field in Portugal by actively promoting awareness of this new field and disseminating information regarding infertility risks and FP options, both to reproductive-age patients and healthcare professionals. Several communication and research activities have been accomplished in the past years by the members of the CFP's team, often in partnership with other institutions, of which can be highlighted:

- Development of information resources;
- Promotion and dissemination of the oncofertility concept and challenges in social media, medical and cancer patients' events;
- Education activities about oncofertility (courses, workshops) intended for health professionals;
- Multidisciplinary meetings;

The Centre is also, since 2013, a global partner of the above mentioned *Oncofertility Consortium*, and contributes to its aims of expanding research about fertility loss in patients with cancer, accelerating clinical translation of FP techniques and addressing the complex health care and quality-of-life issues that concern young patients with cancer whose fertility may be threatened by their disease or its treatment (Ataman, Rodrigues et al. 2016). More recently, during the years of 2015 and 2016, oncofertility has gained a new dimension in Portugal with the organization by the *Portuguese Society of Reproductive Medicine* (Sociedade Portuguesa de Medicina da Reprodução, SPMR) of the 1st and 2nd Portuguese Oncofertility Meetings. These meetings had the purpose of initiating the discussion for future implementation of an integrated national practice concerning FP in cancer patients. Other medical societies involved in this process were the *Portuguese Oncology Society*, *Portuguese Haematology Society*, *Portuguese Andrology, Sexual Medicine and Reproduction Society* and the *Portuguese Gynaecology Society*. Recently, all the above mentioned Portuguese scientific societies endorsed the published *Recommendations for Preserving the Reproductive Potential of Cancer Patients* (Almeida-Santos, Sousa et al. 2016), as a final outcome of the above mentioned national oncofertility meetings, a document herein reproduced and for which this investigation gave foremost contributes, namely by providing summarized, up-to-date and evidence-based information regarding the gonadotoxicity of cancer treatments and the available FP options.

In addition, during the time period from 2013 to 2017, this research team participated in a number of initiatives, which are detailed in the second part of this chapter, in order to disseminate the oncofertility concept in Portugal.

Almost six years after the beginning of this work, we believe that oncofertility is now a much more acknowledged concept by Portuguese healthcare professionals. As a consequence, an increasing number of young patients facing a cancer diagnosis will be aware of the risks for their fertility that come with exposure to a variety of cancer treatments.

References

Almeida-Santos, A. T., G. Sousa, A. Teixeira, P. Cardoso, C. Melo, A. Teixeira, J. Andrade, C. Silva, E. Gouveia, I. Bravo, I. Augusto, J. Magalhães, N. Louro, B. Pereira, R. Ramalho, V. Patrício and S. Pinelo (2016). "Recommendations for the preservation of the reproductive potential in cancer patients." Revista Portuguesa de Oncologia **2**(I): 5-24.

Ataman, L. M., J. K. Rodrigues, R. M. Marinho, J. P. Caetano, M. B. Chehin, E. L. Alves da Motta, P. Serafini, N. Suzuki, T. Furui, S. Takae, Y. Sugishita, K. I. Morishige, T. Almeida-Santos, C. Melo, K. Buzaglo, K. Irwin, W. H. Wallace, R. A. Anderson, R. T. Mitchell, E. E. Telfer, S. K. Adiga, A. Anazodo, C. Stern, E. Sullivan, Y. Jayasinghe, L. Orme, R. Cohn, R. McLachlan, R. Deans, F. Agresta, B. Gerstl, W. L. Ledger, R. L. Robker, E. S. J. M. de Meneses, L. H. Silva, F. O. Lunardi, J. R. Lee, C. S. Suh, M. De Vos, E. Van Moer, D. Stoop, V. Vloeberghs, J. Smitz, H. Tournaye, L. Wildt, K. Winkler-Crepaz, C. Y. Andersen, B. M. Smith, K. Smith and T. K. Woodruff (2016). "Creating a Global Community of Practice for Oncofertility." J Glob Oncol **2**(2): 83-96.

Woodruff, T. K. (2010). "The Oncofertility Consortium--addressing fertility in young people with cancer." Nat Rev Clin Oncol **7**(8): 466-475.

2.1. Recommendations for the preservation of the reproductive potential in cancer patients.

[*Recomendações para a preservação do potencial reprodutivo no doente oncológico*.]

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Abstract

Objective: To provide updated information about the preservation of reproductive potential in adult cancer based on the available evidence.

Methods: A review of literature published after 2006 was performed using the Medline and PubMed. The evidence was analyzed by specialists from several medical societies; and reflected upon on their clinical practice. After discussion on several working groups, a series of recommendations were made.

Results: Scientific literature was reviewed and analyzed, as well as the recommendations and / or guidelines published by international scientific societies. After reviewing the best scientific evidence, the selected panel of experts prepared the recommendations to be implemented in the Portuguese population. Recommendations were reviewed by the participating medical societies, and comments incorporated when appropriate.

Recommendations: All health professionals should address with cancer patients, the risk of infertility and preservation of their reproductive potential. The individual risk for reproductive function of each patient depends on the type of cancer, age and treatment plan. It is very important to define the infertility risk, and fertility evaluation before starting any preservation technique of reproductive potential. In men, the most common and most effective option is sperm cryopreservation, which can be obtained in 24-48 h. In women, the preferred option is cryopreservation of oocytes that can be completed in 2 two to 4 four weeks. Referral must take place as early as possible, right after a cancer diagnosis is established and the need to sue a treatment with potentially detrimental effects on reproductive function is considered.

Resumo

Objetivo: Proporcionar informação atualizada baseada na evidência disponível sobre a preservação do potencial reprodutivo em adultos com cancro.

Métodos: Revisão da literatura publicada após 2006, com recurso à Medline e PubMed. A evidência foi analisada por especialistas das várias sociedades médicas e após reflexão baseada na prática clínica diária e discussão em grupos de trabalho foram elaboradas as recomendações.

Resultados: Foram revistos e analisados os artigos científicos, bem como as recomendações e/ou orientações publicadas por sociedades científicas internacionais. Após revisão da melhor evidência científica, o painel de peritos selecionado para o efeito elaborou as recomendações a aplicar na população portuguesa. O texto esteve disponível para consulta pública e contributos dos elementos das sociedades envolvidas, que foram integrados quando adequado.

Recomendações: Todos os profissionais de saúde devem abordar com o doente oncológico, o risco de infertilidade e as possibilidades de preservação do potencial reprodutivo. O risco individual de cada doente poder vir a sofrer alterações na sua função reprodutiva depende do tipo de cancro, idade e do plano de tratamento. É muito importante proceder à estratificação do risco de infertilidade e à avaliação da fertilidade antes de iniciar qualquer técnica de preservação do potencial reprodutivo. Nos homens, a opção mais comum e mais eficaz é a criopreservação de esperma que pode ser conseguida em 24-48 h. Nas mulheres, a opção preferencial é a criopreservação de ovócitos que pode ser completada em duas a quatro semanas. A referenciação deve ser o mais precoce possível, assim que esteja feito o diagnóstico da doença oncológica e se estabeleça a necessidade de terapêutica potencialmente lesiva da função reprodutiva.

1. Introdução

Nas últimas décadas tem-se assistido a um aumento das taxas de sobrevivência de doença oncológica, apesar do aumento do número de novos casos de cancro por ano[1]. De facto, os avanços no diagnóstico precoce e no tratamento têm aumentado significativamente a esperança de vida dos doentes oncológicos, permitindo um interesse crescente na promoção da qualidade de vida na sobrevivência. Em Portugal, em 2009, a taxa de incidência de doença oncológica era de 426,5 casos por 100000 indivíduos, o que corresponde ao valor mais elevado alguma vez registado[2]. No entanto, Portugal é também indicado como um dos países europeus com a taxa de sobrevivência aos cinco anos mais elevada em diferentes tipos de neoplasias, como o melanoma e o cancro do cólon[3].

Neste contexto, importa ter em conta que o tratamento sistémico do cancro prolonga a esperança média de vida dos doentes, mas tem efeitos deletérios na sua função reprodutiva. É de notar também que é cada vez mais provável encontrarmos um doente oncológico em idade reprodutiva, dado o aumento da incidência de certos tumores em idades mais jovens[4] e a tendência atual para o adiamento do nascimento do primeiro filho para idades mais tardias[5]. Mais ainda, muitas das neoplasias diagnosticadas em idades mais jovens necessitam, além do tratamento cirúrgico, de tratamento sistémico ou de radioterapia. Em alguns casos, como nas neoplasias hematológicas, a quimioterapia e a radioterapia são a base do tratamento.

Tendo em conta esta multiplicidade de fatores, o futuro reprodutivo dos jovens com doença oncológica está ameaçado e é neste contexto que têm sido desenvolvidas e aprimoradas técnicas de preservação da fertilidade. A oncofertilidade surge assim como uma nova área de intervenção e investigação, que estabelece uma ponte entre a oncologia e a medicina da reprodução e que a partir de "uma rede integrada de recursos clínicos, se foca no

desenvolvimento de métodos para poupar ou restaurar a função reprodutiva dos doentes diagnosticados com cancro"[6]. Apesar da tomada de decisão em relação à preservação do potencial reprodutivo ser muitas vezes complexa e ocorrer num contexto particularmente difícil e emocionalmente exigente perante o recente diagnóstico de cancro, os doentes revelam a importância de poderem ter um papel ativo nesta decisão[7,8], num contexto particularmente pautado por falta de controlo. Paralelamente, a parentalidade biológica após a doença oncológica parece assumir muita importância para os sobreviventes. Por um lado, apresentam mais motivações positivas para a parentalidade do que as pessoas saudáveis[9] e, por outro, perante o diagnóstico de infertilidade resultante dos tratamentos da doença oncológica, evidenciam níveis elevados de sintomatologia psicopatológica, como ansiedade[10] e depressão[11], por vezes, tão dolorosos como a notícia do diagnóstico da neoplasia[12]. De notar que os doentes que tiveram a oportunidade de tomar uma decisão relativamente à preservação da sua fertilidade junto de um especialista em medicina da reprodução revelam uma melhor adaptação individual na sobrevivência[13].

Apesar da importância que a fertilidade parece assumir para os doentes e sobreviventes, estes revelam carências de informação sobre os riscos dos tratamentos da doença oncológica na fertilidade, as formas de os tentar contornar e as preocupações associadas à gravidez após o tratamento, o que pode levar a que não tomem uma decisão em relação à preservação da sua fertilidade[14]. O oncologista tem sido apontado pelos doentes como a figura de suporte mais valorizada neste processo e a informação por si transmitida é considerada crucial8. Por seu lado, os oncologistas indicam diversos factores para a ausência ou insuficiência de debate da fertilidade com doentes oncológicos, tais como tempo reduzido para a consulta, urgência no tratamento da doença oncológica e a existência prévia de filhos[15]. No caso particular das mulheres, a falta de confiança na eficácia das técnicas de preservação do potencial reprodutivo é indicada pelos clínicos como desmotivadora do debate com as doentes sobre a possível preservação da sua fertilidade[8].

Neste sentido, as recomendações clínicas de várias sociedades de oncologia mundiais (e.g., *American Society of Clinical Oncology*, *Clinical Oncological Society of Australia*, *European Society for Medical Oncology*, *The European Society of Breast Cancer Specialists*) têm vindo a realçar a responsabilidade dos profissionais de saúde da área da oncologia em informar todos os doentes oncológicos, aquando do seu diagnóstico, sobre o risco de infertilidade associado aos tratamentos a que irão ser submetidos e sobre as formas de preservar o seu potencial reprodutivo, devendo encaminhá-los, em caso de interesse, para médicos especialistas em medicina da reprodução a fim de tomarem uma decisão em relação à preservação do seu potencial reprodutivo [16,17,18,19].

Em Portugal, os resultados preliminares de um estudo ainda em curso (Projeto 2ReproChoose) estão de acordo com a literatura internacional e realçam a importância atribuída pelas mulheres com cancro em idade reprodutiva, à discussão com o seu oncologista sobre o seu futuro reprodutivo, nomeadamente acerca dos riscos de infertilidade futura e das opções de preservação do potencial reprodutivo. Destaca-se também a valorização da preservação do potencial reprodutivo por parte destas doentes[20]. Para além disto, os resultados deste projecto salientam a importância atribuída pelos oncologistas à formação em oncofertilidade e a escassa referenciação dos doentes para centros especializados[21].

Paralelamente, é de realçar que a 7 de agosto de 2015 foi publicada no Diário da República (1ª série-nº 153) a Resolução da Assembleia da República n.º 112/2015, que recomenda que o Serviço Nacional de Saúde assegure a preservação de gâmetas de doentes que correm risco de infertilidade secundária aos tratamentos oncológicos.

Neste contexto surgiu a necessidade do estabelecimento de recomendações clínicas que suportem a discussão por parte dos profissionais de saúde da área da oncologia com os doentes oncológicos em idade reprodutiva acerca da sua fertilidade futura, facilitando a sua referenciação para centros que disponibilizem técnicas de preservação do potencial reprodutivo masculino e feminino.

2. Impacto dos tratamentos oncológicos na fertilidade

Diversos estudos internacionais investigaram a fertilidade em sobreviventes de doença oncológica, face a controlos saudáveis, e demonstraram que a probabilidade de estes doentes produzirem descendência é significativamente inferior[1,2,3,4]. A fertilidade no sobrevivente de cancro é influenciada por um conjunto de fatores inerentes ao próprio doente ou à sua doença, mas também relacionados com o tratamento. Têm sido desenvolvidas algumas ferramentas, para o cálculo estimado de risco de infertilidade, com base no tipo de neoplasia e nos esquemas terapêuticos mais frequentemente utilizados (Tabela 2.1). Considera-se risco elevado de falência gonadal permanente quando a probabilidade é superior a 80%, intermédio quando entre 20 e 80% e baixo quando inferior a 20%[5]. Contudo, e apesar de estas ferramentas permitirem aferir o impacto dos tratamentos na função reprodutiva, é importante salientar que, mesmo para terapêuticas com baixo risco ou risco desconhecido, a preservação do potencial reprodutivo deve ser discutida previamente ao início do tratamento oncológico, de forma a permitir a implementação de técnicas para a sua preservação antes de qualquer procedimento terapêutico.

Mecanismos

Os tratamentos usados no contexto da doença oncológica (cirurgia, radioterapia e terapêutica sistémica) podem ter influência sobre a fertilidade através de um ou mais dos seguintes mecanismos.[6,7]

• Gonadotoxicidade direta, quando há lesão direta do ovário ou do epitélio seminífero no testículo.

• Gonadotoxicidade indireta, quando as alterações interferem com o funcionamento do eixo hipotálamo-hipófise-gónadas, sobretudo através do efeito na função endócrina.

• Alterações ao nível da função uterina na mulher, e das funções erétil ou ejaculatória no homem, que podem ser causadas por irradiação pélvica ou por alguns tipos de cirurgia do aparelho reprodutor.

Impacto por tipo de tratamento

Cirurgia

O local da cirurgia é o fator mais relevante, na medida em que as cirurgias dos aparelhos reprodutores masculino (orquidectomia, sobretudo se bilateral, amputação do pénis e prostatectomia) e feminino (histerectomia e ooforectomia bilateral), têm impacto direto na função reprodutora, com possibilidade de infertilidade permanente. Também cirurgias realizadas noutras zonas anatómicas poderão influenciar indirectamente a capacidade fértil (ex.: linfadenectomia retroperitoneal nos doentes com neoplasia do testículo).

A cirurgia conservadora/preservadora de fertilidade deve ser oferecida, sempre que possível, aos doentes em idade fértil e que manifestem desejo de vir a ter descendência. [8]

Radioterapia

A radioterapia é utilizada no curso do tratamento de diversas neoplasias e pode ser aplicada em campos que afetem os órgãos reprodutores8 (linfoma de Hodgkin, sarcoma de Ewing, e outros).

As gónadas são muito sensíveis à radioterapia cujos efeitos gonadotóxicos dependem da dose, do esquema de fraccionamento e sobretudo do campo de irradiação.[8] Na mulher a radioterapia pélvica, para além do risco de induzir falência ovárica, acarreta também o risco de lesão uterina e/ou tubar, que podem ter implicações no sucesso de técnicas de procriação medicamente assistida (PMA) e aumentam o risco de uma gravidez futura, a nível obstétrico e neonatal.[9] O risco de lesão uterina é maior em mulheres mais jovens. Por estes motivos, deve ser sempre realizada proteção dos órgãos reprodutores não envolvidos pela doença oncológica.[8]

Os espermatozoides são extremamente sensíveis à radiação, independentemente da idade. As células de Leydig, por outro lado, são altamente sensíveis à radiação antes do início da puberdade8 enquanto no adulto se tornam mais resistentes. Após a radioterapia os homens adultos podem manter a função das células de Leydig e a produção hormonal testicular, apesar de se tornarem azoospérmicos. O elevado risco a nível da função testicular verifica-se quando é efetuada irradiação corporal total (como acondicionamento para transplante de medula óssea ou de células estaminais), e se a irradiação testicular é superior a 2,5 Gy no homem adulto ou superior a 6 Gy nos rapazes pré-púberes. Para doses de radiação entre 1 a 6 Gy o risco é intermédio. A radioterapia cranioespinhal tem efeito sobre a hipófise, e em doses superiores a 2,5 Gy tem também um risco intermédio de afetar a função reprodutora, com possível alteração na produção hormonal, nomeadamente hormonas sexuais ou gonadotrofinas.[10]

Tratamento sistémico

Quimioterapia

A quimioterapia sistémica na mulher pode originar depleção direta do pool folicular (no caso dos agentes alquilantes), toxicidade celular por stress oxidativo (ciclofosfamida e antraciclinas) ou lesão vascular (no caso da doxorrubicina)[11].

No homem, os antineoplásicos causam predominantemente lesões no epitélio seminífero com consequentes alterações da espermatogénese, embora também possam danificar as células de Leydig, responsáveis pela produção de testosterona. Adicionalmente, o tratamento sistémico da doença oncológica é potencialmente causador de mutações nas células germinativas[12].

Terapêuticas biológicas

Existe ainda pouca informação sobre o impacto das **terapêuticas biológicas** na fertilidade.[13] A utilização de anticorpos monoclonais e de inibidores da tirosina-cinase na gravidez está condicionada pelo seu risco teratogénico. O bevacizumab, anticorpo monoclonal anti-VEGF, usado frequentemente em associação com quimioterapia sistémica em neoplasias do cólon, condiciona taxas de falência ovárica na ordem dos 34%[14].

Hormonoterapia

Na mulher a indução de amenorreia prolongada tem impacto na fertilidade devendo ter-se em consideração que o próprio tratamento hormonal (cancro da mama) se associa inevitavelmente ao envelhecimento ovárico.

No homem, apesar de ser maioritariamente utilizada em contexto de terapêuticas paliativas num tumor que acomete maioritariamente homens mais velhos (cancro da próstata), existe uma subpopulação em que a hormonoterapia pode ser utilizada como tratamento adjuvante à

radioterapia prostática com intenção curativa. Nestes doentes, esta castração química leva quase inevitavelmente à azoospermia, o que se poderá tornar relevante se o doente tiver projetos de parentalidade futura.

Imunoterapia

Nos últimos anos tem-se assistido a um significativo desenvolvimento nesta área terapêutica. Na prática clínica atual, a imunoterapia apenas tem sido usada no contexto da doença avançada, irressecável ou metastizada. Contudo, dado ser uma área terapêutica emergente, com a possibilidade de aplicação mais precoce num futuro próximo, entendemos fazer uma reflexão sobre os dados existentes na literatura acerca do impacto dos vários tratamentos no sistema reprodutor. Atualmente existem três grupos principais de terapêuticas dirigidas ao sistema imune:

- Citocinas (interferon e interleucinas) – usadas há alguns anos, sobretudo no tratamento do carcinoma de células renais e no melanoma metastizado.
- Anticorpos monoclonais ou pequenas moléculas inibidoras da tirosina-cinase (TKI's) dirigidos às chamadas moléculas *checkpoints* imunes (CTLA4) que desempenham um importante papel na resposta imune mediada pelas células-T (ipilimumab, aprovado no tratamento do melanoma avançado ou metastizado) ou anti-PD-1 (vemurafenib, pembrolizumab).
- Terapias celulares (linfócitos T ativados, linfócitos T citotóxicos, células natural killer ou células dendríticas).

O impacto destas novas moléculas na fertilidade é mal conhecido[15], contudo um dos mecanismos que pode interferir com a fertilidade é a endocrinopatia imune.

Tratamentos combinados

A utilização concomitante de quimioterapia (QT) e radioterapia (RT) aumenta o risco de infertilidade. Quando usados em conjunto (p. ex. no sarcoma de Ewing), a RT tem-se mostrado a principal responsável pelo risco elevado de infertilidade.[6]

O transplante de medula óssea acarreta taxas de falência ovárica entre 72-100%, de acordo com vários estudos.14 Estas taxas devem-se à utilização de irradiação corporal total ou associação de ciclofosfamida e busulfano.[16]

Os tratamentos com iodo radioativo não são causadores de infertilidade (masculina ou feminina), ou problemas durante a gravidez, nomeadamente abortos ou prematuridade. No entanto, por precaução, deve ser evitada a gravidez durante o ano seguinte ao tratamento.[17]

3. Técnicas de preservação e de protecção do potencial reprodutivo

Diversas estratégias foram propostas nos últimos anos para proteger e/ou preservar o potencial reprodutivo nas mulheres e homens com cancro. A eficácia de algumas destas técnicas está comprovada (técnicas estabelecidas), enquanto de outras ainda se encontra por esclarecer (técnicas experimentais).

Mulher

Técnicas para preservação do potencial reprodutivo:

1. Criopreservação de embriões

A preservação do potencial reprodutivo através da criopreservação de embriões compreende uma fase inicial de estimulação hormonal, seguida de punção folicular para recolha de ovócitos e posterior inseminação por fecundação *in vitro* (FIV) ou por microinjeção de espermatozoides (ICSI). Os embriões obtidos são então criopreservados. Quando o casal assim o pretender, os embriões são descongelados e transferidos para o útero.

A opção pela criopreservação de embriões, apesar de clinicamente estabelecida e de eficácia largamente comprovada (embora menor do que a taxa de gravidez por transferência a fresco de embrião, que ronda os 40% em mulheres até aos 35 anos)[1] pode limitar a autonomia reprodutiva da mulher, já que os embriões resultantes só poderão ser utilizados pelo casal que lhes deu origem. Por este motivo entendemos que esta opção não seja considerada em primeira instância.

Em julho de 2015 o Conselho Nacional de Procriação Medicamente Assistida emitiu uma recomendação que desaconselha a criopreservação de embriões para preservação do potencial reprodutivo.

2. Criopreservação de ovócitos

Em outubro de 2012 a Sociedade Americana de Medicina da Reprodução retirou o rótulo de "experimental" à criopreservação de ovócitos[2], passando a ser considerada uma técnica de preservação do potencial reprodutivo com eficácia bem estabelecida, depois de se ter demonstrado que a vitrificação dos ovócitos permite taxas de sobrevivência após desvitrificação superiores a 90%, não existindo diferenças nas taxas de fecundação, no número de embriões de boa qualidade ou na taxa de gravidez clínica por ciclo relativamente aos ovócitos utilizados a fresco[2].

A preservação do potencial reprodutivo através da criopreservação de ovócitos compreende uma fase inicial de estimulação hormonal, seguida de punção folicular e criopreservação por vitrificação (congelação ultrarrápida). A técnica de vitrificação veio melhorar

significativamente a sobrevivência dos ovócitos, as taxas de fecundação e a proporção de embriões de elevada qualidade, face à congelação lenta.

A evidência mais recente indica que as taxas de fecundação e gravidez resultantes das técnicas de Fertilização *in vitro* (FIV) e Injeção intracitoplasmática de espermatozoides (ICSI) são similares quando se utilizam ovócitos frescos ou ovócitos vitrificados/desvitrificados, o que atesta a eficácia da técnica de vitrificação[3]. Esta técnica tem uma eficácia aceitável e evita os problemas éticos decorrentes do armazenamento de embriões de casais em que um dos elementos sofre de doença oncológica.

Recentemente, a utilização de protocolos com antagonista da hormona libertadora de gonadotrofina (GnRH) e de protocolos *random start* que permitem iniciar a estimulação ovárica em qualquer fase do ciclo menstrual, tem-se revelado uma estratégia útil quando há constrangimentos de tempo por necessidade de iniciar rapidamente tratamento gonadotóxico[4,5]. Neste caso, todo o processo (estimulação hormonal, punção folicular e criopreservação) pode ser concluído em cerca de duas semanas. Este protocolo pode mesmo permitir a realização de dois ciclos de estimulação ovárica antes da quimioterapia, com o consequente aumento do número de ovócitos criopreservados.

Para a criopreservação de ovócitos ou embriões é necessário realizar uma estimulação ovárica, para recrutamento multifolicular, seguida de punção folicular para recolha de ovócitos.

Estes tratamentos acarretam um aumento suprafisiológico dos níveis circulantes de estrogénios que impõem alguma cautela no caso de cancro da mama hormonossensível (com expressão de recetores hormonais> 1%) (ver secção de situações clínicas). Existem, no entanto, protocolos que associam às gonadotrofinas, o letrozol (um inibidor da aromatase) com o intuito de reduzir os níveis plasmáticos de estrogénios nestas doentes[6].

3. Criopreservação de tecido ovárico

Para a técnica de criopreservação de tecido ovárico deve ser realizada colheita de vários fragmentos ou da totalidade do ovário por cirurgia laparoscópica. Não é consensual a realização de ooforectomia ou mesmo salpingooforectomia, havendo autores que optam pela realização de biópsias ováricas múltiplas.

A utilização do tecido criopreservado implica o transplante no ovário restante (transplante ortotópico). Tem sido descrita a possibilidade de realizar o transplante em bolsa peritoneal ou mesmo noutras localizações (transplante heterotópico).

Esta técnica, ainda considerada experimental, é a única que não exige estimulação ovárica nem punção folicular, não implicando por isso qualquer adiamento do tratamento da doença oncológica.

De notar que a criopreservação de tecido ovárico não será uma boa opção nas doentes com mutação germinativa patogénica *BRCA* ou outras mutações com risco associado de cancro (Síndrome de Lynch, p53, ou outras), assim como em doentes com leucemia (ver secção de situações clínicas). No futuro poderá recorrer-se a estratégias alternativas, como o isolamento de folículos existentes no tecido criopreservado com subsequente maturação *in vitro* e fecundação.

As maiores desvantagens da técnica são a necessidade de cirurgia e o risco de existência de células tumorais no tecido criopreservado.

A criopreservação de tecido ovárico não deve ser efetuada após os 38 anos (dado que a reserva ovárica nesta idade já será muito menor condicionando o sucesso de uma gravidez futura).

Nota: O processo de tomada de decisão relativa à preservação do potencial reprodutivo é particularmente complexo nas mulheres comparativamente com o dos homens, pois os procedimentos são mais invasivos e um deles ainda experimental (em muitos casos o único executável face à urgência de iniciar os tratamentos da doença oncológica) e é necessário ponderar rapidamente, antes do início dos tratamentos da doença oncológica, vários fatores de natureza sociodemográfica e clínica[7].

4. Maturação ovocitária *in vitro*

Alguns centros realizam a colheita de ovócitos imaturos (sem recurso à estimulação ovárica e, portanto, sem risco de elevação dos estrogénios plasmáticos) para posterior maturação *in vitro* antes ou depois da vitrificação. Trata-se de uma alternativa cuja eficácia é difícil de definir já que não existe uma ampla utilização desta técnica em doentes oncológicas. No entanto, a eficácia da técnica tem sido demonstrada no contexto da realização de técnicas de PMA em mulheres com síndroma dos ovários micropoliquísticos.

Técnicas para proteção do potencial reprodutivo:

1. Transposição ovárica (ooforopexia)

A transposição ovárica pode ser oferecida quando é necessário recorrer à radioterapia pélvica. Contudo, existe o perigo da dispersão de radiação e os ovários podem não ficar completamente protegidos. As mulheres devem ser avisadas que esta técnica nem sempre é eficaz na preservação do potencial reprodutivo.

Esta técnica deve ser realizada o mais próximo possível da data planeada para o início do tratamento devido ao risco de migração dos ovários[8].

2. Cirurgia ginecológica conservadora

A cirurgia conservadora do colo do útero (traquelectomia radical) pode ser oferecida a mulheres com cancro cervical em estádios precoces e com lesões ≤ 2 cm, que pretendam preservar a sua fertilidade.

No cancro do ovário em estádio inicial, a cirurgia conservadora (laparotomia exploradora, anexectomia unilateral e estadiamento completo) pode ser considerada.

No tratamento de outras neoplasias ginecológicas, as intervenções para preservação do potencial reprodutivo têm de respeitar o compromisso entre um adequado resultado oncológico e a cirurgia menos radical possível com a intenção de preservar os órgãos reprodutores tanto quanto possível.

3. Supressão da função ovárica

Têm sido publicados vários estudos acerca da eficácia dos análogos da GnRH e outras formas de supressão ovárica para preservação do potencial reprodutivo com resultados contraditórios[9,10].

Estes ensaios têm tido dificuldades de recrutamento (amostras pequenas e heterogéneas em termos de tratamento citostástico), definição controversa dos objetivos, além de que não é consensual a segurança da administração de análogos da GnRH concomitante com a quimioterapia[10].

Recentemente, foi publicado o estudo POEMS (*Prevention of Early Menopause Study*), um ensaio de fase III que, apesar de não ter concluído o recrutamento previsto, reforça a possível eficácia dos análogos da GnRH na prevenção da falência ovárica associada à quimioterapia em doentes com cancro da mama com recetores hormonais negativos[11]. Neste ensaio, as doentes foram aleatorizadas em dois grupos: um grupo realizou terapêutica com agonista da GnRH (Goserelina) em associação com a quimioterapia *standard* e outro grupo apenas quimioterapia. As doentes realizaram esquemas de quimioterapia com ciclofosfamida, sendo o esquema da escolha do investigador. As doentes incluídas no braço do agonista da GnRH receberam goserelina na dose de 3,6 mg, por via subcutânea a cada quatro semanas, iniciando uma semana antes da primeira dose de quimioterapia. A terapêutica foi mantida até duas semanas antes ou após, a última dose de quimioterapia. Apesar das falhas metodológicas do ensaio, foram publicados os resultados finais relativos às 218 doentes que puderam ser avaliadas. Neste grupo de doentes, a gravidez ocorreu em mais mulheres no grupo tratado com goserelina, de forma estatisticamente significativa (21% versus 11%, p = 0,03).

A evidência publicada até à data não é suficiente para recomendar a utilização de análogos da GnRH em detrimento de outras técnicas de preservação do potencial reprodutivo. Mesmo que seja considerada a sua utilização durante a quimioterapia, nomeadamente em doentes com

carcinoma da mama com recetores hormonais negativos, mantém-se a recomendação de referenciação da doente a consulta de Medicina da Reprodução.

Em situação de emergência ou em raras circunstâncias, quando as opções validadas não possam ser utilizadas, a proteção da função ovárica com análogos da GnRH pode ser considerada.

Homem

Técnicas para preservação do potencial reprodutivo:

1. Criopreservação de espermatozoides

A recolha e armazenamento de espermatozoides para preservação do potencial reprodutivo masculino é um procedimento bem estabelecido e na maioria das vezes simples, principalmente em idade pós-pubertária. Esta técnica tem apresentado resultados satisfatórios, com uma taxa de gravidez por transferência de embriões resultantes de fertilização *in vitro* com gâmetas congelados na ordem dos 40-50%.[12,13].

A criopreservação de espermatozoides foi reportada com sucesso em rapazes com idade superior a 13 anos, com espermogramas normais[14,15]. É o método recomendado em adultos e rapazes pós-púberes. A Sociedade Americana de Medicina da Reprodução recomenda que se efetuem três colheitas com um período de abstinência mínimo de 48 horas entre cada amostra. Desta forma, apesar de ser um procedimento simples e rápido, os doentes devem ser referenciados atempadamente de modo a maximizar as taxas de sucesso. Quando não é possível efetuar colheita de espermatozoides por masturbação (ex.: ansiedade, fadiga, hipogonadismo, diabetes, traumatismo vertebro-medular, doença neurológica, iatrogenia medicamentosa - antidepressivos ou medicação opioide) recomenda-se o recurso a vibroestimulação, electroejaculação ou técnicas de biópsia testicular.

2. Criopreservação de tecido testicular

Esta é uma técnica de preservação do potencial reprodutivo que poderá ser oferecida a doentes que não conseguem obter uma amostra de esperma adequada ou a rapazes pré-púberes (sendo nesta idade considerada experimental). O objetivo final é a utilização dos espermatozoides isolados do tecido biopsado em técnicas de PMA, ou a transplantação deste tecido após a cura (no caso da colheita em crianças), com a possibilidade de restaurar a espermatogénese a partir das espermatogónias criopreservadas. Apesar de já ter sido demonstrada a eficácia do transplante de epitélio germinativo em modelos animais, tal evidência carece de confirmação em humanos.

Técnicas para proteção da fertilidade:

1. Cirurgia conservadora

Em doentes com neoplasia do testículo a espermatogénese e esteroidogénese podem ser mantidas através do recurso a orquidectomia parcial. Esta abordagem conservadora deve ser especialmente considerada em doentes com tumores bilaterais ou tumores em testículo único se o volume tumoral é inferior a 30% da gónada com valores de testosterona pré-operatórios normais[16].

Nestes casos, devem ser feitas biópsias do parênquima restante para excluir a presença de neoplasia germinativa intratubular.

2. Terapêutica com análogos da GnRH

A terapêutica com análogos da GnRH pode ser utilizada para suprimir o eixo hipotálamo-hipófise-gonádico durante a administração de quimioterapia numa tentativa de proteger o epitélio germinativo. Alguns estudos em animais sugeriram eficácia desta técnica, mas, no homem, os estudos falharam na demonstração de preservação do potencial reprodutivo ou na obtenção de um retorno mais rápido da espermatogénese após quimioterapia[17].

3. Proteção gonadal

Em doentes que necessitam de radioterapia abdominal ou pélvica, a proteção gonadal com material blindado é o procedimento *standard* para reduzir a exposição à radiação dos órgãos reprodutores e proteger a função reprodutora.

4. Contextualização portuguesa

As técnicas de preservação do potencial reprodutivo feminino e masculino estão disponíveis no âmbito do Serviço Nacional de Saúde (SNS) e em diferentes instituições privadas de saúde.

No âmbito do SNS, os procedimentos associados à realização das técnicas e à congelação dos gâmetas recolhidos não comportam custos para além dos inerentes à medicação necessária nas mulheres submetidas a estimulação ovárica.

5. Situações clinicas específicas

Na Europa as neoplasias mais frequentes em adultos jovens entre os 15-24 anos são o linfoma de Hodgkin, o cancro do testículo e o melanoma maligno[1,2]. Na faixa etária dos 25-49 anos, as neoplasias mais frequentemente diagnosticadas são o cancro da mama, o carcinoma colorretal, o carcinoma do colo do útero e o melanoma maligno.

Estas neoplasias, se diagnosticadas em fases iniciais, têm elevada probabilidade de cura, com sobrevivências aos cinco anos que ultrapassam os 80%[3].

A preservação do potencial reprodutivo no sexo feminino reveste-se de diferentes especificidades, que pela complexidade e morosidade, devem merecer particular atenção.

Mulher

Cancro ginecológico

Sempre que exequível, em casos selecionados, em que não se comprometa a eficácia do tratamento oncológico, deve considerar-se preservar o útero e os ovários.

Nas doentes que necessitem de radioterapia pélvica, a transposição ovárica (ooforopexia) deverá ser efetuada previamente ao tratamento, com o intuito de diminuir a exposição direta do ovário às radiações ionizantes.

Colo do útero

A cirurgia conservadora do colo do útero pode ser oferecida a mulheres com cancro cervical em estádios precoces ≤ 2 cm (IA1, IA2 e IB1). As taxas de recorrência e mortalidades descritas são semelhantes aos casos tratados com histerectomia radical ou radioterapia. É de referir que a traquelorrafia apresenta morbilidade associada importante (necessidade de cerclagem uterina; infertilidade (14-41%); estenose cervical; aborto tardio; parto pré-termo (20-25% abaixo das 32 semanas)[4].

A criopreservação de ovócitos ou embriões deve ser abordada nestas doentes, previamente à realização de quimioterapia.

Em doentes que necessitem de radioterapia, a cirurgia para transposição ovárica, com ou sem criopreservação de ovócitos e/ou tecido ovárico, poderá ser uma alternativa. Deve ser referido à doente que após radioterapia pélvica o útero irradiado pode contribuir para aumento da infertilidade[5].

Cancro do endométrio

O carcinoma do endométrio é raro na mulher com idade inferior a 40 anos.

Perante um diagnóstico de cancro do endométrio em idade reprodutiva devem ser excluídas as situações de causa hereditária (síndrome de Lynch ou carcinoma colorretal hereditário não polipótico). O tratamento convencional consiste na histerectomia total e anexectomia bilateral com sobrevida aos cinco anos de 93,8 a 98%.

Na mulher que deseje preservar a fertilidade, e apresente carcinoma do endométrio tipo endometrioide, G1 e ressonância magnética negativa para invasão miometrial (estádio IA sem invasão do miométrio), o tratamento médico com progestativo, intrauterino ou oral, pode ser uma opção por um período de três meses, seguido de nova biópsia endometrial. A gravidez deve então ser permitida, com recurso ou não à procriação medicamente assistida, que não parece ter qualquer impacto na sobrevivência destas doentes.[6,7]

Idêntica atitude deve ser tida em relação à hiperplasia atípica, já que cerca de 30% dos casos de hiperplasia complexa atípica evolui para carcinoma endometrial se não forem tratados.[6]

As doentes devem ser informadas da necessidade de repetição periódica de biópsias endometriais bem como da necessidade de completar cirurgia após gravidez.

Tumor borderline do ovário

Mais de 30% dos tumores *borderline* do ovário atingem mulheres com idade inferior aos 40 anos. Estas doentes apresentam excelente sobrevivência, podendo haver tumor bilateral em 25-35% dos casos. A taxa de recorrência é de 0-5 % após tratamento cirúrgico com histerectomia total e anexectomia bilateral ou de 20-30% após cirurgia conservadora (anexectomia unilateral). Nestes casos, a recidiva é geralmente sob a forma de tumor *borderline*, pelo que o tratamento conservador não apresenta assim impacto negativo na sobrevivência[8,9,10].

Nos tumores *borderline* de tipo seroso, pode ainda ser considerada a cistectomia, principalmente se forem bilaterais, com taxa de recorrência de 25%. Os tumores *borderline* de tipo mucinoso são, porém, exceção, atendendo a que quando recorrem tende a ser na forma de carcinoma invasor[11] devendo aqui realizar-se anexectomia unilateral.

Após a cirurgia, em mulheres com parceiros, poderá haver tentativa de gravidez espontânea; em mulheres solteiras poderá ser proposta a preservação de ovócitos atendendo ao risco aumentado de recorrência após cirurgia conservadora. A estimulação ovárica nestas mulheres apesar de levantar algumas questões, parece ser segura, sem aumento das recorrências em relação às mulheres que fizeram cirurgia conservadora, e sem impacto na sobrevida[12,13].

Cancro epitelial do ovário

Entre 3 a 17% destes tumores atingem mulheres com idade inferior a 40 anos.[14]

A cirurgia conservadora (laparotomia exploradora, anexectomia unilateral e estadiamento completo) pode ser considerada nos estádios precoces em determinadas circunstâncias:

• Ausência de história de cancro hereditário do ovário;

• Possibilidade de vigilância adequada após cirurgia;

• Estádio IA G1/G2, IC G1/G2 unilateral (apenas os casos de rotura intraoperatória)

• Exclusão de G3, incluindo células claras, estádio I bilateral, ou superior ao estádio I.

Em aproximadamente 200 casos de tratamento cirúrgico conservador do cancro do ovário IA G1, a taxa de recorrência foi de 5% (sobreponível ao tratamento cirúrgico convencional). No estádio IA G2, em 45 casos, a taxa de recorrência foi de 20%.[15,16]

No caso de mulheres tratadas com cirurgia conservadora e infertilidade, ou, em mulheres cujo diagnóstico de cancro epitelial do ovário foi um achado operatório e que necessitem de completar o estadiamento cirúrgico, pode colocar-se a indicação de estimulação ovárica para

tentativa de gravidez ou preservação da fertilidade. Também aqui a literatura é escassa, já existindo, porém, casos relatados de sucesso com a estimulação ovárica controlada e subsequente gravidez[17,18].

Cancro não epitelial do ovário

Apesar de neoplasias raras, atingem maioritariamente mulheres jovens.

A cirurgia conservadora, em particular nos tumores de células germinativas, parece ser segura com taxas de cura de 90-95%[19].

Apesar de a literatura ser escassa, a estimulação ovárica controlada parece segura.

Devem ser tomadas precauções na realização de estimulação ovárica com o intuito de criopreservação de ovócitos ou embriões nos casos de tumores com algum grau de dependência hormonal, nomeadamente nos carcinomas estrogénio-dependentes do endométrio e epiteliais do ovário. A estimulação ovárica com letrozol deverá nestas situações deverá ser a estratégia a adotar.

A criopreservação de tecido ovárico nas situações de cancro epitelial e não epitelial do ovário, apesar de já terem sido relatadas com sucesso bem como o seu subsequente transplante[20,21], apresenta risco indiscutível de reintrodução de células tumorais pelo que ainda não deve ser recomendada nestas situações.

Cancro da mama

Evidência recente sugere que 40-50% das mulheres com história de cancro da mama desejam uma futura gravidez[22] mas no entanto apenas 4-7% consegue engravidar[23].

Para a criopreservação de embriões ou de ovócitos é necessário realizar uma estimulação ovárica para recrutamento multifolicular, seguida de punção folicular para recolha de ovócitos, o que acarreta um aumento suprafisiológico dos níveis circulantes de estrogénios, cujo impacto no prognóstico é desconhecido. No entanto, nas mulheres com cancro da mama com expressão de recetores hormonais, a estimulação ovárica é feita com recurso a inibidores da aromatase que permitem manter os estrogénios em níveis fisiológicos[23,24,25]. O inibidor mais estudado neste contexto é o letrozol[26]. São necessários mais dados para confirmar a segurança deste procedimento. Os estudos publicados sugerem que doentes submetidas a estimulação ovárica com letrozol e gonadotrofinas tiveram sobrevivência semelhante às doentes com cancro da mama não submetidas a este tratamento[27].

A utilização de protocolos de tratamento *random start*, que permitem iniciar a estimulação ovárica em qualquer fase do ciclo menstrual, não compromete o início do tratamento oncológico[28] e tem-se revelado uma estratégia útil quando há constrangimentos de tempo por necessidade de iniciar rapidamente tratamento sistémico, potencialmente gonadotóxico28.

Neste caso, todo o processo (estimulação hormonal, punção folicular e criopreservação) pode ser concluído em cerca de duas semanas. Este protocolo pode mesmo permitir a realização de dois ciclos de estimulação ovárica antes da quimioterapia, com o consequente aumento do número de ovócitos criopreservados.

Neoplasias digestivas

Carcinoma do cólon, reto e canal anal

Os dados são escassos, mas, dado o aumento significativo da taxa de sobrevivência destas neoplasias, as mulheres que necessitem de QT ou RT pélvica deverão ser informadas do risco de falência ovárica (principalmente as mulheres com cancro do reto) e da possibilidade de transposição ovárica e preservação de gâmetas, embriões ou tecido ovárico[29,30]. A preservação de tecido ovárico poderá acarretar risco de reintrodução de células tumorais.

Carcinoma gástrico

Não existem dados publicados sobre preservação de gâmetas/embriões ou tecido ovárico em doentes com esta patologia, de prognóstico reservado. O tratamento atual do carcinoma gástrico com intenção curativa (quimiorradioterapia adjuvante ou QT peri-operatória) inclui esquemas citostáticos com antraciclinas e cisplatina, com risco intermédio de infertilidade, para além do fluouracilo (5-FU), que acarreta menor risco.

Estão descritas metástases ováricas entre 7 a 54% dos casos diagnosticados na mulher.[31,32]

Outras neoplasias:

Tumores do SNC (gliomas e oligoastrocitomas)

Numa análise de 15 anos de prática de técnicas de preservação da fertilidade de um centro, estes tumores corresponderam a 4,9% dos casos (19/391). Estão reportados 11 casos de mulheres com neoplasias do SNC submetidas a técnicas de PMA com congelação de embriões com resultados sobreponíveis a mulheres não doentes.[33]

Os doentes com resseção macroscópica total que tenham de fazer terapêutica complementar com temozolamida (agente alquilante) deverão ser informados do seu potencial gonadotóxico.

Sarcomas

Um estudo englobando 27 jovens com sarcoma de Ewing submetidas a QT ou QT e RT pélvica, demonstrou uma taxa de falência ovárica precoce de 67% (incluindo todas as doentes que fizeram RT pélvica), pelo que as pacientes deverão ser informadas dos riscos de infertilidade futura. Os ovários deverão ser protegidos da radioterapia pélvica por transposição.

Melanoma maligno

Não existem casos descritos de preservação do potencial reprodutivo em mulheres com melanomas malignos. A doença disseminada tem muito mau prognóstico e é tratada por imunoterapia e terapêuticas-alvo, cuja implicação na fertilidade é mal conhecida.[34] O Ipilimumab pode interferir com a fertilidade por via de endocrinopatia imune.

A terapêutica com quimioterapia, baseada em agentes alquilantes, não altera a sobrevida destes doentes.[35]

Doentes portadoras de mutações BRCA

A salpingooforectomia bilateral profilática apenas deve ser realizada depois de completado o projeto reprodutivo da mulher.[36]

Estas doentes podem apresentar reserva ovárica diminuída previamente à realização de qualquer tratamento, pelo que poderão ser mais suscetíveis a apresentar falência gonadal induzida pela quimioterapia.[37] Nestes casos não está indicada a preservação de tecido ovárico.

A preservação de gâmetas pode permitir a seleção posterior de embriões sem mutação.

Homem

Como referido previamente (ver capítulo do impacto do tratamento oncológico na fertilidade) as gónadas são extremamente sensíveis aos efeitos da quimioterapia e da radioterapia.

Assim, dada a simplicidade e a rapidez inerentes à colheita de esperma para criopreservação, esta possibilidade deve ser abordada em todos os doentes que vão iniciar tratamento oncológico, antes de qualquer procedimento terapêutico.

Cancro do testículo

O cancro do testículo apresenta elevadas taxas de cura mesmo quando diagnosticado em fases avançadas da doença. Geralmente, os doentes submetidos a quimioterapia (esquema BEP ou carboplatina) recuperam rapidamente a sua fertilidade após finalizarem o tratamento. Não obstante, alguns doentes apresentam alterações da espermatogénese e subfertilidade prévias ao diagnóstico e a qualquer manobra terapêutica. A infertilidade pode mesmo, em casos pontuais, servir de alerta para o diagnóstico de cancro do testículo.

Cirurgia

Nos doentes com cancro do testículo, sobretudo nos casos de neoplasia intraepitelial, a produção hormonal e de espermatozoides pode ser mantida através do recurso a uma orquidectomia parcial.[37] (ver **Técnicas para proteção da fertilidade)**.

Esta abordagem cirúrgica conservadora deve ser especialmente considerada em doentes com tumores bilaterais ou tumores em testículo único se o volume tumoral é inferior a 30% da gónada e as regras cirúrgicas oncológicas são respeitadas[38]. Nestes casos, devem ser feitas

biópsias do parênquima restante para excluir a presença de neoplasia germinativa intratubular.

A linfadenectomia retroperitoneal também acarreta potencial morbilidade ejaculatória. Aconselha-se a criopreservação de esperma e se possível, realizar sempre uma técnica de "nerve-sparing".

Outras neoplasias:

Tumores germinativos extragonadais, neoplasias primárias do SNC, tumores da nasofaringe, tumores digestivos ou outros

Os dados existentes referentes a cada uma destas situações são escassos. Contudo, deve ser avaliado o prognóstico da doença e geridas as expectativas do doente. Importante salientar que os doentes que irão necessitar de terapêuticas pélvicas (cirurgia ou radioterapia) devem ser informados das possíveis alterações na função erétil e ejaculatória.

Como referido e dado que o método mais generalizado para preservação do potencial reprodutivo no homem é a criopreservação de esperma, esta possibilidade deve ser oferecida aos doentes que manifestem desejo de preservar a sua fertilidade, sem prejuízo do início do tratamento.

Doenças hematológicas

As doenças hematológicas malignas mais frequentemente encontradas em idade fértil e nas crianças são:

• Leucemias agudas.

• Linfomas, sobretudo doença de Hodgkin.

• Leucemias mieloides crónicas.

• Trombocitemia essencial (TE).

Em regra, a apresentação inicial é clinicamente muito agressiva, a necessitar de tratamento urgente e intensivo, consistindo em vários ciclos de quimioterapia com a associação de vários citostáticos, nalguns casos (linfomas) podendo ter de ser completados por tratamentos de radioterapia.

Frequentemente, na altura do diagnóstico, não há condições para manobras ou intervenções cirúrgicas, em consequência da trombocitopenia ou trombocitose, neutropenia ou risco de disseminação da doença (sobretudo leucemias agudas). A agressividade e o atingimento dos órgãos reprodutores, em consequência do tratamento, é variável:

• Máximo nas leucemias agudas e linfomas não Hodgkin.

• Intermédio para os linfomas de Hodgkin.

• Menor para as LMC e TE.

Todavia, no caso específico do linfoma de Hodgkin e nos homens, a própria doença poderá determinar redução da fertilidade. As taxas de remissão completa e de cura são bastante elevadas nas leucemias agudas e linfomas podendo persistir por bastante tempo (habitualmente considera-se cinco anos) uma probabilidade significativa de recaída da doença, mais elevada nos dois primeiros anos de remissão.

O tratamento das recaídas obriga quase sempre a quimioterapias intensivas de alta dose e/ou o recurso a transplante, com efeito significativo, eventualmente definitivo, sobre a fertilidade.

No caso das LMC e TE há necessidade de tratamentos de manutenção (imatinib e/ ou outros inibidores da tirosina-cinase (anagrelide, por exemplo) de longa duração (anos) o que, no caso dos homens, poderá determinar uma diminuição da espermatogénese. Nas mulheres existe um risco elevado de gravidezes inesperadas sob tratamento com fármacos cujos efeitos teratogénicos são ainda insuficientemente conhecidos.

Preservação do potencial reprodutivo em doenças hematológicas

Leucemias agudas e linfomas

No homem deve realizar-se a criopreservação de esperma, logo após o diagnóstico, desde que não haja suspeita de invasão testicular.

Na mulher

Linfomas: Este nome designa de forma genérica diferentes doenças dos linfócitos com forma de apresentação, sintomatologia, agressividade da doença e prognóstico muito diferentes.

A idade de apresentação, a invasão na apresentação ou na evolução da doença dos órgãos reprodutores e a necessidade ou não de urgência no tratamento são por isso mesmo também muito variáveis. Por outro lado, a terapêutica clássica vai sofrendo modificações com o aparecimento de novas terapêuticas e a publicação na comunidade científica de novos resultados de ensaios clínicos que vão sendo realizados. Por tudo isto, propomos nas doentes em idade fértil, uma reunião entre o hematologista médico da doente e a equipa de Medicina de Reprodução, para elaboração do plano a seguir para a doente no que respeita a preservar o seu potencial reprodutivo, tendo em conta a vontade desta, após o diagnóstico e antes do início do tratamento de indução.

Leucemias: Devem considerar-se os efeitos definitivos sobre a fertilidade dos tratamentos das recaídas, e recolher e conservar ovócitos a todos as doentes em remissão completa (mês +6 após último tratamento de radioterapia ou de quimioterapia, para efeito de "washout" dos fármacos citostáticos utilizados).

A gravidez deve ser adiada até, pelo menos, dois anos após a remissão completa, com a finalidade de minimizar o risco da necessidade de tratamento de recaídas durante a gravidez.

Leucemia mieloide crónica

Considerando os efeitos definitivos sobre a fertilidade dos tratamentos das recaídas, devem recolher-se ovócitos/espermatozoides a todos os doentes em remissão completa.

Assim, nas mulheres, em situação de remissão molecular sustentada e após interrupção temporária de tratamento com TKIs ("washout" de três meses, embora podendo-se considerar, no intervalo, manutenção com interferão alfa) e nos homens sem qualquer restrição, desde que em remissão hematológica.

Embora não se definam restrições temporais para o momento da gravidez, considera-se preferível esperar remissão molecular sustentada, pela necessidade de suspender tratamento com TKIs (mesmo podendo ser feito tratamento de manutenção com interferão alfa, idealmente apenas no segundo e terceiro trimestres).

Trombocitemia essencial

A trombocitemia essencial parece não se acompanhar de alterações definitivas da fertilidade, mesmo em situação de progressão; todavia, na altura da recolha de ovócitos/espermatozoides e em mulheres durante a gravidez, é recomendado, se possível, o tratamento apenas com aspirina e/ou heparina, eventualmente, em situações de risco elevado (trombocitoses extremas e superiores a 1 X $10^6/mm^3$, antecedentes trombóticos ou hemorrágicos, complicações em gestações anteriores) manutenção com interferão alfa (no caso de gravidez, idealmente só no segundo e terceiro trimestres).

Figura 2.1 Recomendações clínicas para a preservação/proteção do potencial reprodutivo na mulher.

Cirurgia

Sempre que possível, garantindo o melhor resultado oncológico, o tratamento cirúrgico de órgãos que comprometam a função reprodutora da mulher deve ser o mais conservador possível.

Radioterapia

Sempre que seja necessário efetuar radioterapia pélvica, como complemento do tratamento cirúrgico (adjuvante) ou com intenção curativa (quimiorradioterapia concomitante) deve considerar-se:

– A transposição ovárica.

– Proteção gonadal com material blindado (se não for possível nenhuma das técnicas de preservação do potencial reprodutivo).

Sempre que seja necessária a realização de radioterapia cranioespinhal deve considerar-se a realização de técnicas de preservação do potencial reprodutivo.

Tratamento sistémico

Idealmente, todas as mulheres que desejem preservar o seu potencial reprodutivo devem ser referenciadas a unidades de Medicina da Reprodução antes do início de qualquer tratamento sistémico.

Se necessidade de tratamento imediato, mas com condições para a realização de técnicas de preservação do potencial reprodutivo, pode proceder-se à criopreservação de tecido ovárico ou aspiração de folículos antrais com maturação *in vitro.*

Se possibilidade de diferir o inicio do tratamento ≥ 2 semanas: Se a doente não quiser ser submetida a estimulação hormonal – ponderar criopreservação de tecido ovárico (técnica experimental). A estimulação hormonal pode ser utilizada em doentes com cancro da mama com indicação para quimioterapia neoadjuvante, adjuvante e/ou hormonoterapia durante 5-10 anos. Nas situações em que os recetores hormonais são positivos deve ser considerada a estimulação hormonal associada a letrozol.

Figura 2.2 Indicações gerais para a preservação do potencial reprodutivo na mulher.

Mulheres com idade inferior a 40 anos.

Adequada reserva ovárica (contagem de folículos antrais, níveis de Hormona Anti-Mulleriana).

Projeto reprodutivo incompleto.

Prognóstico favorável.

Figura 2.3 Contraindicações gerais para a preservação do potencial reprodutivo na mulher.

Mulheres com idade ≥ 40 anos.
Falência ovárica.
Projeto reprodutivo completo.
Doença metastizada ou prognóstico muito reservado.

Figura 2.4 Recomendações clínicas de preservação/proteção do potencial reprodutivo no homem e jovens pós-púberes.

Colheita e criopreservação de esperma - deve ser realizada aquando do diagnóstico e antes de qualquer procedimento terapêutico, desde que não haja suspeita de invasão testicular, ou que não comprometa a realização e/ou o início do tratamento.
Cirurgia
Sempre que possível o tratamento cirúrgico de órgãos que comprometam a função reprodutora do homem, deve ser o mais conservador possível, garantindo o melhor resultado oncológico.
Radioterapia
Sempre que o recurso à radioterapia pélvica ou cranioespinhal for necessário e possa comprometer a função gonadal, deve ponderar-se criopreservação de esperma.
Também poderá ser necessário recorrer à proteção gonadal, se adequado.
Tratamento sistémico
É recomendado que antes de qualquer tratamento sistémico que possa comprometer o potencial reprodutivo, se deva proceder à colheita e criopreservação de esperma.

6. Considerações finais

Os profissionais de saúde devem abordar com o doente (ou com os seus pais ou representantes legais) o risco de infertilidade e as possibilidades de preservação do potencial reprodutivo, tendo em atenção os seguintes aspetos:

Informação sobre o risco individual

• Alguns tratamentos podem causar infertilidade ou menopausa precoce.

• O risco individual de cada doente pode vir a sofrer alterações em função do tipo de cancro, idade e do plano de tratamento.

• Deve estratificar-se o risco de infertilidade (alto, intermédio, baixo, inexistente, desconhecido).

• A avaliação da reserva reprodutiva antes do diagnóstico da neoplasia pode ser determinante na indicação para realização de técnicas de preservação do potencial reprodutivo.

Opções para a preservação de fertilidade

• Nos homens, a opção mais comum e mais eficaz é a criopreservação de esperma. Existem outras opções, caso a criopreservação de esperma não seja possível ou adequada.

• Nas mulheres, a opção preferencial é a criopreservação de ovócitos. Existem outras opções caso esta técnica não seja exequível ou adequada.

Tempo

• O tempo é essencial.

Os tratamentos de preservação do potencial reprodutivo necessitam de estar completos antes do início dos tratamentos potencialmente lesivos da função gonadal.

• Nos homens, a criopreservação de esperma pode ser conseguida em 24-48 h.

• Nas mulheres, a preservação do potencial reprodutivo pode ser completada em duas a quatro semanas. Contudo, alguns procedimentos experimentais podem ser realizados mais rapidamente.

• A referenciação deve ser o mais precoce possível, assim que esteja feito o diagnóstico da doença oncológica e se estabeleça a necessidade de terapêutica potencialmente lesiva da função reprodutiva.

Custo

A Assembleia da República recomendou que o Serviço Nacional de Saúde assegure a preservação de gâmetas de doentes que correm risco de infertilidade devido a tratamentos oncológicos.

O recurso a estas técnicas no âmbito do SNS é gratuito, ficando apenas a cargo das mulheres o custo inerente aos medicamentos necessários para a estimulação ovárica.

Bibliografia

1. Organização Mundial de Saúde (2014). *World Cancer Report 2014*. WHO Press.
2. Direção Geral de Saúde - Programa Nacional para as Doenças Oncológicas (2014). Doenças oncológicas em números 2014. In *Direção Geral de Saúde*.
3. De Angelis, Sant M, Colemanet MP et al. Cancer survival in Europe 1999-2007 by country and age: Results of EUROCARE-5 – A population-based study. *The Lancet Oncology, 15*, (1) 23-34. doi: 10.1016/S1470-2045(13)70546-1
4. Makin, G., & Meyer, S. (2008). Oncology. In N. McIntosh, Helms, P., Smyth, R., & Logan, S. (Eds.), *Forfar and Arneil's textbook of pediatrics* (pp. 991-1038). New York: Churchill Livingstone.
5. Schmidt, L., Sobotka, T., Benzten, J., & Andersen, A. (2012). Demographic and medical consequences of the postponement of parenthood. *Human Reproduction Update, 18*(1), 28-43. doi: 10.1093/humupd/dmr040.
6. Woodruff, T. (2010). The Oncofertility Consortium: Addressing fertility in young people with cancer. *Nature Reviews in Clinical Oncology, 7*(8), 466-475. doi:10.1038/nrclinonc.2010.81.
7. Snyder, K., & Tate, A. (2013). What to do now? How women with breast cancer make fertility preservation decisions. *Journal of Family Planning and Reproductive Health Care, 39*, 172-178. doi: 10.1136/jfprhc-2011-100286.
8. Peddie, V, Porter, M, Barbour, R et al. (2012). Factors affecting decision making about fertility preservation after cancer diagnosis: A qualitative study. *BJOG, 119*, (9) 1049-1057. doi:
10.1111/j.1471-0528.2012.03368.x
9. Zanagnolo, V., Sartori, E., Trussardi, E., Pasinetti, B., & Maggino, T. (2005). Preservation of ovarian function, reproductive ability and emotional attitudes in patients with malignant ovarian tumors. *European Journal of Obstetrics & Gyneacology and Reproductive Biology, 123*(2), 235-243.
10. Loscalzo, M., & Clark, K. (2007). The psychosocial context of cancer-related infertility. In T. Woodruff, & K. Snyder (Eds.). *Oncofertility: Fertility preservation for cancer survivors* (pp. 180-
190). New York: Springer.
11. Carter, J., Rowland, K., Chi, D., Brown, C., Abu-Rustum, N., Castiel, M., & Barakat, R. (2005). Gynecologic cancer treatment and the impact of cancer-related infertility. *Gynecologic Oncology, 97*, 90-95. doi: 10.1016/j.ygyno.2004.12.019
12. Dow, K. (1994). Having children after cancer. *Cancer Practice, 2*(6), 407-413.
13. Letourneau, J, Ebbel, E. Katz et al. (2012). Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. *Cancer, 118* (6), 1710-1717. doi: 10.1002/cncr.26459
14. Balthazar, U., Deal, A., Fritz, M., Kondapalli, L., Kim, J., & Mersereau, E. (2012). The current fertility preservation consultation model: Are we adequately informing cancer patients of their options? *Human Reproduction, 27*(8), 2413-2419. doi: 10.1093/humrep/des188
15. Forman, E., Anders, C., & Behera, M. (2009). Pilot survey of oncologists regarding treatment-related infertility and fertility preservation in female cancer patients. *Journal of Reproductive Medicine, 54*(4), 203-207.
16. Cardoso, F, Loibl, S, Pagani, O et al. (2012). The European Society of Breast Cancer Specialists recommendations for the management of young women with breast cancer. *European Journal of Cancer, 48*, (18) 3355-3377. doi: 10.1016/j.ejca.2012.10.004
17. Clinical Oncological Society of Australia (2012). Fertility preservation for AYAs diagnosed with cancer: Guidance for health professionals. In *Cancer Council Australia*. Disponível em: http://wiki.cancer.org.au/
18. Loren, A, Mangu, P, Beck, L et al. (2013). Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. *Journal of Clinical Oncology, 31*, (19) 2500-10. doi: 10.1200./jco.2013.49.2678
19. Peccatori, F, Azim, H, Orecchia, R et al. (2013). Cancer, pregnancy and fertility: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, 24 suppl 6, vi160-70. doi: 10.1093/annonc/mdt199
20. Melo, Almeida-Santos, & Canavarro (Novembro, 2013). *Having children in survivorship: Are women cancer patients positively motivated to preserve their fertility?* Comunicação oral apresentada no The 3rd World Congress of the International Society for Fertility Preservation, Valencia, Espanha.
21. Melo, Almeida-Santos, & Canavarro (Outubro, 2014). *Oncologist-female patient discussion about the reproductive future: What are the practices of the Portuguese oncologists and what do they want to know about Oncofertility?* Poster apresentado no 16th World Congress of Psycho-Oncology and Psychosocial Academy, Lisboa, Portugal.

Impacto dos tratamentos na fertilidade

1. Green DM, Kawashima T, Stovall M et al. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol 2009. 27(16):2677-85.
2. Green DM, Kawashima T, Stovall M et al. Fertility of male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2010 Jan 10;28(2):332-9
3. Madanat LM, Malila N, Dyba T et al. Probability of parenthood after early onset cancer: a population-based study. Int J Cancer. 2008; 123: 2891–2898.

4. Schover LR. Premature ovarian failure and its consequences: vasomotor symptoms, sexuality, and fertility. J Clin Oncol. 2008; 26: 753–758.
5. Cancer and fertility preservation: Barcelona consensus meeting. F. Martínez et al. Gynecol Endocrinol. 2013.
6. Wallace WH, Kelsey TW. Human ovarian reserve from conception to menopause. PLoS ONE 2010;5:e8772.
7. Ovarian function in female survivors after multimodal Ewing sarcoma therapy. Raviborska A. et al. Pediatric Blood Cancer, 2014 Nov 15. doi:10.1002
8. Rodrigues et al. Fertility preservation during cancer treatment: clinical guidelines. Cancer Management and Research 2014: 6 105-117.
9. Wo JY, Viswanathan AN. Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients. *Int J Radiat Oncol Biol Phys*. 2009;73(5):1304–1312.
10. The effects of cancer treatment on reproductive functions: guidance on management. Royal College of Physicians, The Royal College of Radiologists, Royal College of Obstetricians and Gynaecologists. (2007)
11. Ben-Aharon I. Shalgi R. What lies behind chemotherapy induced ovarian toxicity? Reproduction. 2012; 144: 153-163.
12. Meistrich M.L. Male Gonadal Toxicity. Pediatr Blood Cancer. 2009 August; 53(2): 261-266.
13. Fertility Preservation for Patients With Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. Alison W. Loren et al. JCO. 2013.49.2678
14. Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update 2001.
15. New systemic agents in dermatology with respect to fertility, pregnancy, and lactation, 2015 Deutsche Dermatologische Gesellschaft (DDG). Published by John Wiley & Sons Ltd. | JDDG | 1610-0379/2015/1304
16. National Cancer Institute: FDA approval for bevacizumab. Disponível em: http://www.cancer.gov/cancertopics/druginfo/fda-bevacizumab
17. Chow SM, Yau S, Lee SH et al. Pregnancy outcome after diagnosis of differentiated thyroid carcinoma: no deleterious effect after radioactive iodine treatment. Int J Radiat Oncol Biol Phys 2004;59:992–1000.

Técnicas de preservação e/ou de proteção do potencial reprodutivo

1. Westphal LM, Massie JAM. Oncofertility Medical Practice: Clinical Issues and Implementation; Chapter 4: Embryo and Oocyte Banking. C. Gracia and T.K. Woodruff (eds.). Springer 2012. Disponível em: http://oncofertility.northwestern.edu/sites/default/files/uploadedfilecontent/embryo_and_oocyte_banking_-_lynn_m._westphal_and_jamie_a.m._massie.pdf
2. The Practice Committee of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. 2012. Mature oocyte cryopreservation: a guideline. Fertil Steril 2012.
3. Cobo A, Diaz C. Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril 2011; 96: 277–85.
4. Nayak SR, Wakim AN. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles with GnRH agonist trigger for fertility preservation. Fertil Steril. 2011 Jul;96(1):e51-4.
5. von Wolff M, Thaler CJ, Frambach T et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. Fertil Steril 2009; 92: 1360-1365.
6. Rodriguez-Wallberg KA, Oktay K. Fertility Preservation and Pregnancy in Women With and Without BRCA Mutation–Positive Breast Cancer. The Oncologist 2012; 17: 1409–1417.
7. Chang, H., & Suh, C. (2008). Fertility preservation for women with malignancies: Current developments of cryopreservation. Journal of Gynecologic Oncology, 19(2),99-107, doi: 10.3802/jgo.2008.19.2.99
8. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K; American Society of Clinical Oncology. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013 Jul 1;31(19):2500-10.
9. Roness H, Kalich-Philosoph L, Meirow D. Prevention of chemotherapy-induced ovarian damage: possible roles for hormonal and non-hormonal attenuating agents. Hum Reprod Update 2014.20(5): 759–774.
10. Blumenfeld Z, Katz G, Evron A. 'An ounce of prevention is worth a pound of cure': the case for and against GnRHagonist for fertility preservation. Ann Oncol 2014.25: 1719–1728, 2014
11. Moore H, Unger JM, Phillip KA et al. Goserelin for Ovarian Protection during Breast-Cancer Adjuvant Chemotherapy. N Engl J Med 2015;372:923-32.
12. van Casteren NJ, van Santbrink EJ, van Inzen W, Romijn JC, Dohle GR. Use rate and assisted reproduction technologies outcome of cryopreserved semen from 629 cancer patients. Fertil Steril. 2008 Dec;90(6):2245-50. doi: 10.1016/j.fertnstert.2007.10.055. Epub 2008 Jan 14.
13. Selk A, Belej-Rak T, Shapiro H, Greenblatt E. Use of an oncology sperm bank: a Canadian experience. Can Urol Assoc J. 2009 Jun;3(3):219-222.
14. Bahadur G, Ling KL, Hart R et al. Semen quality and cryopreservation in adolescent cancer patients. Hum Reprod. 2002 Dec;17(12):3157-61.
15. Menon S, Rives N, Mousset-Siméon N et al. Fertility preservation in adolescent males: experience over 22 years at Rouen University Hospital. Hum Reprod. 2009 Jan;24(1):37-44.
16. P. Albers, W. Albrecht, F. Algaba et al. Guidelines on Testicular Cancer, European Association of Urology, 2015.

17. Meistrich ML, Shetty G. Hormonal Suppression for Fertility Preservation in Males and Females. Reproduction. 2008 Dec;136(6):691-701.

Situações clínicas especiais

1. Martin I, Valls C, Paviac, et al. Gonadal function and puberty assessment in a cohort of spanish pediatric survivors of childhood cancer. Endocrinologist 2009; 19:133-41.
2. Cancer Research UK. Cancer Incidence by Age 2009. Available at: http://info.cancerresearchuk.org/cancerstats (Last accessed 27 Dec 2014)
3. Ries LAG, Eisner MP, Kosary CL, et al. SEER Cancer Statistics Review, 1975-2002. National Cancer Institute. Bethesda, MD. Available from: http://seer.cancer.gov (last accessed 27
Dec 2014)
4. Plante M, Gregoire J, Renaud MC, Roy M: The vaginal radical trachelectomy: an update of a series of 125 cases and 106 pregnancies. Gynecol Oncol. 2011;121(2):290.
5. Teh WT Stern C, Chander S, Hickey M. et al. The impact of uterine radiation on subsequent fertility and pregnancy outcomes. Biomed Res Int. 2014. Epub 2014 Aug 6. Review. PMID: 25165706.
6. Ichinose M et al. The influence of infertility treatment on the prognosis of endometrial cancer and atypical complex endometrial hyperplasia. Int J Gynecol Cancer. 2013 Feb; 23(2):288-93. PMID 23314282.
7. Park JY, Kim DY, Kim TJ et al. Hormonal therapy for women with stage IA endometrial cancer of all grades. Obstet Gynecol 2013; 122 (1): 7-14. PMID: 23743459.
8. Morice P et al. Borderline tumours of the ovary and fertility. Eur J Cancer 2006; 42:149-58.
9. Zanetta G et al. Behavior of borderline tumors with particular interest to persistence, recurrence, and progression to invasive carcinoma: a prospective study. J Clin Oncol 2001;
19: 2658-64.
10. Alvarez R et al. Fertility sparing treatment in borderline ovarian tumours: ecancer 2015; 9:507.
11. Khunamornpong S et at. Mucinous tumor of low malignant potencial ("borderline" or "atypical proliferative" tumor) of the ovary: a study of 171 cases with the assessment of intraepithelial carcinoma and microinvasion. Int J Gynecol Pathol 2011; 30: 218-30.
12. Park CW, Yang KM, Kim HO et al. Outcomes of controlled ovarian hyperstimulation for infertile patients with borderline ovarian tumor after conservative treatment. J Korean Med Sci.2007 Sep 22 Suppl: S134-8. PMID: 17923740.
13. Denschlag D. Clinical recommendation on fertility preservation in borderline ovarian neoplasm: ovarian stimulation and oocyte retrieval after conservative surgery. Gyn Obst Inv 2010; vol 70:160-165.
14. Theodoros M. Reproduction beyond cancer: A message of hope for young women. Gynecologic Oncology 2006, 103; 3, 1109-1121.
15. Fruscio R et al. Conservative management of early stage epithelial ovarian cancer: results os a large retrospective series. Ann Oncol 2013, 24 (1), 138-144.
16. Zapardiel I, Diestro MD, Aletti G. Conservative treatment of early stage ovarian cancer: oncological and fertility outcomes. Eur J Surg Oncol 40(4), 387-393.
17. Alvarez M; Solé M, Devesa M et al. Live birth using vitrified-warmed oocytes in invasive ovarian cancer: case report and literature review. Rep Biom Online (2014) 28, 663-668.
18. Abu-Musa A, Seoud M, Hannoun A. In vitro fertilization in a patient with ovarian cancer (stage IC) following conservative surgery and chemotherapy: a case report. Eur J Gynaecol Oncol 2008. 29, 408-410.
19. Tomao F et al (2015). Crit Rev Oncol Hemato. Special issues in fertility preservation for gynecologic malignancies.
20. Jensen AK et al. Outcomes of ovarian tissue transplantations of cryopreserved ovarian tissue to 41 women in Denmark. Human Reprod, 2015 Dec 30 (12): 2838-45.
21. Domans MM et al. Ovarian tissue cryopreservation followed by controlled ovarian stimulation and pick-up of mature oocytes does not impair the number or quality of retrieved oocytes. J Ovarian Research 2014; 7:80.
22. Litton JK. Breast cancer and fertility. Curr Treat Options Oncol 13:137-145, 2012.
23. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol. 1; 26(16):2630-5, 2008.
24. Oktay K. Further evidence on the safety and success of ovarian stimulation with letrozole and tamoxifen in breast cancer patients undergoing in vitro fertilization to cryopreserve their embryos for fertility preservation.J Clin Oncol. 1; 23(16):3858-9, 2005.
25. Oktay K, Buyuk E, Libertella N, Akar M, Rosenwaks Z. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol.1; 23 (19):4347-53, 2005.
26. Mitwally MF, Casper RF. Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. Fertil Steril ;77:776–780, 2002.
27. Azim AA, Costantini-Ferrando M, LostrittoK, et al. Relative potencies of anastrozole and letrozole to suppress estradiol in breast cancer patients undergoing ovarian stimulation before in vitro fertilization. J Clin Endocrinol Metab; 92:2197–2200, 2007.

28. Sönmezer M, Türkçüoğlu I, Coşkun U, Murat S, Oktay K. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. Fertil Steril.95(6):2125.9-11, 2011.
29. Spanos C, Mamopoulos A, Tsapas A, Syrakos A, Syrakos T, Kiskinis D.Female fertility and colorectal cancer, Int J Colorectal Dis. 2008 Aug;23(8):735-43.
30. O´Neill MT, Ni Dhonnchu T, Brannigan AE. Topic update: effects of colorectal cancer treatments on female fertility and potential methods for fertility preservation. Dis Colon Rectum. 2011 Mar; 54(3):363-9.
31. Bastings L, Beerendonk CM, Westphal JR et al. Autotransplantation of cryopreserved ovarian tissue in cancer survivors and the risk of reintroducing malignancy: a systematic review, Human Rep Update. 2013; 19 (5):483-506.
32. Earle C, Mamon H. Adjuvant and neoadjuvant treatment of gastric cancer, Uptodate Feb 2015.
33. Courbiere et al. Emergency IVF for embryo freezing to preserve female fertility: a French multicentre cohort study. Hum Reprod 2013; 28 (9): 2381-2388.
34. Grunewald S, Jank A. New systemic agents in dermatology with respect to fertility, pregnancy, and lactation, 2015 Deutsche Dermatologische Gesellschaft (DDG). Published by John Wiley & Sons Ltd. | JDDG | 1610-0379/2015/1304
35. Sosman JA. Overview of the management of advanced cutaneous melanoma. , MD Uptodate® Feb 2015.
36. Loren AW, Mangu PB, Nohr Beck L, et al. Fertility Preservation for Patients With Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. JCO.2013.49.2678.
37. Titus S, Li F, Stobezki R, et al. Impairment of BRCA1-related DNA double-strand break repair leads to ovarian aging in mice and humans. *Sci Transl Med*. 2013;5(172):172ra21.
38. P. Albers, W. Albrecht, F. Algaba et al. Guidelines on Testicular Cancer, European Association of Urology, 2015.

2.2 Collaboration in the development and accomplishment of other national education and information initiatives

In the course of this research, a number of other initiatives was planned and implemented to promote the field of oncofertility and to engage Portuguese healthcare professionals in the provision of information and discussion of these topics with patients, in one hand, and in practices of timely and effective patient referral, on the other. The main promoters of these initiatives were the LPCC, the CFP and the SPMR. Through participation in these events, courses and seminaries, it was possible to share information with oncologists and other health professionals about the specific effects of cancer and cancer treatments in fertility, to create awareness on the tools available to support infertility risk assessment and also to disseminate some important results of this investigation.

A summary of these initiatives, including their promotors, target audience and objectives, is presented below in chronological order.

Initiative	Target audience and objectives	Contribution
Course "Fertility preservation: a multidisciplinary approach" 5th Congress of the SPMR; Pre-congress course Portuguese Society of Reproductive Medicine (SPMR) October 2013	Oncologists, embryologists, gynaecologists, reproductive medicine specialists and other healthcare professionals The course intended to inform, educate and stimulate a debate regarding the value of this new intervention area and of patient's participation in the decision process.	Oral presentation **Section 2: Infertility risk in oncology patients: the contribution of information for supporting the fertility preservation decision.**
IV Conference of the Center for Pharmaceutical Studies Center for Pharmaceutical Studies (CEF), Faculty of Pharmacy of the University of Coimbra November 2013	Researchers and academics in pharmaceutical sciences To disseminate research projects from the CPS	Poster presentation **Effects of antineoplastic therapy on fertility: contribution of new markers in risk identification and production of information to support the decision to preserve fertility**
5th APFH Oncology Meeting Portuguese Association of Hospital Pharmacists (APFH) May 2014	Hospital pharmacists To inform and raise awareness on the subject of infertility risks and FP options in cancer patients	Oral presentation **Cancer treatments and fertility**

Initiative	Target audience and objectives	Contribution
Public session of presentation of the project "Oncofertility: a new approach to young patients with cancer" Portuguese League Against Cancer in partnership with the Centre for Fertility Preservation and the Regional Health Administration of the Centre February 2015	Cancer care clinicians To highlight the importance of providing maximum information to cancer patients about the possibility of preserving their fertility and of their rapid referral to reproductive medicine specialists.	Oral presentation **Risk of infertility in cancer patients: Determinants and online tools**
Faculty Meetings Faculty of Pharmacy of Coimbra University May 2015	PhD students, Professors and Researchers from the Faculty of Pharmacy of Coimbra University	Oral presentation PhD project
SIF 2015 - 10th International Pharmacy Seminary Racine Institute September 2015	Portuguese and Brazilian Pharmacists To promote scientific and cultural exchange between pharmacists.	Oral presentation **(in)Fertility in Oncology**
IX NEF/AAC Scientific Congress "Cancer: From Prevention to Therapeutics" Students' Association of the Faculty of Pharmacy of Coimbra University (NEF/AAC) November 2015	Students, Professors and Researchers of the Faculty of Pharmacy of Coimbra University	Oral presentation **(in)Fertility in Oncology**
"It's time to talk about your fertility!" **The challenges of pediatric oncofertility** Reproductive Medicine and Pediatric Oncology Units of CHUC, EPE November 2016	Specialists and interns of Gynecology and Obstetrics, Pediatrics, Hematology and Oncology To highlight the importance of providing information to pediatric cancer patients and/or their parents about the possibility of FP and of their rapid referral to reproductive medicine specialists	Oral presentation **Why are we going to talk about my fertility? - The impact of cancer treatments on future fertility**

Chapter III

Factors associated with ovarian function recovery after chemotherapy for breast cancer: a systematic review and meta-analysis

Factors associated with ovarian function recovery after chemotherapy for breast cancer: a systematic review and meta-analysis.

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Abstract

Study question: Which factors related to patient, treatment or disease are associated with ovarian function recovery after chemotherapy in premenopausal women with breast cancer?

Summary answer: Younger age and GnRH agonist (GnRHa) administration during chemotherapy were significantly associated with menses recovery, but this recovery was less likely in patients exposed to taxanes.

What is already known: To date, published meta-analyses have only assessed GnRHa administration as a possible factor for ovarian function recovery, and their results were conflicting. Current guidelines present distinct recommendations regarding the use of GnRHa for fertility preservation in women with breast cancer.

Study design, size, and duration: A systematic review and meta-analysis of published studies in the English, Portuguese, French or Spanish languages (1990–2015), ongoing trials or completed trials (1990–2015) and conference proceedings (2000–2015) were performed.

Participants/materials, setting, and methods: We searched the Medline, Embase, Lilacs, Scielo, Toxline and DART databases, online trial registries and conference proceedings. Studies were eligible if they included premenopausal women with early breast cancer treated with chemotherapy, reported ovarian function recovery data and identified factor(s) associated with recovery. Two authors independently screened the studies, extracted data and assessed the risk of bias. an odds ratio (or) was estimated from the number of recovery events. a meta-analysis was conducted using a random-effects model.

Main results and the role of chance: Fifteen articles were included. Five different factors were analysed: younger age and baseline levels of anti-Mullerian hormone (patient-related factors), co-administration of GnRHa, addition of taxanes to anthracycline-based chemotherapy and addition of endocrine therapy to chemotherapy (treatment-related factors). Menses recovery was the most used marker. Younger age (≤40 years) and exposure to GnRHa were positively associated with menses recovery (or 6.07 and 2.03, respectively) but exposure to taxanes adversely affected recovery (or 0.49). Significant heterogeneity among studies was found.

Limitations, reasons for caution: A general limitation of the included studies is the use of menses as the main recovery marker. Regarding GnRHa, the substantial heterogeneity and conflicting results limit the interpretation of our results. Studies that use additional markers and have a longer follow-up are needed.

1. Introduction

Breast cancer is one of the most frequently diagnosed malignancies in young women worldwide. Approximately 1 in every 200 women will develop breast cancer before the age of 40 years (Jemal et al., 2010).

Adjuvant chemotherapy has significantly improved disease-free and overall survival, but a considerable number of these young patients eventually develop premature ovarian failure, which is one of the possible long-term adverse effects of chemotherapy regimens, especially when alkylating agents such as cyclophosphamide are included.

Consequently, many women with breast cancer may present with diminished reproductive potential after treatment.

In an attempt to overcome these effects, fertility preservation (FP) methods such as oocyte or ovarian tissue cryopreservation are available and can be offered but may not be suitable for all patients. Each method presents specific limitations, which makes FP a complex decision, particularly for very young patients or those presenting aggressive or hormonal-dependent disease.

Typically, the effects of chemotherapy on female reproductive potential have been analysed using amenorrhoea, but this may not be an accurate marker. Infertility, and also reduced ovarian reserve, is typically observed in women who resume menses after treatment with chemotherapy (Partridge et al., 2010). On the other hand, spontaneous pregnancies can still occur in women with chemotherapy induced amenorrhoea (Hamre et al., 2012; van der Kaaij et al., 2012).

Measures of ovarian reserve, including ultrasound (e.g. antral follicle count (AFC) and mean ovarian volume) and hormonal measures (e.g. anti-Müllerian hormone (AMH), oestradiol (E2) and FSH), are useful surrogate markers for fertility potential. AMH appears to be the most promising hormonal marker because its levels show low intercycle variation and correlate strongly with the number of antral follicles and follicle depletion at an earlier stage. The potential use of AFC as a surrogate measure of ovarian reserve in this population has also been demonstrated (Nelson, 2013).

The individual risk of infertility after treatment for women with breast cancer is difficult to estimate because it depends on several factors such as age, baseline fertility and the type and dose of chemotherapy received (Ben-Aharon and Shalgi, 2012). Owing to the influence of all of these variables, the incidence of amenorrhoea can vary from 30 to 70% (Kasum et al., 2014). Nevertheless, the effects on fertility can be reversible. A variable proportion of women recover their menstrual or ovarian function after presenting with amenorrhoea or menopausal hormone

levels during or shortly after chemotherapy (Hickey et al., 2009; Hamy et al., 2014). In this context, it will be important to identify the potential factors associated with that recovery because they can influence both treatment and FP decisions.

Until now, GnRH agonist (GnRHa) administration has been the only aspect evaluated in relation to ovarian function recovery (Vitek et al., 2014; Shen et al., 2015; Munhoz et al., 2016), even though the results of these studies were conflicting. Therefore, it is crucial to gather additional knowledge on GnRHa and the impact of other potential recovery factors.

The aim of this systematic review and meta-analysis was to identify factors related to patient, treatment or disease associated with ovarian function recovery after chemotherapy in young women with breast cancer.

2. Methods

This study was conducted in agreement with the guidelines of the Cochrane Collaboration, and our findings were reported according to the PRISMA statement (Moher et al., 2009).

Protocol and registration

The reviewers (C.S. and O.C.) and two mentors (T.A.-S. and A.C.R.R.) established the protocol for this systematic review, which was published in the International Prospective Register of Systematic Reviews (PROSPERO) in April 2015 (registration number CRD42015013494, available in http://www.crd.york.ac.uk/prospero/display_record.asp?ID=CRD42015013494).

Eligibility criteria

A number of eligibility criteria were established for the studies (PICOD methodology).

Population (P): Premenopausal women with early, non-metastatic (excluding axillae) breast cancer, who have developed amenorrhoea and/ or present decreased ovarian reserve after CT using anthracycline- or taxane-based regimens.

Intervention/exposure (I): Reported patient, treatment or disease-related factors (e.g. age, CT regimen and pre-chemotherapy ovarian reserve).

Comparators/controls (C): Non-exposed or differently exposed groups (e.g. different age, different chemotherapy regimens or patients not exposed to GnRHa).

Outcomes (O): Data on ovarian function recovery up to 2 years after the end of chemotherapy with at least one of the following markers: menstrual cycle recovery, increase in ovarian reserve markers to premenopausal levels, time for menses or ovarian reserve marker recovery, pregnancy and/or live birth.

Study design (D): Analytical studies including a comparison of groups exposed and not/differently exposed with any design (e.g. interventional or observational, prospective, retrospective or cross-sectional).

Literature search

The following databases were searched: MEDLINE (through PubMed), Embase, LILACS, Scielo, Toxline and DART. The search strategies were built using free terms or medical descriptors when available (e.g. MeSH terms) for each PICOD synonym (search equation used for PubMed is provided in Supplementary Data). The search was limited to articles published after 1990 because anthracycline-based chemotherapy regimens were introduced in the 1990 s (Verrill, 2009). One reviewer (C.S.) performed the electronic search, piloted it in PubMed MEDLINE (1990–2014) and adapted it to run in all the other databases (1990–2014). The last search was performed on 25 September 2014 with weekly automatic e-mail updates until the end of 2015. Online trial registries (i.e. the Cochrane Central Register of Controlled Trials and the World Health Organization International Clinical Trials Registry Platform) were searched for ongoing or completed trials with available results (1990–2014). The online conference proceedings of the American Society for Clinical Oncology (ASCO) annual meetings were searched from 2000 onwards.

Study selection

Studies were screened for inclusion over three phases, using EndNote®s oftware: we searched and deleted duplicates; two authors (C.S. and O.C.) independently assessed the results, screening first by title and then by abstract. When a title seemed relevant, the abstract was reviewed for eligibility; if any doubt remained, the full text of the article was retrieved and discussed. Arbitration by the third author was applied in cases of disagreement. Reasons for exclusion were recorded after full text screening.

Data collection

The data extraction form was validated by C.S. and O.C. and then pilot tested for feasibility and comprehensiveness with three studies. Minor consensual adjustments were made. The data were independently extracted by C.S. and O.C., and then compared and discussed until an agreement was reached. Two authors were contacted to obtain additional information regarding statistical data. We collected the following data: Study (authors, year, country); Methods (study design, duration of follow-up, inclusion and exclusion criteria, number of participants included/evaluated, outcomes of recovery assessed, exposure/intervention analysed as a factor); Participants (mean age, treatments) and Results (intervention/exposure associated with recovery, risk measure, significance).

Risk of bias assessment

For each study included, the risk of bias was independently assessed by C.S. and O.C. using the Cochrane Collaboration Tool for assessing risk of bias in randomized trials (Higgins et al., 2011) and the QUIPS Tool for Assessing Risk of Bias in Prognostic Factor Studies (Hayden et al., 2013)

to assess the risk of bias in RCTs and observational studies, respectively. Disagreements were discussed and solved by a third researcher (T.A.-S. or A.C.R.R.) when needed.

Data analysis

A study-specific odds ratio (OR) was estimated indirectly from the number of patients and the number of recovery events in each arm (exposed/not or differently exposed). If only a risk measure was reported with a measure of precision (confidence interval (CI) or standard error) it was used.

MedCalc Software® version 16.1 (https://www.medcalc.org/) was used for statistical calculations and forest-plot generation. Meta-analysis was conducted using a random-effects model. Statistical heterogeneity was assessed with a chi-square test.

3. Results

Search results

After removal of duplicates, a total of 5222 references were identified (Fig. 3.1). A total of 60 were selected for detailed review and 46 of these were excluded. Of the excluded studies, 17 did not assess any outcome of recovery, 11 had no comparator/control group, five did not identify any factor associated with recovery, four were published in Chinese or Czech, three included non-eligible participants, one was a letter to the editor and five did not provide sufficient data. We identified one additional article through saved search e-mail updates. A total of 15 articles were finally included, from which 13 were included in the meta-analysis (Fig. 3.1).

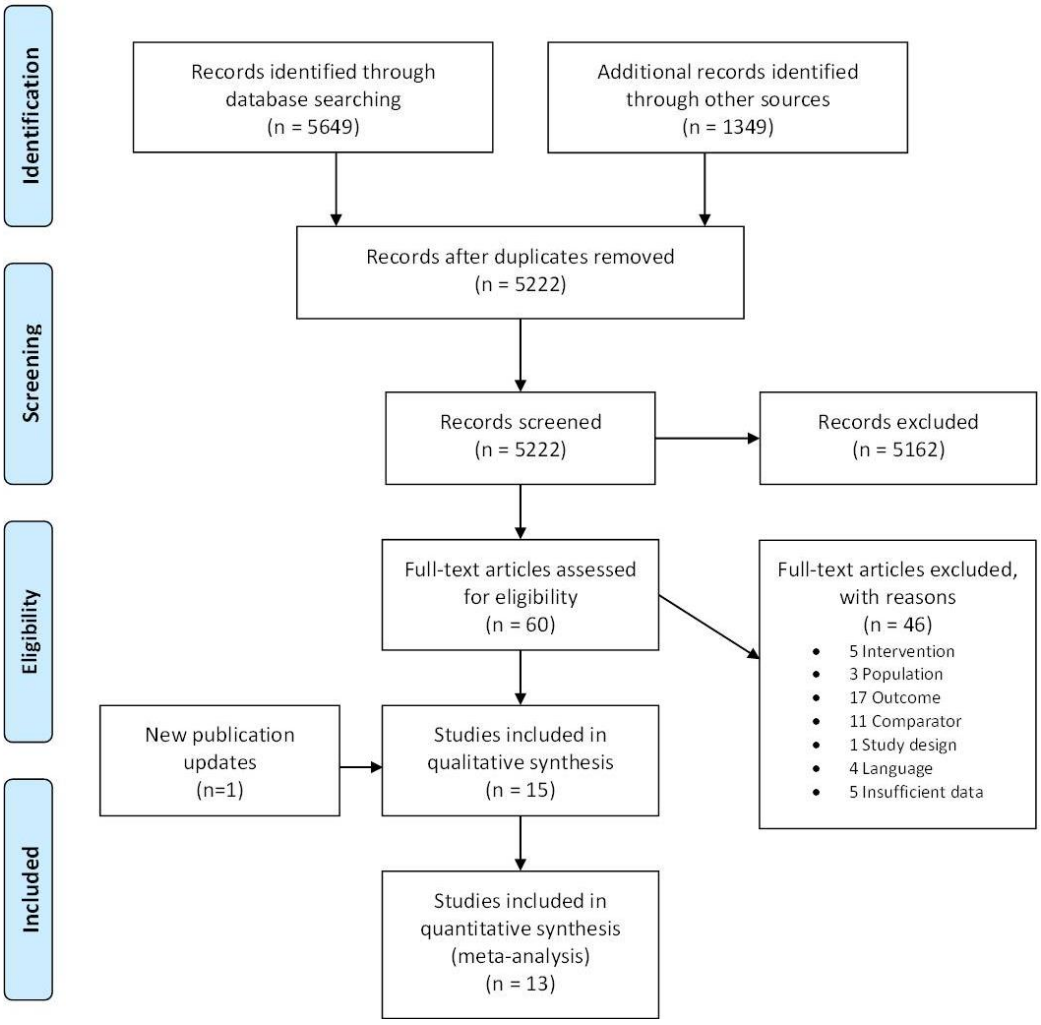

Figure 3.1 PRISMA flow diagram of the literature search.

Studies, risk factors and recovery outcomes

From the 15 studies, 8 were RCTs (Table 3.1) and 7 were observational studies (Table 3.2). Of these seven, four were retrospective and three had a prospective design. The general characteristics of the included studies are summarized in Tables 3.1 and 3.2.

Five different factors potentially associated with ovarian function recovery were subject to analysis in the included studies. The factors included young age and baseline levels of AMH (patient-related risk factors), co-administration of GnRHa, addition of taxanes to anthracycline-based chemotherapy regimens and addition of endocrine therapy (treatment-related factors). No studies assessed the influence of disease-related factors on ovarian function recovery. Four of the included studies assessed more than one risk factor (Table 3.3).

Menses recovery was the most frequently used marker but recovery time was also assessed in five studies. One study evaluated return of ovulation through ultrasound examination. Re-

occurrence of premenopausal FSH values and pregnancy rates was assessed in one and two studies, respectively.

Patient-related factors

Young age. In our meta-analysis, younger women (≤40 years) had a significantly higher rate of menses recovery after treatment (OR 6.07; 95% CI, 2.70–13.61; P < 0.001) (Fig. 3.2). Significant heterogeneity among studies was observed (I2 = 68.15%; P = 0.014). The study by Lee et al. (2009) also found a significantly shorter menses recovery time (P = 0.03) for younger women.

Baseline AMH. In a small prospective observational study (n = 27) by Henry et al. (2014), detectable levels of baseline AMH (≥0.16 ng/ml) were significantly associated with ovarian function recovery in univariate analysis (OR 108; 95%CI, 5.80–2.000; P < 0.002), but this association was not confirmed in multivariate analysis.

Treatment-related factors

Administration of GnRHa. (1) Administration of GnRHa and menses recovery: The administration of GnRHa was associated with a higher rate of menses recovery (OR 2.03; 95%CI, 1.18–3.47; P = 0.01) (Fig. 3.3) although a substantial heterogeneity among trials was observed (I2 = 60.91%; P = 0.018). Recovery time was assessed in four trials (Del Mastro et al., 2011; Gerber et al., 2011; Munster et al., 2012; Song et al., 2013) but significant differences were only found in the study by Del Mastro et al. (2011) (P = 0.07). (2) Administration of GnRH agonists and ovulation: In the RCT by Badawy et al. (2009), administration of GnRHa was associated with a higher rate of recovery of ovulation (OR 6.53; 95%CI, 2.43–17.55; P = 0.0002). (3) Administration of GnRH agonists and pregnancy: Two trials evaluated the association of GnRHa administration with pregnancy rate (Lambertini et al., 2015; Moore et al., 2015). Only the study by Moore et al. (2015) found a significant association (OR 2.23; 95%CI, 1.04–4.77; P = 0.034).

Addition of taxanes

(1) Addition of taxanes and menses recovery: Taxane exposure was a negative factor for recovery (OR 0.49; 95% CI, 0.30–0.80; P = 0.004) (Fig. 3.4). Women exposed to these agents showed a lower rate of menses recovery compared with women exposed to chemotherapy regimens without taxanes.

(2) Addition of taxanes and premenopausal hormone levels: In the study by Berliere et al. (2008), exposure to taxanes increased recovery of premenopausal FSH levels even though the effect was not significant (OR 1.63; 95% CI, 0.71–3.72; P = 0.247).

Addition of endocrine therapy. One retrospective study assessed the addition of endocrine therapy as a factor for recovery (Lee et al., 2009). Menses recovery was lower in patients exposed although this result was not significant (OR 0.67; 95% CI, 0.28–1.62; P = 0.38).

Risk of study bias

Global results for risk of bias concerning RCTs are presented in Fig. 3.5. In summary assessments, one trial was classified as 'low risk of bias', two were classified as 'unclear risk of bias' and four as 'high risk of bias'. For observational studies (Fig. 3.6), study participation, attrition and confounders were the domains in which more studies presented moderate or high risk of bias.

Table 3.1 Main characteristics of the eight included Randomized Controlled Trials (RCTs).

Study and country	Inclusion criteria	Follow-up (months)	Participants included/ evaluated	Mean age	Hormone-receptor status (n) HR+/HR-	Use of Tamoxifen (n)	Chemotherapy regimens	Marker(s) of recovery assessed	Exposure/Intervention (factor)
Badawy, A., et al. (2009); Egypt	18 - 40 years; premenopausal status with basal [FSH] levels <10 mIU/mL	8	80/78	Study group: 30,0 Control group: 29.2 (p=0,76)	Not reported	Not reported	FAC regimen (5-Fluorouracil 500 mg/m2 iv, Doxorubicin 500 mg/m2 iv, Cyclophosphamide 500 mg/m2 iv)	**Menses recovery** **Ovulation recovery**	**GnRH agonist (Goserelin)** 3,6 mg subcutaneous, 2 weeks before CT and then every 28 days for 6 months
Del Mastro, L., et al. (2011); Italy PROMISE-GIM6 study	18 - 45 years; stage I through III; premenopausal status with active menstrual cycles in 6 weeks before CT	12	281/260	Study group: 39 Control group: 39 (ns)	226/51	226	Anthracycline-based (n=118) Anthracycline plus taxane (n=151) CMF (n=12)	**Menses recovery** **Time to menses recovery**	**GnRH agonist (Triptorelin)** 3,75 mg intramuscular at least 1 week before starting CT and then every 4 weeks for the duration of the treatment

Study and country	Inclusion criteria	Follow-up (months)	Participants included/ evaluated	Mean age	Hormone-receptor status (n) HR+/HR-	Use of Tamoxifen (n)	Chemotherapy regimens	Marker(s) of recovery assessed	Exposure/Intervention (factor)
Elgindy, E. A., et al. (2013); Egypt	18 - 40 years; stage I – IIIa; history of regular menstrual periods	12	100/100	Study group1: 33.28 Control1: 32.32 Study group2: 33 Control2: 32.84 (ns)	**0/100**	**NA**	FAC regimen (5-flourouracil (500 mg/m2), adriamycin (50 mg/m2), and cyclophosphamide (500 mg/m2) IV every 21 days) for six cycles	**Menses recovery**	**GnRH agonist (Triptorelin)** 3.75 mg 1 week before and then every 4 weeks until the end of CT
Gerber, B., et al. (2011); Germany ZORO study	18 - 45 years; premenopausal status with regular and spontaneous menstrual periods and FSH below 15 mIU/mL in the follicular phase	24	60/52	Study group: 35,0 Control group: 38,5 (p=0,092)	**0/52**	**NA**	FEC + Docetaxel (n=10) EC + Docetaxel (n=7) FEC/FAC (n=28) TAC (n=13) Other (n=2)	**Menses recovery Time to menses recovery**	**GnRH agonist (Goserelin)** 3.6 mg at least 2 weeks before start of CT and then every 4 weeks until the end of CT

Study and country	Inclusion criteria	Follow-up (months)	Participants included/ evaluated	Mean age	Hormone-receptor status (n) HR+/HR-	Use of Tamoxifen (n)	Chemotherapy regimens	Marker(s) of recovery assessed	Exposure/Intervention (factor)
Munster, P. N., et al. (2012); USA	<45 years; FSH level less than 40 mIU/mL and at least two menstrual periods in the preceding 6 months.	18	49/47	Study group: 39 (median) Control group: 38 (median) (p=0,99)	**36/32**	**36**	AC, 4 cycles (n=23) AC, 4 cycles, then taxane, 4 cycles (n=13) FEC/FAC, 6 cycles (n=13); ER+ tumours: tamoxifen for 5 years (n=36)	**Menses recovery Time to menses recovery**	**GnRH agonist (Triptorelin)** administered every 28 to 30 days by IM injection starting no sooner than 4 weeks and at least 7 days before the first cycle of CT and continued throughout CT duration
Lambertini M. (2015); Italy	18 - 45 years; stage I to III; premenopausal (menstrual cycles/normal menses during the 6 weeks before the start of CT	87	281/246	Study group: 39 Control group: 39 (ns)	**226/51**	**182**	Anthracycline plus taxane-based (n=148) Anthracycline-based (n=113) CMF (n=12)	**Menses recovery Pregnancy rate**	**GnRH agonist (Triptorelin)** 3.75 mg administered intramuscularly at least 1 week before chemotherapy and then every 4 weeks for the duration of CT

Study and country	Inclusion criteria	Follow-up (months)	Participants included/ evaluated	Mean age	Hormone-receptor status (n) HR+/HR-	Use of Tamoxifen (n)	Chemotherapy regimens	Marker(s) of recovery assessed	Exposure/Intervention (factor)
Song, G., et al. (2013); China	18 - 45 years; histological stage I–III; premenopausal status with basal FSH<10 mIU/ml and regular menstrual cycle	12	220/183	Study group: 40.3 Control group: 42.1 (p>0,05)	**150/33**	**150**	AC for 6 cycles (600 mg/m2 cyclophosphamide and 60 mg/m2 doxorubicin) (n=126) AC + Paclitaxel for 4 cycles (n=57) Tamoxifen was given to all patients with HR+ tumours (n=150)	**Menses recovery Time to menses recovery**	**GnRH agonist (Leuprorelin)** subcutaneous injections of 3.75 mg, before CT until ovarian suppression; during CT, patients were given leuprolide acetate at same dosage every 4 weeks.
Moore, H. C. F., et al. (2015); USA POEMS study	18 - 49 years; operable stage I to IIIA; premenopausal	48	257/218	Study group: 37,6 Control group: 38,7	**0/257**	**Not Applicable**	3–4 cycles of anthracycline-based therapy (ABT) (n=46); 3–4 cycles of non-ABT (n=12); 6–8 cycles of ABT (n=152) 6–8 cycles of non-ABT (n=8)	**Pregnancy rate**	**GnRH agonist (Goserelin)** 3.6 mg subcutaneously every 4 weeks beginning 1 week before CT and continued to within 2 weeks before or after the final CT dose.

Abbreviations: AC, doxorubicin (adriamycin) and cyclophosphamide; ACT, doxorubicin (adriamycin), cyclophosphamide and taxane; AI, aromatase inhibitor; AMH, anti-Müllerian hormone; BSO, bilateral salpingo-oophorectomy; CMF, cyclophosphamide, methotrexate and 5-flourouracil; CRA, chemotherapy-related amenorrhea; CT, chemotherapy; dd, dose-dense; EC, epirubicin and cyclophosphamide; ER, estrogen receptor; FAC/FEC, 5-flourouracil, doxorubicin (adriamycin)/epirubicin and cyclophosphamide; FSH, follicle-Stimulating Hormone; GnRH, gonadotropin-releasing hormone; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; ns, non-significant; T, taxane; TAC/TEC, taxane, doxorubicin (adriamycin)/epirubicin and cyclophosphamide.

Table 3.2 Main characteristics of the seven included observational studies.

Study	Study design (follow-up)	Inclusion criteria	Number participants included/ evaluated	Mean age (significance)	Hormone receptor status (n) HR+/HR-	Use of Tamoxifen (n)	Chemotherapy regimens	Markers of recovery assessed	Exposure/ Intervention (factor)
Berliere, M., et al. (2008); Belgium and France	Prospective controlled (12 months follow-up)	Premenopausal patients with node-positive operable breast cancer	154/154 (101 for hormonal values)	Study group: 44 Control group: 43.5	112/42	112		**Menses recovery Premenopausal FSH (FSH <45.7 mIU/mL)**	Addition of taxanes to anthracycline-based chemotherapy regimens (6FEC vs 3FEC+3Docetaxel) Younger age (≥ or < 40 years)
Henry, N. L., et al. (2014); USA	Prospective (12 months follow-up; 13.6 on average)	25–50 years; one menstrual cycle within 3 months prior to study entry; stage I–III	29/27 4 age cohorts: 25–34 years (n=6) 35–39 years (n=8) 40–44 years (n=7) 45–50 years (n= 8)	Median of 41	21/8	14 (AI plus GnRH agonist or BSO in 6)	ddAC or ddAC- ddpaclitaxel or ddAC-weekly paclitaxel or Docetaxel/ cyclophosphamide or Docetaxel/carboplatin Trastuzumab n=12 AI plus GnRH agonist or BSO n=6	Menses recovery and/or Oestradiol > 10 pg/mL	Baseline levels of AMH and inhibin B
armadha, M. P., et al. (2012); India	Retrospective	Premenopausal women; hormone sensitive early breast cancer	50 FAC/FEC (n=36) TAC/TEC (n=14)	FAC/FEC group: 41 TAC/TEC group: 38 (ns)	50/0	50	All patients received endocrine adjuvant therapy with tamoxifen.	Menses recovery	Addition of taxanes to anthracycline-based chemotherapy regimens (FAC/FEC x 6 *versus* TAC/TEC x 6) Younger age (≥ or <40 years)

Study	Study design (follow-up)	Inclusion criteria	Number participants included/ evaluated	Mean age (significance)	Hormone receptor status (n) HR+/HR-	Use of Tamoxifen (n)	Chemotherapy regimens	Markers of recovery assessed	Exposure/ Intervention (factor)
Perez-Fidalgo, J. A., et al. (2010); Spain	Retrospective	Pre- or peri-menopausal women, diagnosed with hormone-sensitive early breast cancer	305 (Anthracyclines group n=212; Anthracyclines and taxanes group n=93)	Anthracyclines group: 44 (29–53) Anthracyclines and taxanes group: 43 (29–53) (ns)	305/0	305	Anthracyclines group: ACx6/FACx6 Anthracyclines and taxanes group: FACx4+Paclitaxelx8w/ATx4+ CMFx4/TACx6 All patients received adjuvant endocrine therapy.	Menses recovery	Younger age (< 40, 40-45 or >45 years)
Reh, A., et al. (2008); USA	Prospective (mean of 28 months of follow-up)	Women who had a history of breast cancer of stages I-IIIa	45 (AC group n=28; ACT group n=17)/ 25 in second follow-up	ACT group: 38.4 ±5.6 AC Group: 41.4 ±3.3 (ns)	Not reported	Not reported		Menses recovery	Addition of taxanes to anthracycline-based chemotherapy regimens (AC *versus* ACT)
Tham, Y. L., et al. (2007); USA	Retrospective	Less than 50 at the start of chemotherapy; premenopausal	191	≤ 40 years; n=157 > 40 years; n= 34	Not reported	106		Menses recovery	Addition of taxanes to anthracycline-based chemotherapy regimens (AC *versus* ACT) Younger age (≤ 40 years)

Study	Study design (follow-up)	Inclusion criteria	Number participants included/ evaluated	Mean age (significance)	Hormone receptor status (n) HR+/HR-	Use of Tamoxifen (n)	Chemotherapy regimens	Markers of recovery assessed	Exposure/ Intervention (factor)
Lee, S., et al. (2009); Korea	Retrospective	Premenopausal, newly diagnosed with localized breast carcinoma and less than 50 at time of diagnosis	326 <40 n=128 ≥40 n=198	42 (22-50)	246/78	238 (adjuvant endocrine therapy, not specified)	CT with and without taxanes; some received tamoxifen	Temporary CRA Time to menses recovery	Addition of endocrine therapy to chemotherapy Younger age (≥ or < 40 years)

Abbreviations: AC, doxorubicin and cyclophosphamide; ACT, doxorubicin, cyclophosphamide and taxane; AI, aromatase inhibitor; AMH, anti-Müllerian hormone; BSO, bilateral salpingo-oophorectomy; CMF, cyclophosphamide, methotrexate and 5-flourouracil; CRA, chemotherapy-related amenorrhea; CT, chemotherapy; dd, dose-dense; EC, epirubicin and cyclophosphamide; ER, estrogen receptor; FAC/FEC, 5-flourouracil, adriamycin/epirubicin and cyclophosphamide; FSH, follicle-Stimulating Hormone; GnRH, gonadotropin-releasing hormone; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; ns, non-significant; T, taxane; TAC/TEC, taxane, adriamycin/epirubicin and cyclophosphamide.

Table 3.3 Factors associated with recovery and markers assessed in the included studies.

Factor	Recovery marker	Studies
Administration of GnRH agonist	Menses recovery	Badawy (2009) Del Mastro (2011) Elgindy (2013) Gerber (2011) Munster (2012) Song (2013) Lambertini (2015)
	Ovulation	Badawy (2009)
	Time to menses recovery	Del Mastro (2011) Gerber (2011) Munster (2012) Song (2013)
	Pregnancy rate	Moore (2015) Lambertini (2015)
Addition of taxanes	Menses recovery	Berliere (2008) Narmadha (2012) Reh (2008) Tham (2007)
	Premenopausal hormone values	Berliere (2008)
Younger age	Menses recovery	Narmadha (2012) Pérez-Fidalgo (2010) Tham (2007) Lee (2009) Berliere (2008)
	Premenopausal hormone values	Berliere (2008)
	Time to menses recovery	Lee (2009)
Addition of endocrine therapy	Menses recovery	Lee (2009)
Baseline AMH	Menses recovery	Henry (2014)

	GnRH agonist		Control		Odds Ratio (OR) 95% (CI)
	Events	Total	Events	Total	
Badawy et al, 2009	35	39	13	39	17.50 (5.11-59.88)
Del Mastro et al, 2011	88	139	60	121	1.75 (1.07-2.88)
Elgindy et al, 2013	41	46	40	47	1.44 (0,42-4.90)
Gerber et al, 2011	28	30	28	30	1.00 (0.13-7.60)
Munster et al, 2012	23	26	19	21	0.81 (0.12-5.34)
Song et al, 2013	53	89	39	94	2.08 (1.15-3.74)
Lambertini et al, 2015	116	148	96	133	1.40 (0.81-2.41)
Total		517		485	2.03 (1.18 to 3.47)
Total Events	384		295		
Heterogeneity (I^2)	60.91 (p=0.018)				
Test for overall effect	Z=2.58 (p=0.01)				

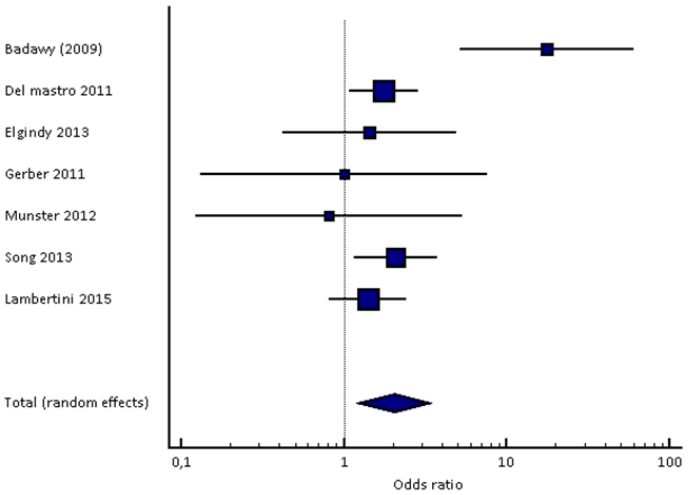

Figure 3.2 Younger age (≤40 years) and menses recovery.

	Study group		Control		Odds Ratio (OR) (95% CI)
	Events	Total	Events	Total	
Narmadha et al., 2012	11	15	8	26	6.19 (1.50-25,48)
Pérez-Fidalgo et al., 2010	10	39	5	198	13.30 (4.25-41.71)
Tham et al., 2007	35	87	5	28	3.10 (1.07-8.92)
Lee et al., 2009	13	55	19	168	2.42 (1.11-5.32)
Berliere et al., 2008	28	39	17	115	14.67 (6.17 to 34.92)
Total		196		420	6.07 (2.70 to 13.61)
Total Events	69		37		
Heterogeneity (I^2)	68.15 (p=0.014)				
Test for overall effect	Z=4.37 (p<0,001)				

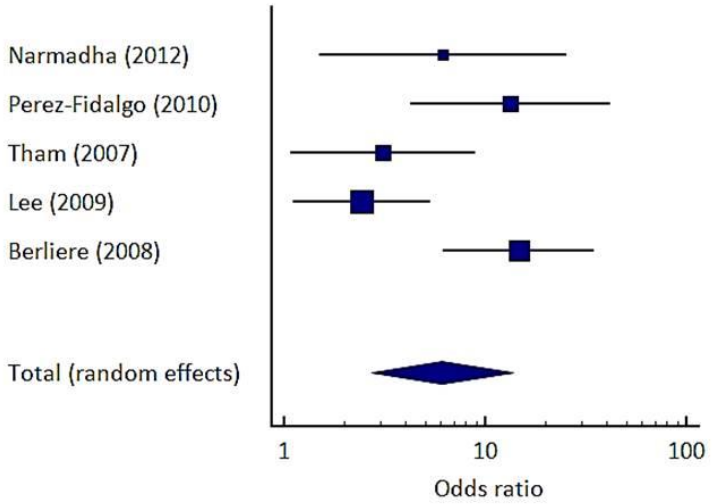

Figure 3.3 GnRH agonist exposure and menses recovery.

	Study group		Control		Odds Ratio (OR) 95% (CI)
	Events	Total	Events	Total	
Berliere et al, 2008	25	70	20	84	0.52 (0.25 to 1.05)
Tham et al, 2007	28	74	12	41	0.36 (0.14 to 0.92)
Narmadha et al, 2012	5	14	14	27	0.52 (0.14 to 1.94)
Reh et al, 2008	13	23	9	14	0.72 (0.18 to 2.84)
Total		181		166	0.49 (0,30 to 0,80)
Total Events	71		55		
Heterogeneity (I^2)	0,00 (p=0.857)				
Test for overall effect	Z=2.58 (p=0.004)				

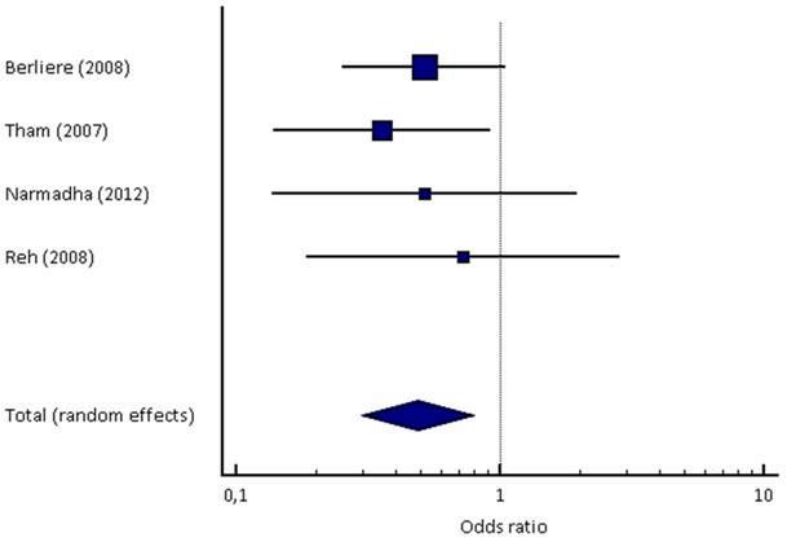

Figure 3.4 Taxane exposure and menses recovery.

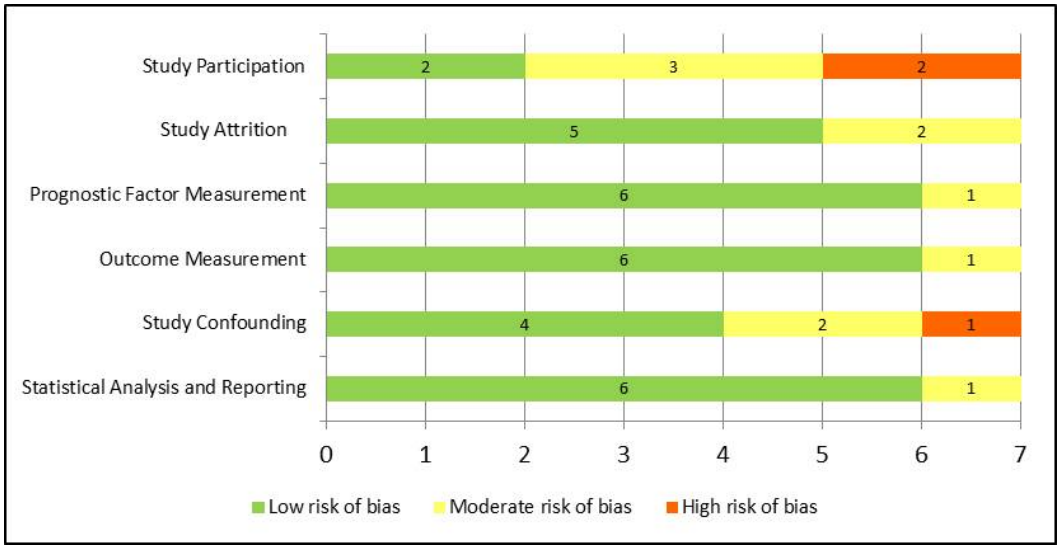

Figure 3.5 Risk of bias assessment in the included RCTs.

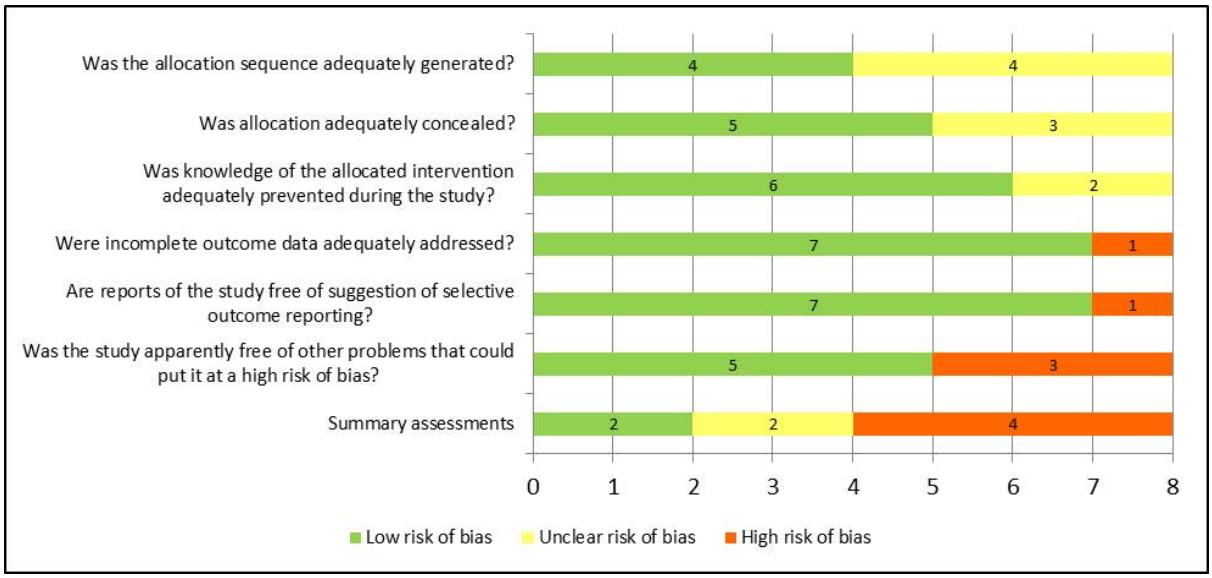

Figure 3.6 Risk of bias assessment in the included observational studies.

4. Discussion

Our results confirmed that younger age (<40 years) is a major factor for recovery of ovarian function in women who received CT for breast cancer because this factor was the most significant factor for menses recovery (pooled OR of 6.07; Fig. 3.2).

The use of GnRHa was also identified as a significant factor for menses recovery regardless of type of agonist used. However, we found substantial heterogeneity across studies (I2 = 60.91%, P = 0.0177) (Fig. 3.3). Clinical and methodological sources of heterogeneity were identified and limited the applicability of results. For instance, it is not clear which patient subgroups for hormone receptor status could benefit most from this intervention. In addition, inconsistency was evident concerning the definitions of ovarian dysfunction and recovery as well as time for recovery assessment. For GnRHa exposure, other markers of recovery were evaluated. Although the return of ovulation was significantly higher in women exposed to GnRHa (Badawy et al., 2009), the results for pregnancy and recovery time outcomes were conflicting. Some studies reported a significant beneficial effect of this exposure on ovarian function recovery (Song et al., 2013; Moore et al., 2015), but others did not (Del Mastro et al., 2011; Gerber et al., 2011; Munster et al., 2012; Lambertini et al., 2015). The published consensus statements and guidelines also include distinct recommendations regarding the use of GnRHa for FP. The latest guidelines from ASCO (Loren et al., 2013) and the European Society for Medical Oncology (Peccatori et al., 2013) still consider this approach to be experimental, but St. Gallen's consensus recommends GnRHa for premenopausal women with estrogen receptor-negative tumours (Coates et al., 2015), and another consensus states that GnRHa administration is a reliable strategy to preserve ovarian function and fertility (Lambertini et al., 2016). Considering the conflicting results regarding GnRHa use, other FP strategies should also be considered.

Another factor that was assessed was the addition of taxanes (Berliere et al., 2008; Tham et al., 2007; Reh et al., 2008; Narmadha et al., 2012). Despite the possible advantage of a lower number of cycles with cyclophosphamide, taxane exposure was not associated with an increase in menses recovery. Meta-analysis results for this outcome indicate that taxane exposure can inclusively be regarded as a negative factor for recovery (OR 0.488; 95% CI, 0.299–0.796; P = 0.004) (Fig. 3.4). In contrast, the only study evaluating recovery of premenopausal FSH levels (Berliere et al., 2008) showed that exposure to taxanes increased recovery, although the effect was not significant and the number of patients assessed for this outcome was low.

Only one small study (Henry et al., 2014) evaluated baseline AMH as a factor for menses recovery. AMH is known to be an important marker of ovarian reserve and is considered a promising predictor of future fertility in young cancer patients (Broer et al., 2014; Dewailly et al., 2014). However, the very small sample may have limited the statistical power for detecting

associations in multivariate analysis and did not allow for the identification of a cut-off value for recovery. Other indirect ovarian reserve markers, such as AFC, were rarely assessed.

Concerning endocrine therapy exposure, more data from prospective controlled studies are needed. In the study by Lee et al. (2009), the type of endocrine therapy used was not uniform, and the short duration of follow-up may have underestimated recovery in the exposed group.

To the best of our knowledge, this is the first published study that evaluates a broad range of potential factors associated with ovarian function recovery. Our search was not restricted to any particular factor but intended to find every published study assessing one or more factors related to recovery. We believe that the identification of these factors will be important to support counselling for young breast cancer patients regarding their infertility risks and available FP options.

Other publications on this subject only assessed the impact of the addition of GnRHa to chemotherapy (Vitek et al., 2014; Shen et al., 2015; Munhoz et al., 2016). Similar to our results, two of them (Shen et al., 2015; Munhoz et al., 2016) have shown a higher rate of menses recovery with GnRHa. However, in the study by Shen et al. (2015), the

effect was only significant in the subgroups of older (age >35 years) and hormone receptor-negative patients. In a review by Vitek et al. (2014), which included only breast cancer patients who did not receive tamoxifen, no significant differences in the rate of menses recovery were found. In the study by Munhoz et al. (2016), the beneficial effect was confirmed by a higher number of pregnancies in the group exposed to GnRHa (OR 1.85; 95% CI, 1.02–3.36; P = 0.04). However, as stated by the authors, this outcome was not a primary end point for the included studies, and the number of attempted pregnancies was not uniformly reported.

There are some potential limitations to our review. Our search did not include unpublished literature and some articles were excluded because they were written in languages such as Chinese or Czech. Concerning the meta-analysis, the substantial heterogeneity limits the interpretation of results. Furthermore, we did not have access to individual patient information. Limitations were also found in most of the included studies. Recovery was mainly evaluated using menses, which is a marker that is not considered reliable for ovarian function and fertility assessment. Diverse definitions for amenorrhoea and menses recovery were used across studies. Moreover, when other markers, such as FSH or LH values, were assessed the data were often incomplete. It would be important in future studies to include additional markers, such as AMH and AFC. Pregnancy was also used as a marker but not always as a primary end point. Moreover, the number of attempted pregnancies and the pregnancy outcomes were not consistently reported.

Regarding study design, potential methodological limitations were found. The observational studies were mainly retrospective. Only one was a large prospective controlled study (Berliere et al., 2008).

Furthermore, participation bias (small samples) and attrition bias (patients lost to follow-up) were seen in prospective studies and the presence of confounders, i.e. unbalanced age groups or heterogeneous CT regimens, was also found. Other limitations affected the interpretation of the results. Some studies (Gerber et al., 2011; Elgindy et al., 2013; Moore et al., 2015) included only hormone receptor negative patients and eliminated the potential confounding effect of tamoxifen but many others included both hormone receptor-positive and -negative patients or this information was absent (Tham et al., 2007; Reh et al., 2008; Badawy et al., 2009). Concerning patient's age, in the studies by Song et al. (2013), Berliere et al. (2008), Munster et al. (2012) and Pérez-Fidalgo et al. (2010), the mean patient age was 41, 44, 45 and 43 years, respectively, which may have contributed to diminished recovery. Furthermore, five of the included studies followed patients for 12 months or less. Longer follow-up times may be needed to appropriately assess recovery.

A high risk of bias was found in four RCTs, including lack of outcome data, selective outcome reporting and other, such as the absence of hormone receptor or endocrine therapy status (Fig. 3.5). Additionally, most studies did not assess other factors that may influence fertility, such as smoking or BMI.

Conclusion

An age <40 years was the most relevant factor associated with ovarian function recovery after chemotherapy for breast cancer. Our results favoured the use of GnRHa for FP but we have to keep in mind the substantial heterogeneity and conflicting results for some recovery markers. Therefore, other FP strategies should also be considered in premenopausal women with breast cancer. Concerning chemotherapy regimens, our results showed that the decision to use taxanes must take into account potential adverse effects on female fertility.

Additional trials are needed to reach definitive conclusions about factors such as pre-chemotherapy AMH levels, different chemotherapy regimens and endocrine therapy in recovery. Furthermore, future studies should track ovarian function recovery over longer time periods and consider the use of ovarian reserve markers.

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Authors' roles

O.C. made substantial contributions to the acquisition, analysis or interpretation of data, writing the manuscript, critical revision and final approval; T.A.-S. made substantial contributions to the conception and design of the study as well as analysis and interpretation of data, critical revision of the manuscript and final approval; A.C.R.R. made substantial contributions to the conception and design of the study and analysis as well as interpretation of data, critical revision of the manuscript and final approval.

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Conflict of interest

The authors have no conflicts of interest to declare.

Supplementary File I

Search Strategy in PubMed MEDLINE

((("breast cancer"[Title/Abstract] OR "breast neoplasm"[Title/Abstract] OR "breast carcinoma"[Title/Abstract] OR "breast tumor"[Title/Abstract] OR "breast tumour"[Title/Abstract] OR breast neoplasm[MeSH])) AND (chemotherapy[Text Word] OR antineoplastic[Text Word] OR "Antineoplastic Agents"[Mesh] OR "Antineoplastic Combined Chemotherapy Protocols"[Mesh])) AND ((amenorrhea[Text Word] OR "Amenorrhea"[Mesh] OR "Primary Ovarian Insufficiency"[Mesh] OR menstruation OR menses OR menstrual OR "Menstruation"[Mesh] OR "Menstrual cycle"[Mesh] OR "Menopause, Premature"[Mesh] OR "ovarian function" OR "ovary function" OR ovarian reserve OR ovarian markers OR "ovary failure" OR "ovarian failure" OR Ovary/drug effects[MeSH] OR Ovarian diseases/chemically induced[MeSH] OR "Ovary/injuries"[Mesh] OR "Ovary/physiology"[Mesh] OR mullerian OR "antral follicle" OR Ovarian Function Tests[Mesh] OR "Follicle Stimulating Hormone"[Mesh] OR "Anti-Mullerian Hormone"[Mesh] OR conception OR motherhood OR offspring OR Pregnancy[MeSH] OR Pregnancy rate[MeSH] OR "Time-to-Pregnancy"[Mesh] OR "Fertility"[Mesh] OR "Infertility"[Mesh] OR "Live Birth"[Mesh])). Publication date from 1990/01/01

References

Badawy A, Elnashar A, El-Ashry M, Shahat M. Gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage: prospective randomized study. Fertil Steril 2009;91:694–697.

Ben-Aharon I, Shalgi R. What lies behind chemotherapy-induced ovarian toxicity? Reproduction 2012;14:153–163.

Berliere M, Dalenc F, Malingret N, Vindevogel A, Piette P, Roche H, Donnez J, Symann M, Kerger J, Machiels JP. Incidence of reversible amenorrhea in women with breast cancer undergoing adjuvant anthracycline-based chemotherapy with or without docetaxel. BMC Cancer 2008;8:56.

Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 2014;20:688–701. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, Thürlimann B, Senn HJ, Panel Members. Tailoring therapies–improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol 2015;26:1533–1546.

Del Mastro L, Boni L, Michelotti A, Gamucci T, Olmeo N, Gori S, Giordano M, Garrone O, Pronzato P, Bighin C et al. Effect of the gonadotropin-releasing hormone analogue triptorelin on the occurrence of chemotherapy-induced early menopause in premenopausal women with breast cancer: a randomized trial. JAMA 2011;306:269–276.

Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La Marca A, Lambalk C et al. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 2014;20:370–385.

Elgindy EA, El-Haieg DO, Khorshid OM, Ismail EI, Abdelgawad M, Sallam HN, Abou-Setta AM. Gonadatrophin suppression to prevent chemotherapy-induced ovarian damage: a randomized controlled trial. Obstet Gynecol 2013;121:78–86.

Gerber B, von Minckwitz G, Stehle H, Reimer T, Felberbaum R, Maass N, Fischer D, Sommer HL, Conrad B, Ortmann O et al. Effect of luteinizing hormone-releasing hormone agonist on ovarian function after modern adjuvant breast cancer chemotherapy: the GBG 37 ZORO study J ClinOncol 2011;29:2334–2341.

Hamy AS, Porcher R, Cuvier C, Giacchetti S, Schlageter MH, Coussieu C, Gronier H, Feugeas JP, Adoui N, Lacorte JM et al. Ovarian reserve in breast cancer: assessment with anti-Müllerian hormone. Reprod Biomed Online 2014;29:573–580.

Hamre H, Kiserud CE, Ruud E, Thorsby PM, Fossa SD. Gonadal function and parenthood 20 years after treatment for childhood lymphoma: a cross-sectional study. Pediatr Blood Cancer 2012;59:271–277.

Hayden JA, Danielle A, Jennifer LC, Pierre C, Claire B Assessing bias in studies of prognostic factors Ann Intern Med 2013;158:280–286.

Henry NL, Xia R, Schott AF, McConnell D, Banerjee M, Hayes DF. Prediction of postchemotherapy ovarian function using markers of ovarian reserve. Oncologist 2014;19:68–74.

Hickey M, Peate M, Saunders CM, Friedlander M. Breast cancer in young women and its impact on reproductive function. Hum Reprod Update 2009;15:323–339.

Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials BMJ 2011;343:d5928.

Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277–300.

Kasum M, Beketić-Orešković L, Peddi PF, Orešković S, Johnson RH. Fertility after breast cancer treatment. Eur J Obstet Gynecol Reprod Biol 2014;173:13–18.

Lambertini M, Boni L, Michelotti A, Gamucci T, Scotto T, Gori S, Giordano M, Garrone O, Levaggi A, Poggio F et al. Ovarian suppression with triptorelin during adjuvant breast cancer chemotherapy and long-term ovarian function, pregnancies, and disease-free survival: a randomized clinical trial. JAMA 2015;314:2632–2640.

Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim HAJr, Peccatori FA, Costa M, Revelli A, Salvagno F, Gennari A. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med 2016;14:1.

Lee S, Kil WJ, Chun M, Jung YS, Kang SY, Kang SH, Oh YT. Chemotherapy-related amenorrhea in premenopausal women with breast cancer. Menopause 2009;16:98–103.

Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K, American Society of Clinical Oncology. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500–2510.

Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009;151:264–269, w64.

Moore HC, Unger JM, Phillips KA, Boyle F, Hitre E, Porter D, Francis PA, Goldstein LJ, Gomez HL, Vallejos CS et al. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy N Engl J Med 2015; 372:923–932.

Munhoz RR, Pereira AA, Sasse AD, Hoff PM, Traina TA, Hudis CA, Marques RJ. Gonadotropin-releasing hormone agonists for ovarian function preservation in premenopausal women undergoing chemotherapy for early-stage breast cancer: a systematic review and meta-analysis. JAMA Oncol 2016;2:65–73.

Munster PN, Moore AP, Ismail-Khan R, Cox CE, Lacevic M, Gross-King M, Xu P, Carter WB, Minton SE. Randomized trial using gonadotropin-releasing hormone agonist triptorelin for the preservation of ovarian function during (neo)adjuvant chemotherapy for breast cancer. J Clin Oncol 2012;30:533–538.

Narmadha MP, Malaikkal V, Rajendran NN. Assessment of chemotherapy induced amenorrhea (CIA) in hormone receptor positive premenopausal women with breast cancer. Res J Pharm Biol Chem Sci 2012;3:97–106.

Nelson SM. Biomarkers of ovarian response: current and future applications. Fertil Steril 2013;99:963–969.

Partridge AH, Ruddy KJ, Gelber S, Schapira L, Abusief M, Meyer M, Ginsburg E. Ovarian reserve in women who remain premenopausal after chemotherapy for early stage breast cancer. Fertil Steril 2010;94:638–644.

Peccatori FA, Azim HAJr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G, ESMO Guidelines Working Group. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24:160–170.

Pérez-Fidalgo JA, Roselló S, García-Garré E, Jordá E, Martín-Martorell P, Bermejo B, Chirivella I, Guzman C, Lluch A. Incidence of chemotherapy-induced amenorrhea in hormone-sensitive breast cancer patients: the impact of addition of taxanes to anthracycline-based regimens.

Breast Cancer Res Treat 2010;120:245–251.

Reh A, Oktem O, Oktay K. Impact of breast cancer chemotherapy on ovarian reserve: a prospective observational analysis by menstrual history and ovarian reserve markers. Fertil Steril 2008;90:1635–1639.

Shen YW, Zhang XM, Lv M, Chen L, Qin TJ, Wang F, Yang J, Liu PJ, Yang J. Utility of gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage in premenopausal women with breast cancer: a systematic review and meta-analysis. Onco Targets Ther 2015;8:3349–3359.

Song G, Gao H, Yuan Z. Effect of leuprolide acetate on ovarian function after cyclophosphamide-doxorubicin-based chemotherapy in premenopausal patients with breast cancer: results from a phase II randomized trial. Med Oncol 2013;30:667.

Tham YL, Sexton K, Weiss H, Elledge R, Friedman LC, Kramer R. The rates of chemotherapy-induced amenorrhea in patients treated with adjuvant doxorubicin and cyclophosphamide followed by a taxane. Am J Clin Oncol 2007;30:126–132.

van der Kaaij MA, Heutte N, Meijnders P, Abeilard-Lemoisson E, Spina M, Moser EC, Allgeier A, Meulemans B, Simons AH, Lugtenburg PJ et al.

Premature ovarian failure and fertility in long-term survivors of Hodgkin's lymphoma: a European Organisation for Research and Treatment of Cancer Lymphoma Group and Groupe d'Etude des Lymphomes de l'Adulte Cohort Study. J ClinOncol 2012;30:291–299.

Verrill M. Chemotherapy for early-stage breast cancer: a brief history. Br J Cancer 2009;101:S2–S5.

Vitek WS, Shayne M, Hoeger K, Han Y, Messing S, Fung C. Gonadotropin-releasing hormone agonists for the preservation of ovarian function among women with breast cancer who did not use tamoxifen after chemotherapy: a systematic review and meta-analysis. Fertil Steril 2014;102:808–815.

Chapter IV

Adverse reproductive health outcomes in a cohort of young breast cancer women exposed to chemotherapy

Adverse reproductive health outcomes in a cohort of young breast cancer women exposed to chemotherapy

1. Introduction

Breast cancer (BC) is the second most frequent cancer in the world and by far the most frequent cancer among women, with an estimated 1.67 million new diagnoses in 2012 (International Agency for Research on Cancer 2012). Although most cases are diagnosed in women older than 40 years of age, it is also the leading type of cancer in younger women, with an incidence around 6.6%, being also the most lethal (Assi, Khoury et al. 2013, Ribnikar, Ribeiro et al. 2015). BC in young women is more likely to be of a more aggressive subtype, such as triple negative or HER_2-positive BC and to be diagnosed at a more advanced stage(Assi, Khoury et al. 2013). Still, in women under the age of 40 with BC, survival rates range from 72-85% (Anders, Johnson et al. 2009, UK Cancer Research 2013). Young BC women are often treated with first-line (neo) adjuvant chemotherapy (CT) regimens that include gonadotoxic agents, like cyclophosphamide and anthracyclines. Additionally, because the mean age of women at childbirth has been increasing in recent decades (OECD - Social Policy Division - Directorate of Employment 2015), it is now more likely that a cancer diagnosis occurs in childless women. Thus, BC survivors have to deal with reproductive concerns and engage in family planning decisions, more and more often.

The identification of (in)fertility in cancer patients has been traditionally based on the presence or absence of amenorrhea. This clinical surrogate marker is currently known to be a poor and late marker of damaged ovarian function (Ruddy and Partridge 2012). Female reproductive potential is mainly dictated by the ovarian reserve (OR), *i.e.* the pool of non-growing (primordial) ovarian follicles present in the ovaries at a specific time point. Although it is not possible to directly measure the OR, various indirect markers are available. The Anti-Mullerian Hormone (AMH), produced by the granulosa cells of growing follicles and the Antral Follicle Count (AFC), assessed by intravaginal ultrasound, are highly inter-correlated measures and are currently recognized as the most specific surrogate markers of OR (Partridge, Ruddy et al. 2010, Su, Chung et al. 2011, Nelson 2013). In comparison to other endocrine markers such as follicle-stimulating hormone (FSH) and oestradiol, AMH presents the additional advantage of little inter and intra-cycle variability (Dewailly, Andersen et al. 2014). The measurement of serum AMH levels is now recognized as a relevant tool for OR assessment and follow-up during treatment in premenopausal women with BC (Freour, Barriere et al. 2017) even though it is not yet accepted as a criteria used to identify women with premature ovarian insufficiency (POI) induced by chemotherapy (van Dorp, Mulder et al. 2016, Webber, Davies et al. 2016).

Much is still to be known about the mechanisms and gonadotoxic effects of specific CT regimens for BC. The more commonly used regimens include a combination of three cytotoxic agents (cyclophosphamide, an anthracycline and a taxane), with the addition of targeted therapy (TT) for Her_2-positive tumours, followed by hormonal therapy (HT) for hormone sensitive tumours. While cyclophosphamide and anthracyclines are recognized gonadotoxics, it is not definitely established if the addition of a taxane contributes to the impact of CT on OR (Freour, Barriere et al. 2017), despite their widely use in current regimens for BC. As for targeted therapy agents, the data currently available is limited although some clinical studies do not indicate ovarian toxicity with exposure to trastuzumab (Abusief, Missmer et al. 2010, Ben-Aharon, Granot et al. 2015). Furthermore, HT with tamoxifen is the mainstay of HT for premenopausal BC patients and is currently recommended for a time period of up to 10 years (Anampa, Makower et al. 2015) which further narrows the reproductive window of BC patients. In this scenario, the assessment of the risk of infertility in BC woman and their reproductive counselling can be challenging tasks. There is a clear need to prospectively collect data on the reproductive health outcomes of BC patients exposed to modern treatment regimens, using reliable fertility markers, in order to support an informed and shared decision on fertility preservation (FP).

In order to gather additional information on the gonadotoxicity of CT regimens for BC and to improve the reproductive counselling of young female patients with BC, this study had the following objectives:

1. To prospectively measure levels of OR markers in a cohort of young women with BC exposed to CT;
2. To identify adverse reproductive health outcomes during and after CT: amenorrhea, decreased OR, ovarian function impairment and POI;
3. To assess the influence of patient and treatment-related factors in those outcomes.

2. Methods

2.1. Patients and study design

This prospective observational study was conducted at the *Center for Fertility Preservation* (CFP) of the Coimbra Hospital and University Centre (CHUC, EPE). Patients included were premenopausal women, aged 18 to 40 years at the time of BC diagnosis and undergoing (neo) adjuvant CT. Exclusion criteria were metastatic BC, pregnancy, levels of AMH below the quantification limit or history of previous gonadotoxic chemo/radiotherapy. Women with BC

that were scheduled for a first consultation for FP counselling in the CFP were invited to participate. Recruitment took place between July 2014 and September 2016 and all participants signed an informed consent. The study received ethical approval by the institutional ethics committee and authorization to collect patient's data from the Portuguese Data Protection Authority. Hormonal (FSH, AMH) and ultrasound (AFC) ovarian reserve markers were assessed at several time points (Figure 4.1):

1) Before CT initiation;

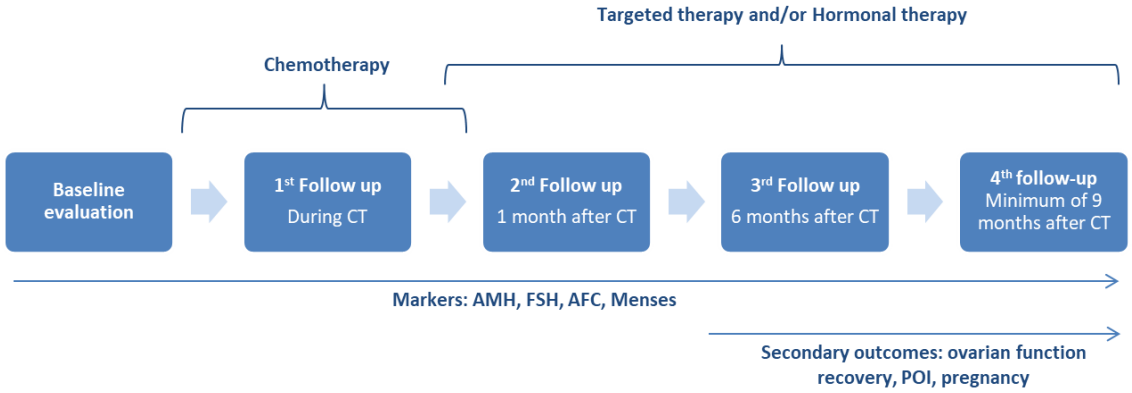
2) During CT, in patients where treatment with taxane was sequential to the anthracycline-based regimen;

3) Up to 1 month after the end of CT;

4) At six months after the end of CT;

5) At a minimum of 9 months after the end of CT.

Demographic, reproductive, menstrual and clinical data were collected at the time of patient recruitment (by interview and review of clinical records) and updated at subsequent appointments during and after CT.



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT – chemotherapy; FSH – follicle-stimulating hormone; POI – premature ovarian insufficiency.

Figure 4.1 Schematic representation of the study design.

2.2. Reproductive health outcomes

Menses and ovarian reserve markers

Self-reported menstrual data was collected at the time of recruitment and updated at subsequent appointments.

Blood samples for hormonal assays were drawn by venous puncture, regardless of the phase of the menstrual cycle. In a few cases, blood samples were collected, and serum was frozen, in a private clinic located in a different country region, for patient convenience. All samples were centrally analysed for AMH and FSH levels at the Clinical Pathology Department of CHUC, EPE. AMH was measured by the UltraSensitive AMH ELISA assay kit (Ansh Labs) with a Limit of Quantification (LoQ) of 0.06 ng/mL. FSH was measured by the ADVIA Centaur® FSH immunoassay, with a LoQ of 0.3 mIU/mL.

Antral follicle count (AFC) by intravaginal ultrasound was performed by experienced gynaecologists at the CFP and the private clinic. The total number of ovarian follicles with 2 to 10 mm of diameter, in both ovaries, was documented (Broekmans, de Ziegler et al. 2010). Ultrasound examinations took place regardless of the patient's phase of the menstrual cycle due to time constraints (pre-CT evaluation) and patient convenience (during and after CT evaluations). Furthermore, several women became amenorrheic during and after CT, making impossible to identify the optimal day of the cycle. At each time point, AFC was not performed if the participants were under ovarian suppression with GnRH agonists.

Recovery of ovarian function

Recovery of ovarian function at the last follow-up was defined with one of the following criteria:

1) Post-CT return of menses *and* recovery of at least one of the measures of OR (FSH level ≤25 mIU/mL *or* AMH level ≥ baseline level/expected median level for age *or* AFC ≥ baseline level/ expected median count for age;

2) The occurrence of pregnancy at any point after the end of chemotherapy.

The expected AMH and AFC levels for age were set based on median results obtained by *Seifer* (Seifer, Baker et al. 2011) and *Almog* (Almog, Shehata et al. 2011), correspondingly.

Published data suggest that patients under tamoxifen therapy may have reduced FSH levels (Rossi, Morabito et al. 2009, Su 2010) and exposure to GnRH agonists (GnRHa) also downregulates the levels of FSH (Kumar and Sharma 2014). Tamoxifen does not seem to interfere with AMH levels (Dezellus, Barriere et al. 2017, Shandley, Spencer et al. 2017, Trapp, Steidl et al. 2017) but the effect of GnRHa in the OR is still poorly understood (Anderson, Themmen et al. 2006, Hagen, Sorensen et al. 2012, Su, Maas et al. 2013). Due to these uncertainties, ovarian function recovery was not assessed in women with premenopausal FSH levels that were exposed to some form of HT.

Premature Ovarian Insufficiency

According to the recommendations from the *European Society of Human Reproduction and Embryology* (Webber, Davies et al. 2016), Premature Ovarian Insufficiency (POI) *i.e.* loss of ovarian activity before the age of 40, was defined as the occurrence of oligo/amenorrhea for at least four months and elevated FSH serum levels (>25 IU/L) on two occasions more than four weeks apart, after CT. Patients under ovarian suppression with a GnRHa and therefore amenorrheic, were not evaluated for the occurrence of POI.

2.3. Statistical analysis

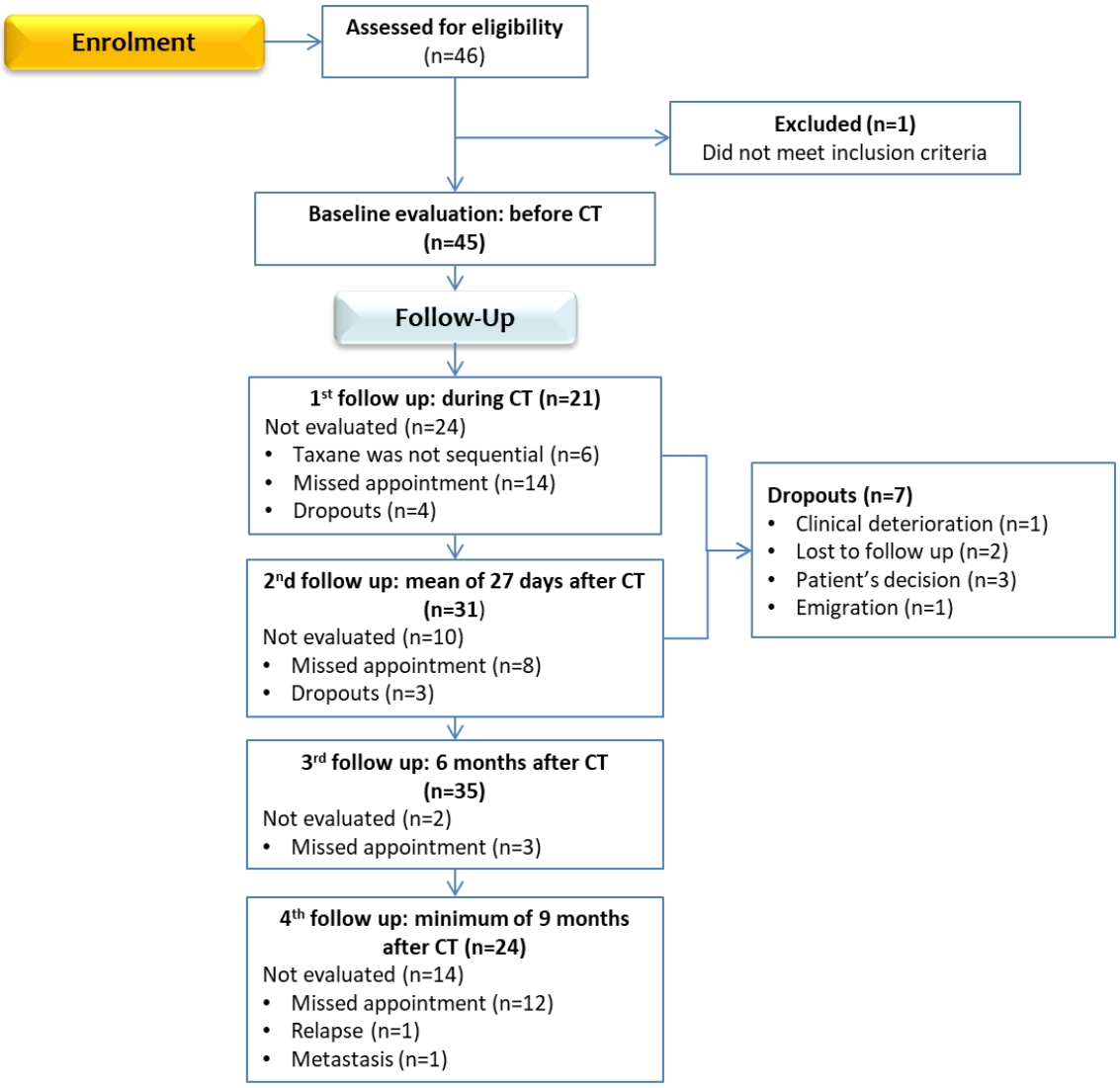
The analysis was performed with the software Statistical Package for Social Sciences (SPSS) version 21.

Non-parametric tests were used due to the small sample size and deviation from normality of most variables. Spearman's Rho (ρ) was used to test the association between variables. *Mann-Whitney* and *Kruskall-Wallis* tests were used to compare 2 and 3 or more groups, respectively. Paired sample analysis was conducted using the Wilcoxon signed-rank test. The significance level was set at 0.05. All measurements of AMH below the limit of quantification (LoQ = 0.06 ng/mL) were assigned the value of 0.06 ng/mL.

3. Results

3.1. Patients' characteristics and cancer treatments

A total of forty six women were recruited. One patient with baseline level of AMH below the LoQ and an FSH of 54 mIU/mL was excluded at enrolment. Seven participants were lost to follow-up. Two of them were unreachable and the other five dropout of the study due to clinical deterioration (n=1), patient's decision (n=3) and emigration (n=1). From the total number of patients included in the study (n=45), thirty six (n=36) were evaluated at six months after the end of CT (Figure 4.2) and twenty-four (n=24) in a subsequent follow-up at a minimum of 9 months after the end of CT. Median age of participants at study inclusion and at the last follow–up was 33 years (mean 32.9 ± 3.5 years; min 25-max 39) and 34 years (mean 34.5 ± 3.7 years; min 27-max 41), respectively. The last follow-up occurred at a mean of 17 months (range 6 – 35 months) after the end of CT and a mean of 2 years (range 1-3 years) after recruitment (Table 4.1).



Legend: CT – chemotherapy

Figure 4.2 Flow diagram of participation in the study.

Table 4.1 Patient's age at recruitment and at the last follow-up and time periods between recruitment, end of chemotherapy and the last follow-up.

Patient	Age at recruitment	Age at the last follow-up	Date of recruitment	Date of end of CT	Date of last follow-up	Time between end of CT and last follow-up (Years/Months)	Time between recruitment and last follow-up (Years/Months)
1	30	32	26/06/2014	18/10/2014	13/09/2017	2Y/11M	3Y/3M
2	34	36	05/09/2014	28/11/2014	22/09/2016	1Y/10M	2Y/1M
3	30	31	11/08/2014	08/12/2014	01/08/2015	8M	1Y
4	34	37	20/06/2014	08/12/2014	13/09/2017	2Y/10M	3Y/3M
5	29	30	23/09/2014	23/01/2015	13/08/2015	7M	11M
6	34	37	11/08/2014	01/02/2015	02/10/2017	2Y/8M	3Y/2M
7	33	34	27/08/2014	09/02/2015	01/08/2015	6M	11M
8	34	36	31/09/2014	13/02/2015	10/11/2016	1Y/9M	2Y/2M
9	36	37	27/10/2014	19/02/2015	12/08/2015	6M	10M
10	39	41	06/11/2014	06/03/2015	09/11/2016	1Y/8M	2Y/1M
11	34	37	25/11/2014	17/03/2015	01/11/2017	2Y/8M	3Y
12	32	33	25/11/2014	20/03/2015	19/10/2015	7M	11M
13	37	38	20/01/2015	07/04/2015	12/10/2015	6M	9M
14	36	39	05/12/2015	10/04/2015	25/10/2017	2Y/7M	1Y/11M
15	31	34	20/01/2015	22/05/2015	10/10/2017	2Y/5M	2Y/9M
16	36	39	27/02/2015	25/05/2015	18/10/2017	2Y/5M	2Y/8M
17	29	31	31/03/2015	12/07/2015	06/06/2017	1Y/11M	2Y/3M
18	38	41	11/03/2015	16/07/2015	20/12/2017	2Y/6M	2Y/10M
19	33	35	30/03/2015	30/07/2015	14/06/2017	1Y/11M	2Y/3M
20	30	33	27/05/2015	07/08/2015	21/11/2017	2Y/4M	2Y/6M
21	27	29	05/08/2015	03/09/2015	16/08/2017	2Y	2Y/1M
22	32	34	17/04/2015	29/09/2015	19/07/2017	1Y/10M	2Y/4M
23	30	32	18/06/2015	05/10/2015	18/10/2017	2Y/1M	2Y/4M
24	30	31	02/07/2015	26/10/2015	06/06/2016	7M	11M
25	39	41	01/09/2015	27/10/2015	13/12/2017	2Y/2M	2Y/4M
26	25	27	02/07/2015	06/11/2015	08/11/2017	2Y	2Y/5M
27	32	33	11/08/2015	13/01/2016	01/10/2017	1Y/9M	2Y/2M
28	32	34	15/10/2015	20/01/2016	20/12/2017	1Y/11M	2Y/3M
29	33	34	15/10/2015	24/01/2016	28/07/2016	6M	10M
30	31	32	22/10/2015	18/02/2016	11/08/2016	6M	10M
31	37	39	10/02/2016	28/03/2016	07/11/2017	1Y/8M	1Y/9M
32	31	32	15/04/2016	12/05/2016	16/02/2017	9M	10M
33	30	31	17/05/2015	10/08/2016	14/02/2017	6M	1Y/9M
34	31	31	01/09/2016	30/08/2016	20/02/2017	6M	6M
35	35	36	06/06/2016	11/09/2016	24/05/2017	9M	1Y
36	38	39	01/09/2016	13/10/2016	07/06/2017	8M	9M
37	27	28	01/09/2016	28/12/2016	28/06/2017	6M	10M
38	39	40	01/09/2016	31/01/2017	07/02/2018	1Y	1Y/6M

Legend: CT – chemotherapy; M – months; Y – years.

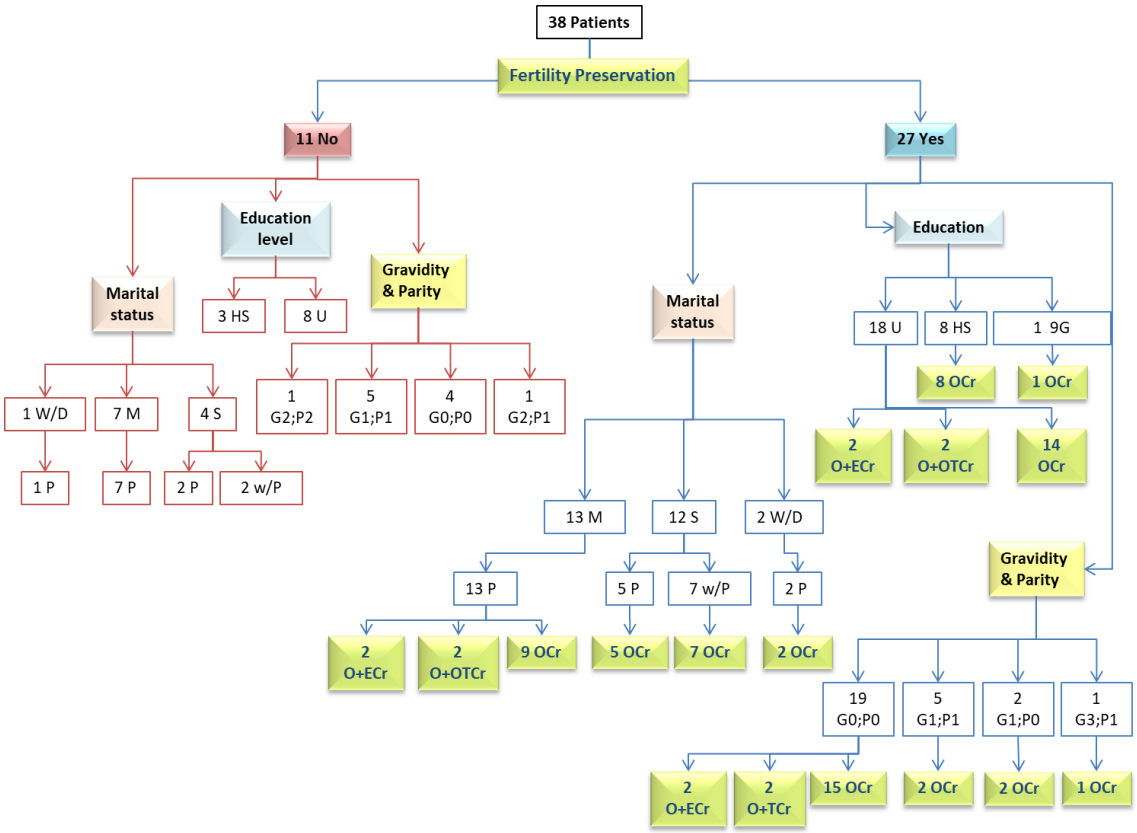
Baseline demographic, obstetric and fertility preservation data for the 38 participants are detailed in Table 4.2. At the time of recruitment, seven of the participants were overweight, three were obese, five were past smokers and two were current smokers. All but one participant had been previously exposed to some form of hormonal contraception (HC). In most of them, HC was discontinued less than 2 months before the baseline evaluation, usually at the time they received the BC diagnosis. The vast majority of participants had a university level of education, (26/38; 68%). Twenty-five patients were childless at diagnosis (25/38; 66%). After their first consultation at the CFP for FP counselling before cancer treatment initiation, twenty-seven decided to undergo FP (27/38; 71%), by freezing oocytes (n =23), oocytes and

embryos (n=2) or ovarian tissue (n=2). From those who did not undergo FP (n=11), seven had already children, three were single and two did not have a partner. Figure 4.3 shows the relations between fertility preservation decision/techniques and patients' education level, marital status and obstetrical history.

Table 4.2 Baseline demographic, obstetric and fertility preservation characteristics of the study participants.

Patient	Age	Marital status	Partner	Education level	BMI Category	Smoking status	HC use; Time since discontinuation	Gravidity & Parity	Fertility Preservation technique
1	30	Single	No	Highschool	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
2	34	Married/ Civil union	Yes	University	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
3	30	Married/ Civil union	Yes	University	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 0, para 0	Oocyte and embryo cryopreservation
4	34	Single	Yes	University	Normal weight	Former smoker	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
5	29	Single	Yes	University	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
6	34	Married/ Civil union	Yes	University	Overweight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte and embryo cryopreservation
7	33	Single	Yes	University	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 0, para 0	No
8	34	Single	Yes	Highschool	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
9	36	Married/ Civil union	Yes	University	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 1, para 1	No
10	39	Single	No	University	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
11	34	Single	No	Highschool	Normal weight	Former smoker	Former user; > 2 months ago	Gravida 1, para 0	Oocyte cryopreservation
12	32	Married/ Civil union	Yes	University	Normal weight	Former smoker	Former user; < 2 months ago	Gravida 0, para 0	Ovarian tissue cryopreservation
13	37	Single	No	University	Obesity	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	No
14	36	Married/ Civil union	Yes	University	Normal weight	Former smoker	Former user; < 2 months ago	Gravida 1, para 1	No
15	31	Married/ Civil union	Yes	Highschool	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 1, para 1	No
16	36	Single	No	University	Normal weight	Former smoker	Former user; > 2 months ago	Gravida 1, para 1	Oocyte cryopreservation
17	29	Married/ Civil union	Yes	9th grade	Normal weight	Current smoker	Former user; > 2 months ago	Gravida 1, para 1	Oocyte cryopreservation
18	38	Single	Yes	University	Overweight	Former smoker	Former user; < 2 months ago	Gravida 2, para 1	No; GnRHa during CT
19	33	Married/ Civil union	Yes	University	Obesity	Never smoked	Former user; < 2 months ago	Gravida 0, para 0	No
20	30	Single	No	University	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
21	27	Married/ Civil union	Yes	Highschool	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
22	32	Widowed/ Divorced	Yes	University	Normal weight	Former smoker	Former user; < 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
23	30	Married/ Civil union	Yes	University	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 1, para 1	Oocyte cryopreservation
24	30	Married/ Civil union	Yes	Highschool	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 1, para 1	Oocyte cryopreservation
25	39	Single	No	University	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
26	25	Single	No	Highschool	Overweight	Former smoker	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
27	32	Married/ Civil union	Yes	University	Overweight	Former smoker	Former user; < 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
28	32	Married/ Civil union	Yes	University	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 1, para 1	No; GnRHa during CT
29	33	Single	No	Highschool	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 0, para 0	No
30	32	Single	Yes	Highschool	Overweight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
31	37	Widowed/ Divorced	Yes	University	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 1, para 0	Oocyte cryopreservation
32	31	Married/ Civil union	Yes	University	Overweight	Never smoked	Former user; < 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
33	30	Married/ Civil union	Yes	University	Normal weight	Never smoked	Former user; < 2 months ago	Gravida 2, para 2	No
34	31	Widowed/ Divorced	Yes	Highschool	Normal weight	Never smoked	Never used	Gravida 1, para 1	No
35	35	Married/ Civil union	Yes	Highschool	Obesity	Never smoked	Former user; < 2 months ago	Gravida 3, para 1	Oocyte cryopreservation
36	38	Married/ Civil union	Yes	University	Normal weight	Current smoker	Former user; < 2 months ago	Gravida 0, para 0	Ovarian tissue cryopreservation
37	27	Single	Yes	University	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 0, para 0	Oocyte cryopreservation
38	39	Married/ Civil union	Yes	University	Normal weight	Never smoked	Former user; > 2 months ago	Gravida 1, para 1	Oocyte cryopreservation

Legend: BMI – Body Mass Index; CT – Chemotherapy; HC – Hormonal contraception; GnRHa – Gonadotropin-Releasing Hormone agonist



Legend: ECr – embryo cryopreservation; G;P – gravidity; parity; 9G -9th grade; HS – high school; M – married; OCr – oocyte cryopreservation; S – single; P – partner; OTCr – ovarian tissue cryopreservation; U – university W/D – widowed/divorced w/P – without partner.

Figure 4.3 Relations between fertility preservation decision/performed techniques and patients' education level, marital status and obstetrical history at baseline.

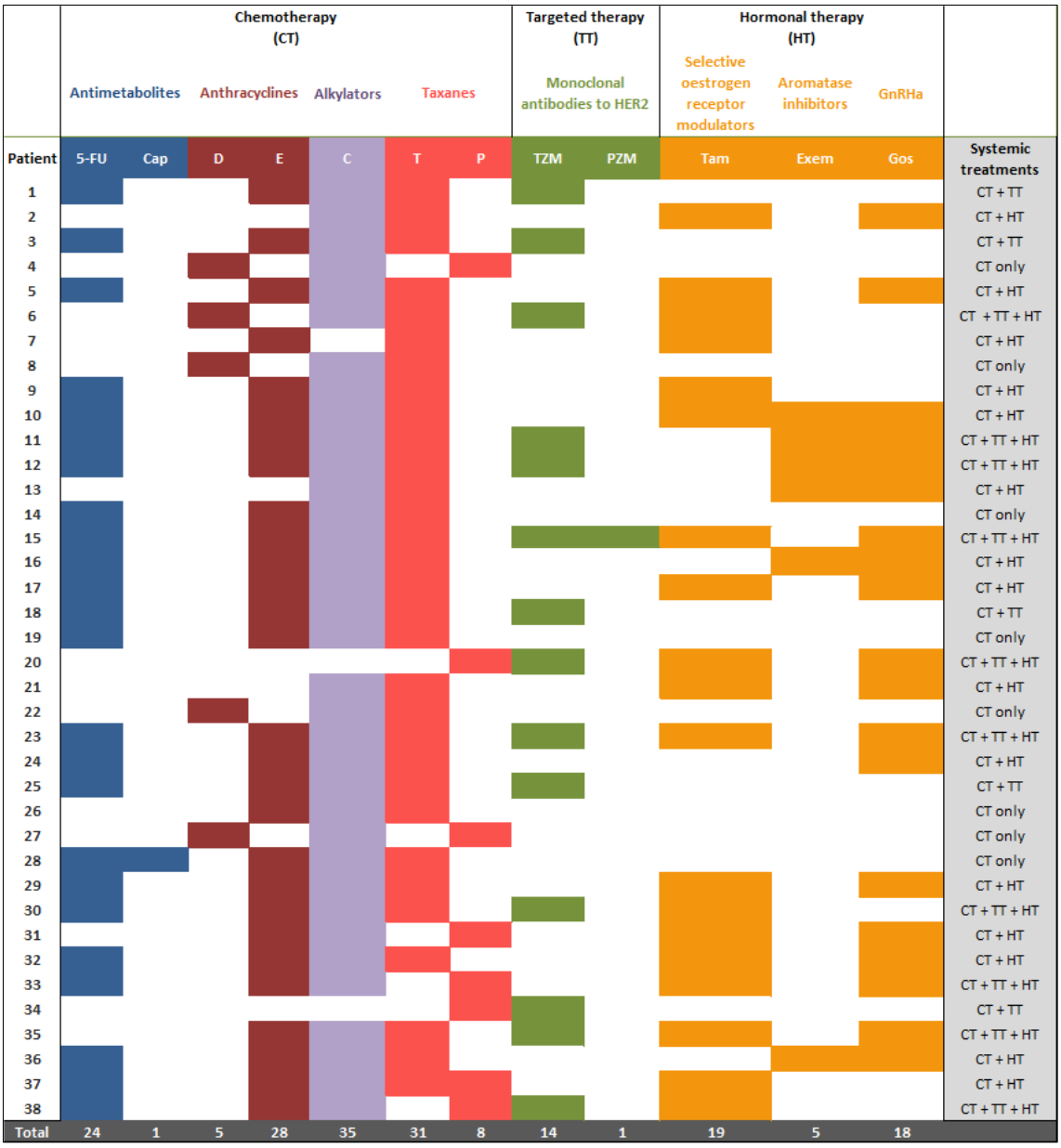
Systemic treatments for BC are presented in Table 4.3, organized according to the tumour biology. Eight patients were treated with cytotoxic CT only (triple-negative tumours), five with CT + TT (Her2-positive tumours), fifteen with CT + some form of HT (HR-positive tumours) and ten patients with CT + TT + HT (triple-positive tumours). Concerning the CT regimens, most women (32/38; 84%) received an anthracycline-based chemotherapy regimen followed by a taxane (FEC, AC or EC and sequential docetaxel and/or paclitaxel) with or without targeted therapy (mainly trastuzumab) and hormonal therapy (tamoxifen, aromatase inhibitor and/or ovarian suppression agents). Tables 4.4 and 4.5 present additional information about the diversity of antineoplastic, targeted therapy and hormonal therapy agents, and the diversity of CT regimens to which the participants were exposed. All regimens included at least one taxane and all but three patients were exposed to cyclophosphamide (35/38; 92%). CT duration varied from 12 to 24 weeks with a median of 18 weeks (Table 4.5).

Table 4.3 Type of systemic treatments for breast cancer in each participant, organized by the corresponding tumour biology.

Tumour biology	Patient	Systemic treatments	CT type	CT regimen type	Targeted therapy (TT)	Hormonal therapy (HT)
HER2 positive	1	CT + TT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	No
HER2 positive	3	CT + TT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	No
HER2 positive	18	CT + TT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	No
HER2 positive	25	CT + TT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	No
HER2 positive	34	CT + TT	Adjuvant	Taxane-based	Yes	No
HR-positive	2	CT + HT	Adjuvant	Taxane-based	No	Yes
HR-positive	5	CT + HT	Adjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	7	CT + HT	Neoadjuvant	Taxane-based	No	Yes
HR-positive	9	CT + HT	Neoadjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	10	CT + HT	Adjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	13	CT + HT	Adjuvant	Taxane-based	No	Yes
HR-positive	16	CT + HT	Adjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	17	CT + HT	Neoadjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	21	CT + HT	Adjuvant	Taxane-based	No	Yes
HR-positive	29	CT + HT	Neoadjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	31	CT + HT	Neoadjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	32	CT + HT	Adjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	33	CT + HT	Neoadjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	36	CT + HT	Neoadjuvant	Anthracycline-based + seq taxane	No	Yes
HR-positive	37	CT + HT	Adjuvant	Anthracycline-based + seq taxane	No	Yes
Triple-negative	4	CT only	Adjuvant	Anthracycline-based + seq taxane	No	No
Triple-negative	8	CT only	Adjuvant	Anthracycline-based + seq taxane	No	No
Triple-negative	14	CT only	Adjuvant	Anthracycline-based + seq taxane	No	No
Triple-negative	19	CT only	Neoadjuvant	Anthracycline-based + seq taxane	No	No
Triple-negative	22	CT only	Adjuvant	Anthracycline-based + seq taxane	No	No
Triple-negative	26	CT only	Neoadjuvant	Anthracycline-based + seq taxane	No	No
Triple-negative	27	CT only	Neoadjuvant	Anthracycline-based + seq taxane	No	No
Triple-negative	28	CT only	Neoadjuvant & Adjuvant	Anthracycline-based + seq taxane	No	No
Triple-negative (3% HR+)	24	CT + HT	Neoadjuvant	Anthracycline-based + seq taxane	No	Yes
Triple-positive	6	CT + TT + HT	Adjuvant	Anthracycline-based + seq taxane	Yes	Yes
Triple-positive	11	CT + TT + HT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	Yes
Triple-positive	12	CT + TT + HT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	Yes
Triple-positive	15	CT + TT + HT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	Yes
Triple-positive	20	CT + TT + HT	Adjuvant	Taxane based	Yes	Yes
Triple-positive	23	CT + TT + HT	Adjuvant	Anthracycline-based + seq taxane	Yes	Yes
Triple-positive	30	CT + TT + HT	Adjuvant	Anthracycline-based + seq taxane	Yes	Yes
Triple-positive	35	CT + TT + HT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	Yes
Triple-positive	38	CT + TT + HT	Neoadjuvant	Anthracycline-based + seq taxane	Yes	Yes

Legend: CT – chemotherapy; HER2 - Human Epidermal growth factor Receptor-type 2; HR – hormonal receptors; HT – hormonal therapy; seq – sequential; TT – targeted therapy.

Table 4.4 Chemotherapy, targeted therapy and hormonal therapy agents to which each participant was exposed.



Legend: C – cyclophosphamide; Cap – capecitabine; CT – Chemotherapy; D – doxorubicin; 5-FU – fluorouracil; E - epirubicin; Exem – exemestane; Gos – goserelin; GnRHa - gonadotrophin-releasing hormone agonists; HT – hormonal therapy; P – paclitaxel; PZM – pertuzumab; T – docetaxel; Tam – tamoxifen; TT – targeted therapy; TZM – Trastuzumab.

Table 4.5 Treatment combinations of chemotherapy, targeted therapy and hormonal therapy and corresponding sequence and length that were administered to each patient.

Patient	Tumour biology	CT type	CT regimen	CT Number of Cycles	AC	T	P	T + TZM	P + TZM	Cap	TE	CT duration (weeks)	TZM	TZM + PZM	Tam + GnRHa	Tam	GnRHa	Exem + GnRHa	Exem
8	Triple-negative	Adjuvant	AC → T	4 → 4	12 W	12 W						24							
22	Triple-negative	Adjuvant	AC → T	4 → 4	12 W	12 W						24							
27	Triple-negative	Neoadjuvant	DD AC → weekly	4 → 12	8 W		12 W					20							
4	Triple-negative	Adjuvant	AC → weekly Paclitaxel	4 → 12	12 W		12 W					24							
6	Triple-positive	Adjuvant	AC → T	4 → 4	12 W			12 W				24	40 W		1 Y	≥ 4 Y			
					EC	T	P					CT duration (weeks)	TZM	TZM + PZM	Tam + GnRHa	Tam	GnRHa	Exem + GnRHa	Exem
31	HR-positive	Neoadjuvant	DD EC → weekly	4 → 4	8 W		4 w					12			0,5 Y	≥ 4,5 Y			
26	Triple-negative	Neoadjuvant	EC → T	4 → 4	12 W	12 W						24							
35	Triple-positive	Neoadjuvant	EC → T	4 → 4	12 W			12 W				24	40 W		≥ 5 Y				
					FEC	T	P					CT duration (weeks)	TZM	TZM + PZM	Tam + GnRHa	Tam	GnRHa	Exem + GnRHa	Exem
3	HER2 positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18	43 W						
15	HER2 positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18	43 W						
16	HER2 positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18	43 W						
17	HER2 positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18	43 W						
18	HR-positive	Adjuvant	FEC → T	3 → 3	9 W	9 W						18						2 Y	≥ 3 Y
19	HR-positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18			2 Y	8 Y			
23	HR-positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18			2 Y	≥ 3 Y			
24	HR-positive	Adjuvant	FEC → T	3 → 3	9 W	9 W						18			2 Y	≥ 3 Y			
25	HR-positive	Adjuvant	FEC → T → weekly	3 → 1 → 3	9 W	3 W	3 w					15				≥ 5 Y			
29	HR-positive	Adjuvant	FEC → T	3 → 3	9 W	9 W						18			2 Y	≥ 3 Y			
30	HR-positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18				≥ 5 Y			
32	HR-positive	Adjuvant	FEC → T	3 → 3	9 W	9 W						18			18 M			≥ 3 Y	
37	HR-positive	Neoadjuvant	FEC → T	4 → 4	12 W	12 W						24						2 Y	≥ 3 Y
1	HR-positive	Neoadjuvant	FEC → weekly Paclitaxel	4 → 5	12 W		5 W					17			2 Y	≥ 3 Y			
5	Triple-negative	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18							
9	Triple-negative	Adjuvant	FEC → T	3 → 3	9 W	9 W						18							
10	Triple-negative	Neoadjuvant & Adjuvant	FEC → D → adjuvant	4 → 3 → 2	12 W	9 W				2 W		23							
11	Triple-negative (3% HR+)	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18					2 Y		
12	Triple-positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18	52 W	60 W	2 Y	≥ 3 Y			
14	Triple-positive	Adjuvant	FEC → T	3 → 3	9 W	9 W						18	43 W		2 Y	≥ 3 Y			
28	Triple-positive	Adjuvant	FEC → T	3 → 3	9 W	9 W						18	52 W			≥ 5 Y			
36	Triple-positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18	43 W						≥ 5 Y
38	Triple-positive	Neoadjuvant	FEC → T	3 → 3	9 W	9 W						18	43 W					2 Y	≥ 3 Y
33	Triple-positive	Neoadjuvant	FEC → weekly Paclitaxel	4 → 12	12 W		12 W					24	52 W			≥ 5 Y			
					TC							CT duration (weeks)	TZM	TZM + PZM	Tam + GnRHa	Tam	GnRHa	Exem + GnRHa	Exem
2	HR-positive	Adjuvant	TC	4	12 W							12			2 Y	≥ 3 Y			
13	HR-positive	Adjuvant	TC	4	12 W							12						2 Y	≥ 3 Y
21	HR-positive	Adjuvant	TC	4	12 W							12			2 Y	≥ 3 Y			
						T					TE	CT duration (weeks)	TZM	TZM + PZM	Tam + GnRHa	Tam	GnRHa	Exem + GnRHa	Exem
7	HR-positive	Neoadjuvant	T → TE	4 → 4		12 W					12 W	24			2 Y	≥ 3 Y			
							P					CT duration (weeks)	TZM	TZM + PZM	Tam + GnRHa	Tam	GnRHa	Exem + GnRHa	Exem
34	HER2 positive	Adjuvant	Weekly Paclitaxel	12			12 W					12	40 W						
20	Triple-positive	Adjuvant	Weekly Paclitaxel	12			12 W					12	40 W		2 Y	≥ 3 Y			

Legend: AC – doxorubicin hydrochloride (Adriamycin) and cyclophosphamide; Cap – capecitabine; CT – Chemotherapy; EC - epirubicin and cyclophosphamide; Exem – exemestane; FEC - Fluorouracil, epirubicin and cyclophosphamide; GnRHa - gonadotrophin-releasing hormone agonists; HR – hormonal receptors; Her2 - Human Epidermal growth factor Receptor-type 2; P – paclitaxel; PZM – pertuzumab; T – docetaxel; Tam – tamoxifen; TC – docetaxel and cyclophosphamide; TE – docetaxel and epirubicin; TZM – Trastuzumab; W – weeks; Y – years.

3.2. Reproductive health outcomes

3.2.1. **Menses**

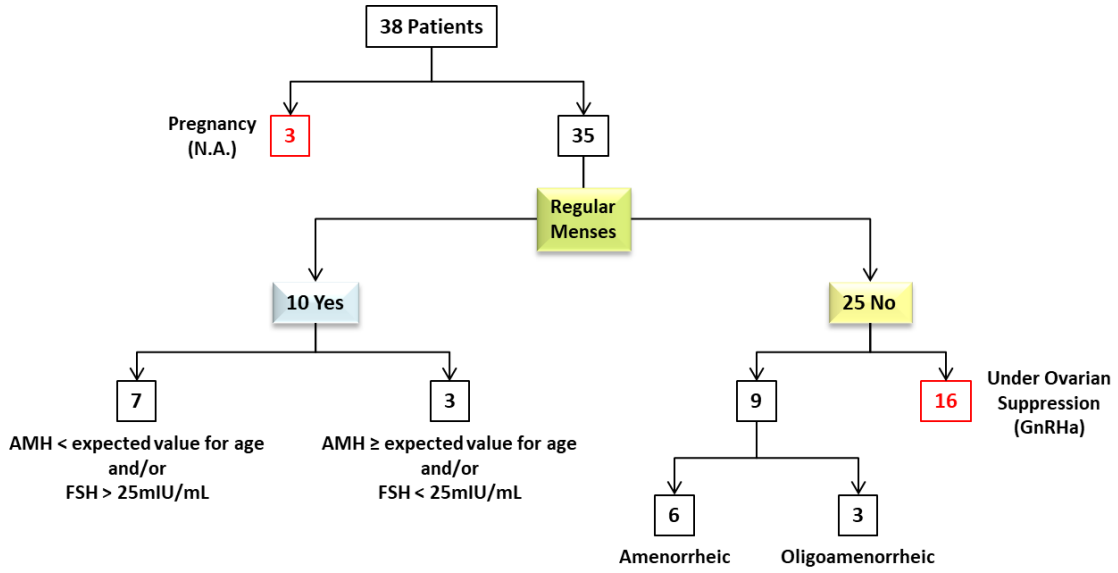
All patients reported regular menses at the time of recruitment. At the first follow-up after the end of CT, only 4 patients reported menses (4/32; 13%). Detailed information on the occurrence of menses, in each patient, at the two follow-ups after the end of CT can be seen in Table 4.6.

Table 4.6 Patient's self-reported regular menses (Yes/No) at baseline, 1 month after the end of CT and at the last follow-up.

Patient	Baseline	1 month after CT	Last follow-up	Observations	GnRHa
1	Y	N	Y		N
4	Y	N	Y	AMH 0,06 ng/mL	N
7	Y	N	Y	AMH 0,06 ng/mL	N
18	Y	N	Y		N
20	Y	N	Y		N
22	Y	N	Y	AMH 0,09 ng/mL; FSH 188 mIU/mL	N
25	Y	Y	Y	FSH 68 mIU/mL	N
26	Y	***	Y	AMH 0,58 ng/mL	N
28	Y	N	Y	AMH 0,68 ng/mL	N
31	Y	N	Y	AMH 0,06 ng/mL	N
2	Y	Y	N		Y
5	Y	Y	N		Y
6	Y	***	N		N
8	Y	N	N	Oligomenorrhea	N
9	Y	N	N		N
10	Y	Y	N		Y
11	Y	***	N		N
12	Y	N	N		Y
13	Y	N	N		Y
14	Y	N	N	Oligomenorrhea	N
15	Y	N	N		Y
16	Y	N	N		Y
17	Y	***	N		Y
19	Y	***	N	Oligomenorrhea	N
21	Y	N	N		Y
23	Y	N	N		Y
24	Y	N	N		Y
29	Y	***	N		Y
30	Y	N	N		N
32	Y	N	N		Y
33	Y	N	N		Y
35	Y	N	N		Y
36	Y	N	N		Y
37	Y	N	N		N
38	Y	N	N		N
3	Y	N	N.A.	Pregnancy	N
27	Y	N	N.A.	Pregnancy	N
34	Y	***	N.A.	Pregnancy	N

Legend: AMH – Anti-Mullerian Hormone; CT – Chemotherapy; FSH – Follicle-stimulating Hormone; GnRHa - GnRHa - gonadotrophin-releasing hormone agonists; N – No; N.A. – not applicable; Y – Yes.

As represented in Figure 4.4, the occurrence of menses at the last follow-up was assessed in a total of 35 patients: ten reported regular menses (n=10/35) and twenty-five (n=25/35) reported amenorrhea (absence of menstrual periods) or oligomenorrhea (i.e. menstrual periods occurring at intervals of greater than 35 days). Most of the amenorrheic patients were under ovarian suppression (n=16). From the nine that were not under therapy with GnRHa, six were amenorrheic and three were oligomenorrheic (9/35; 26%). Moreover, the majority of patients reporting regular menses presented low levels of OR markers (AMH levels below the normal for their age and/or FSH levels >25 mIU/mL), at the last follow-up (7/10; 70%).



Legend: AMH – Anti-Mullerian Hormone; FSH – Follicle-stimulating Hormone; GnRHa - gonadotropin-releasing hormone agonists; N.A. – not applicable.

Figure 4.4 Patient distribution according to menstrual status at the last follow-up and corresponding levels of OR markers.

3.2.2. Ovarian reserve markers

A total of 152 evaluations of hormonal and/or ultrasound markers were made throughout the study: at baseline (n = 45), during CT (n =21), at a mean of 27 days [range 11-55] after the end of CT (n =31), at a mean of 6 months [range 6-8] after the end of CT (n = 32) and at a minimum of 9 months after the end of CT (n = 23). The mean time between the end of CT and each patient's last follow-up was of 18 months.

Ovarian reserve markers at baseline

Table 4.7 presents the baseline levels of AMH, AFC and FSH for each of the participants.

Mean baseline AMH in this cohort was of 3.1 ± 2.9 ng/mL (n=38; median of 2.2 ng/mL) and mean AFC was of 10.6 ± 5.2 (n=35; median of 9). Twenty-two (22/38; 58%) and sixteen women

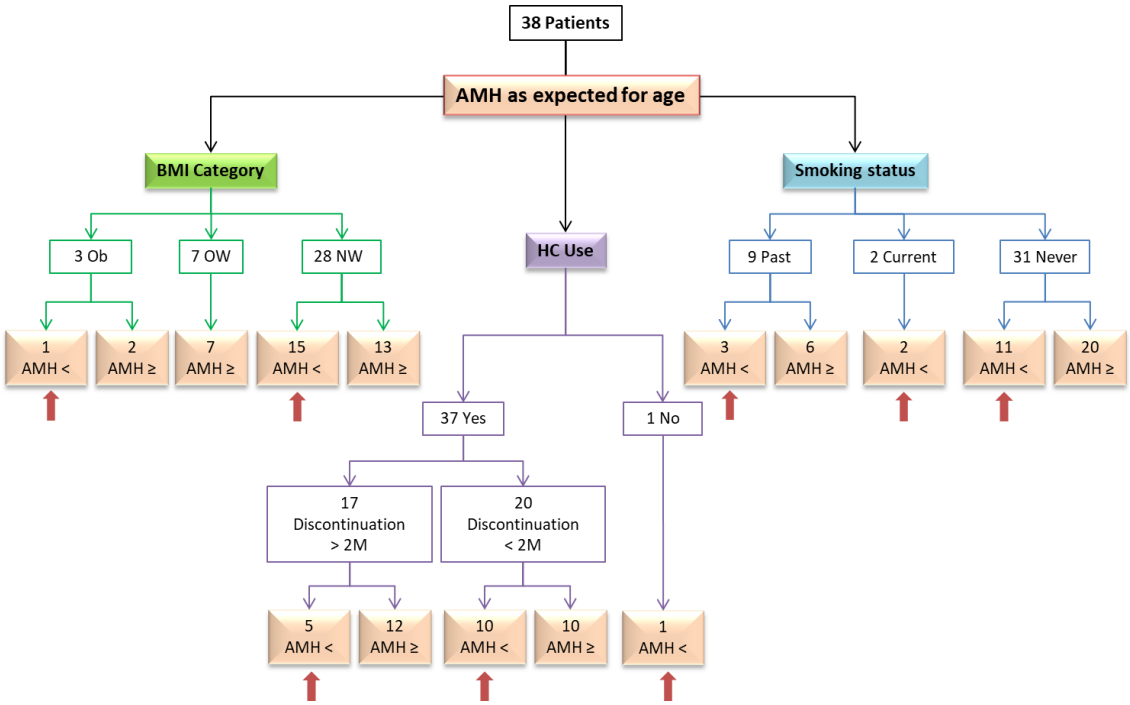
(16/35; 46%) presented AMH levels and AFC that were normal (equal/above) according to the expected values for their age, respectively. In some, AMH levels were consistent with polycystic ovarian syndrome (PCOS), which is a frequent benign condition in young women. In the remaining participants, AMH levels and AFC at recruitment were below the expected normal values for age. The baseline levels of AMH and AFC were strongly inter-correlated (ρ= 0.656; p<0.001) and negatively correlated with patient's age (AMH, ρ= -0.41, p=0.01; AFC, ρ= -0.37, p=0.028). A total of 36 patients had their FSH levels assessed at baseline and mean levels were 7.1 ± 5.6 mIU/mL (with a median of 5.2 mIU/mL). Only one patient presented a baseline FSH level >25 mIU/mL.

Table 4.7 Baseline levels of ovarian reserve markers (AMH, AFC and FSH) and patient's categorization according to expected AMH/AFC values for their age, BMI, smoking status and previous exposure to HC.

Patient	Age	AMH level expected for age (ng/mL)	AMH (ng/mL)	Group according to AMH level expected value for age	AFC expected for age	AFC	Group according to AFC expected for age	FSH (mIU/mL)	BMI Category	Smoking status	HC use; Time since discontinuation
1	30	2,40	15,70	*Equal/above*	12,0	15	*Equal/above*	5,8	Normal weight	Never smoked	Former user; < 2 months ago
2	34	1,60	5,70	*Equal/above*	10,9	12	*Equal/above*	5,3	Normal weight	Never smoked	Former user; > 2 months ago
3	30	2,40	3,50	*Equal/above*	12,0	**		1,7	Normal weight	Never smoked	Former user; < 2 months ago
4	34	1,60	2,10	*Equal/above*	10,9	8	*Below*	**	Normal weight	Former smoker	Former user; > 2 months ago
5	29	2,60	6,30	*Equal/above*	12,3	12	*Equal/above*	4,9	Normal weight	Never smoked	Former user; > 2 months ago
6	34	1,60	4,10	*Equal/above*	10,9	9	*Below*	16,9	Overweight	Never smoked	Former user; > 2 months ago
7	33	1,70	3,70	*Equal/above*	11,2	18	*Equal/above*	5,2	Normal weight	Never smoked	Former user; < 2 months ago
8	34	1,60	0,60	*Below*	10,9	**		17,6	Normal weight	Never smoked	Former user; > 2 months ago
9	36	1,20	0,90	*Below*	10,0	7	*Below*	4,9	Normal weight	Never smoked	Former user; < 2 months ago
10	39	0,80	0,80	*Equal/above*	8,5	3	*Below*	2,0	Normal weight	Never smoked	Former user; > 2 months ago
11	34	1,60	1,40	*Below*	10,9	10	*Below*	2,7	Normal weight	Former smoker	Former user; > 2 months ago
12	32	1,80	8,50	*Equal/above*	11,5	10	*Below*	4,1	Normal weight	Former smoker	Former user; < 2 months ago
13	37	1,10	3,70	*Equal/above*	9,6	9	*Below*	5,8	Obesity	Never smoked	Former user; > 2 months ago
14	36	1,20	0,39	*Below*	10,0	6	*Below*	3,7	Normal weight	Former smoker	Former user; < 2 months ago
15	31	2,20	6,00	*Equal/above*	11,7	16	*Equal/above*	4,5	Normal weight	Never smoked	Former user; < 2 months ago
16	36	1,20	2,30	*Equal/above*	10,0	5	*Below*	12,0	Normal weight	Former smoker	Former user; > 2 months ago
17	29	2,60	2,00	*Below*	12,3	8	*Below*	6,9	Normal weight	Current smoker	Former user; > 2 months ago
18	38	0,90	2,50	*Equal/above*	9,1	9	*Equal/above*	9,0	Overweight	Former smoker	Former user; < 2 months ago
19	33	1,70	1,10	*Below*	11,2	19	*Equal/above*	2,8	Obesity	Never smoked	Former user; < 2 months ago
20	30	2,40	4,70	*Equal/above*	12,0	17	*Equal/above*	3,8	Normal weight	Never smoked	Former user; > 2 months ago
21	27	2,90	0,89	*Below*	12,8	6	*Below*	2,4	Normal weight	Never smoked	Former user; < 2 months ago
22	32	1,80	0,92	*Below*	11,5	14	*Equal/above*	9,5	Normal weight	Former smoker	Former user; < 2 months ago
23	30	2,40	1,80	*Below*	12,0	9	*Below*	6,9	Normal weight	Never smoked	Former user; < 2 months ago
24	30	2,40	4,50	*Equal/above*	12,0	18	*Equal/above*	8,8	Normal weight	Never smoked	Former user; < 2 months ago
25	39	0,80	3,50	*Equal/above*	8,5	8	*Equal/above*	5,0	Normal weight	Never smoked	Former user; > 2 months ago
26	25	3,20	5,40	*Equal/above*	13,6	21	*Equal/above*	5,7	Overweight	Former smoker	Former user; > 2 months ago
27	32	1,80	2,60	*Equal/above*	11,5	7	*Below*	15,0	Overweight	Former smoker	Former user; < 2 months ago
28	32	1,80	1,70	*Below*	11,5	8	*Below*	3,8	Normal weight	Never smoked	Former user; > 2 months ago
29	33	1,70	0,19	*Below*	11,2	3	*Below*	6,0	Normal weight	Never smoked	Former user; < 2 months ago
30	32	1,80	1,80	*Equal/above*	11,5	7	*Below*	8,2	Overweight	Never smoked	Former user; > 2 months ago
31	37	1,10	0,89	*Below*	9,6	11	*Equal/above*	9,2	Normal weight	Never smoked	Former user; < 2 months ago
32	31	2,20	4,50	*Equal/above*	11,7	22	*Equal/above*	4,4	Overweight	Never smoked	Former user; < 2 months ago
33	30	2,40	1,00	*Below*	12,0	4	*Below*	4,4	Normal weight	Never smoked	Former user; < 2 months ago
34	31	2,20	0,59	*Below*	11,7	7	*Below*	**	Normal weight	Never smoked	Never used
35	35	1,30	4,10	*Equal/above*	10,5	**		1,9	Obesity	Never smoked	Former user; < 2 months ago
36	38	0,90	0,67	*Below*	9,1	9	*Equal/above*	11,3	Normal weight	Current smoker	Former user; < 2 months ago
37	27	2,90	5,50	*Equal/above*	12,8	18	*Equal/above*	2,9	Normal weight	Never smoked	Former user; > 2 months ago
38	39	0,80	0,15	*Below*	8,5	6	*Below*	30,0	Normal weight	Never smoked	Former user; > 2 months ago

Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; BMI – Body Mass Index; FSH – Follicle-stimulating Hormone; HC – Hormonal Contraception; ** Not assessed

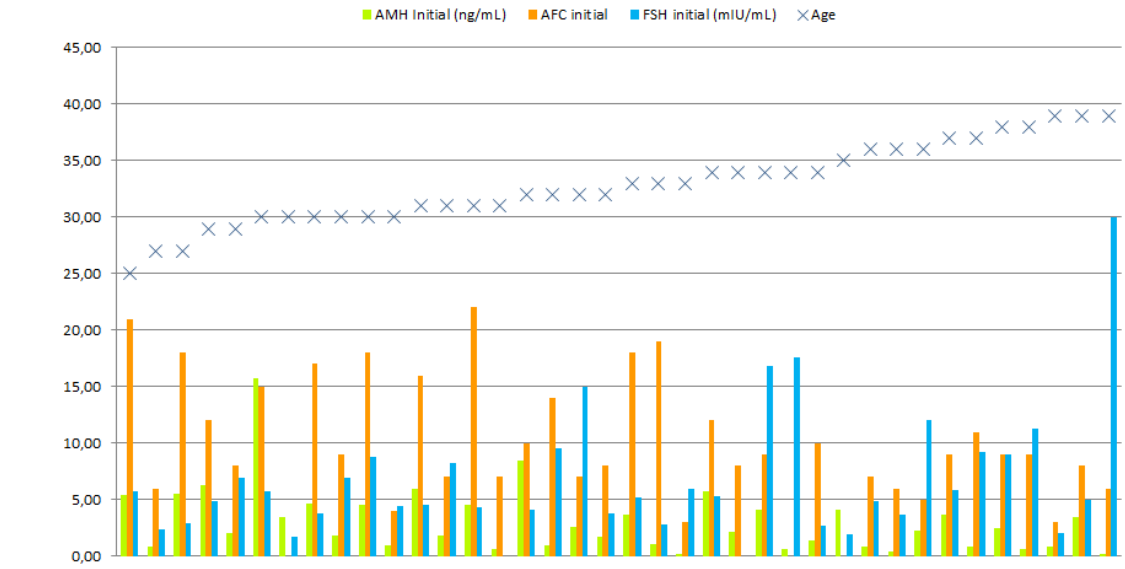
A distribution of patients with normal/high or low AMH levels considering their weight, smoking status and HC use at baseline can be seen in Figure 5. Current smokers (n=2) had lower than expected AMH levels. However, the proportion of patients with lower AMH was similar in the groups of past smokers compared with those who had never smoked. Half of those who had discontinued HC less than 2 months before (10/20) presented low baseline AMH as compared to less than one third (5/17) of those who had discontinued HC more than 2 months before. All women that were overweight (n=7) had normal AMH levels and only one of the obese patients (1/3) presented low baseline AMH.



Legend: AMH – Anti-Mullerian Hormone; BMI – Body Mass Index; HC – Hormonal Contraception; NW- normal weight; Ob – obese; OW - overweight.

Figure 4.5 Distribution of patients with normal/higher and lower AMH levels considering their weight, smoking status and HC use at baseline.

An overview of the levels of the three OR markers at baseline, for each patient, is presented in Figure 4.6. Patients are organized in ascending order with respect to their age at recruitment. Their categorization according to the expected AMH and AFC levels is also included. Most patients with lower than expected AMH levels at baseline had also lower than expected AFC. Yet, ten of the patients with lower than expected AFC had normal/high AMH. A trend towards higher AMH and AFC in younger patients is visible, in accordance with the previously mentioned correlations of baseline AMH and AFC with patient's age.



Patient	26	21	37	5	17	1	3	20	23	24	33	15	30	32	34	12	22	27	28	7	19	29	2	4	6	8	11	35	9	14	16	13	31	18	36	10	25	38
AMH Normal Level for Age	≥	<	≥	≥	<	≥	≥	≥	<	≥	<	≥	≥	≥	<	≥	<	≥	<	≥	<	<	≥	≥	≥	<	<	≥	<	<	≥	≥	<	≥	<	≥	≥	<
AFC Normal Count for Age	≥	<	≥	≥	<	≥	<	≥	<	≥	<	≥	<	≥	**	<	<	<	<	≥	≥	<	≥	**	<	<	<	<	<	<	<	<	≥	<	<	<	<	<
FSH Normal Level (< 25 mIU/mL)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	**	Y	Y	Y	Y	Y	Y	Y	Y	**	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N

Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; FSH – Follicle-stimulating Hormone; N – No; Y – Yes; < - below; ≥ - equal/above; * - on goserelin; ** - not performed.

Figure 4.6 Baseline levels of AMH, AFC and FSH for each patient, presented in ascending order of patient's age at recruitment.

Evolution of ovarian reserve markers

Table 4.8 presents the mean levels of AMH, AFC and FSH measured at baseline, during CT, at a mean of 27 days after the end of CT and at each patient's last available follow-up (mean of 18 months after the end of CT).

Table 4.8 Mean and median levels of ovarian reserve markers (AMH, AFC and FSH) measured at baseline, during CT, 1 month and at a mean of 18 months after the end of CT.

	Baseline	During CT	1 month after CT	Last available follow-up
Mean time since the end of CT [range]	–	–	27 days [11-55]	18 months [6-35]
AMH ng/mL Mean ± SD (median)	3.07 ± 2.95 (2.20) n=38	0.30 ± 0.50 (0.06) n=21	0.15 ± 0.46[1] (0.06) n=30	0.32 ± 0.68[1 3] (0.06) n=34
AFC Mean ± SD (median)	10.6 ± 5.2 (9.0) n=35	4.1 ± 3.5 (4.0) n=19	3.4 ± 6.8[2] (1.0) n=25	2.2 ± 3.0[1] (2.0) n=21
FSH mIU/mL Mean ± SD (median)	7.1 ± 5.6 (5.2) n=36	21.2 ± 24.2 (9.1) n=21	64.3 ± 47.8[1] (64) n=28	21.4 ± 36.9[3] (6.4) n=35

Legend: AFC - antral follicle count; AMH - anti-Mullerian hormone; CT - chemotherapy; FSH - follicle-stimulating hormone; SD - standard deviation. [1] as compared to baseline, p<0.001; [2] as compared to baseline, p=0.001; [3] as compared to the previous follow-up, p=0.03; [4]as compared to the previous follow-up, p<0.001

Anti-Mullerian Hormone (AMH)

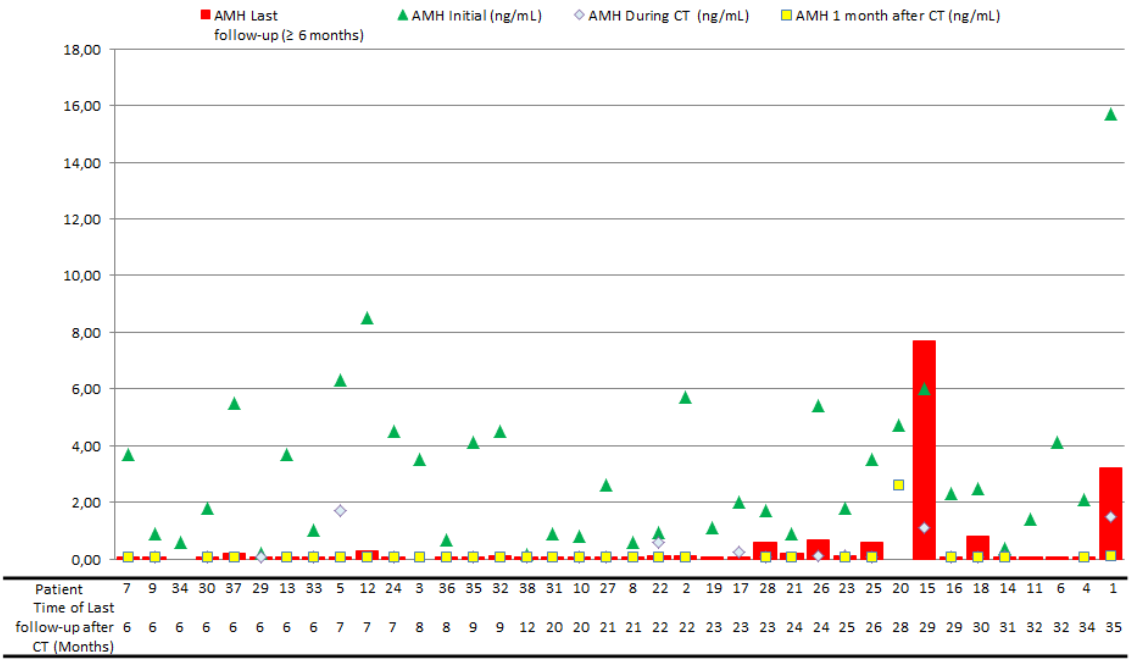
The levels of AMH significantly decreased from baseline to the post-CT follow-ups, both at 1 month after CT (z= -4.78, p<0.001) and at a mean of 18 months after CT (z=-4,9; p<0.001). However, a significant increase of AMH levels between these two last follow-ups was noticed (z=-2.9, p=0.003). A detailed picture of the evolution of AMH in each patient can be seen in Table 4.9. During the course of the study, the number of patients with AMH levels below the LoQ increased from 12 during CT (12/21; 57%) to 28 at 1 month after CT (28/31; 90%) and then decreased to 21 (21/35; 60%), at the last available follow-up for each patient. Still, at the end of the study, 30 patients (30/35; 86%) had AMH levels below the expected values for age and only one had recovered to baseline levels. Among those whose AMH levels re-increased at some point after CT (n=12), the mean monthly recovery rate was of 2.1%.

Table 4.9 Evolution of AMH levels, in each patient, from baseline until the last follow-up.

Patient	AMH level expected for age [1] (ng/mL)	Group according to AMH level expected for age at baseline	Baseline (ng/mL)	During CT (ng/mL)	1 month after CT (ng/mL)	6 months after CT (ng/mL)	Last follow-up	AMH level expected for age [1] (ng/mL)	Group according to AMH level expected for age at the last available follow-up	Time between end of CT and last follow-up (Months)
1	2,40	*Equal/above*	15,70	1,50	0,10	1,70	3,20	1,70	*Equal/above*	35
2	1,60	*Equal/above*	5,70	ŧ	0,06	0,06	0,10	1,20	*Below*	22
3	2,40	*Equal/above*	3,50	**	0,06	Pregnancy		2,20	*Below*	8
4	1,60	*Equal/above*	2,10	**	0,06	0,06	0,06	1,10	*Below*	34
5	2,60	*Equal/above*	6,30	1,70	0,06	0,06		2,40	*Below*	7
6	1,60	*Equal/above*	4,10	**	***	0,06	0,06	1,10	*Below*	32
7	1,70	*Equal/above*	3,70	ŧ	0,06	0,06		1,60	*Below*	6
8	1,60	*Below*	0,60	**	0,06	0,06	0,06	1,20	*Below*	21
9	1,20	*Below*	0,90	0,06	0,06	0,06		1,10	*Below*	6
10	0,80	*Equal/above*	0,80	0,06	0,06	0,06	0,06	0,60	*Below*	20
11	1,60	*Below*	1,40	***	***	0,06	0,06	1,10	*Below*	32
12	1,80	*Equal/above*	8,50	***	0,06	0,28		1,70	*Below*	7
13	1,10	*Equal/above*	3,70	ŧ	0,06	0,06		0,90	*Below*	6
14	1,20	*Below*	0,39	0,10	0,06	0,06	0,06	0,80	*Below*	31
15	2,20	*Equal/above*	6,00	1,10	**	7,70	§	1,60	*Equal/above*	29
16	1,20	*Equal/above*	2,30	0,06	0,06	0,06	0,06	0,80	*Below*	29
17	2,60	*Below*	2,00	0,25	***	0,06	0,06	2,20	*Below*	23
18	0,90	*Equal/above*	2,50	0,08	0,06	0,63	0,78	0,60	*Equal/above*	30
19	1,70	*Below*	1,10	***	***	0,06	0,06	1,30	*Below*	23
20	2,40	*Equal/above*	4,70	ŧ	2,60	***	**	1,70	*Equal/above*	28
21	2,90	*Below*	0,89	ŧ	0,06	0,66	0,19	2,60	*Below*	24
22	1,80	*Below*	0,92	0,60	0,06	0,06	0,09	1,60	*Below*	22
23	2,40	*Below*	1,80	0,12	0,06	0,06	0,10	1,80	*Below*	25
24	2,40	*Equal/above*	4,50	0,06	0,06	0,06		2,20	*Below*	7
25	0,80	*Equal/above*	3,50	0,06	0,06	0,06	0,57	0,60	*Equal/above*	26
26	3,20	*Equal/above*	5,40	0,12	***	0,57	0,68	2,90	*Below*	24
27	1,80	*Equal/above*	2,60	0,06	0,06	0,06	Pregnancy	1,70	*Below*	21
28	1,80	*Below*	1,70	0,06	0,06	0,08	0,58	1,60	*Below*	23
29	1,70	*Below*	0,19	0,06	***	0,06		1,60	*Below*	6
30	1,80	*Equal/above*	1,80	0,06	0,06	0,06		1,80	*Below*	6
31	1,10	*Below*	0,89	***	0,06	0,06	0,06	0,80	*Below*	20
32	2,20	*Equal/above*	4,50	**	0,06	***	0,11	1,80	*Below*	9
33	2,40	*Below*	1,00	0,06	0,06	0,06		2,20	*Below*	6
34	2,20	*Below*	0,59	ŧ	***	Pregnancy		2,20	*Below*	6
35	1,30	*Equal/above*	4,10	0,06	0,06	0,06	0,06	1,20	*Below*	9
36	0,90	*Below*	0,67	***	0,06	0,06		0,80	*Below*	8
37	2,90	*Equal/above*	5,50	***	0,06	0,18		2,80	*Below*	6
38	0,80	*Below*	0,15	0,06	0,06	0,06	0,06	0,70	*Below*	12

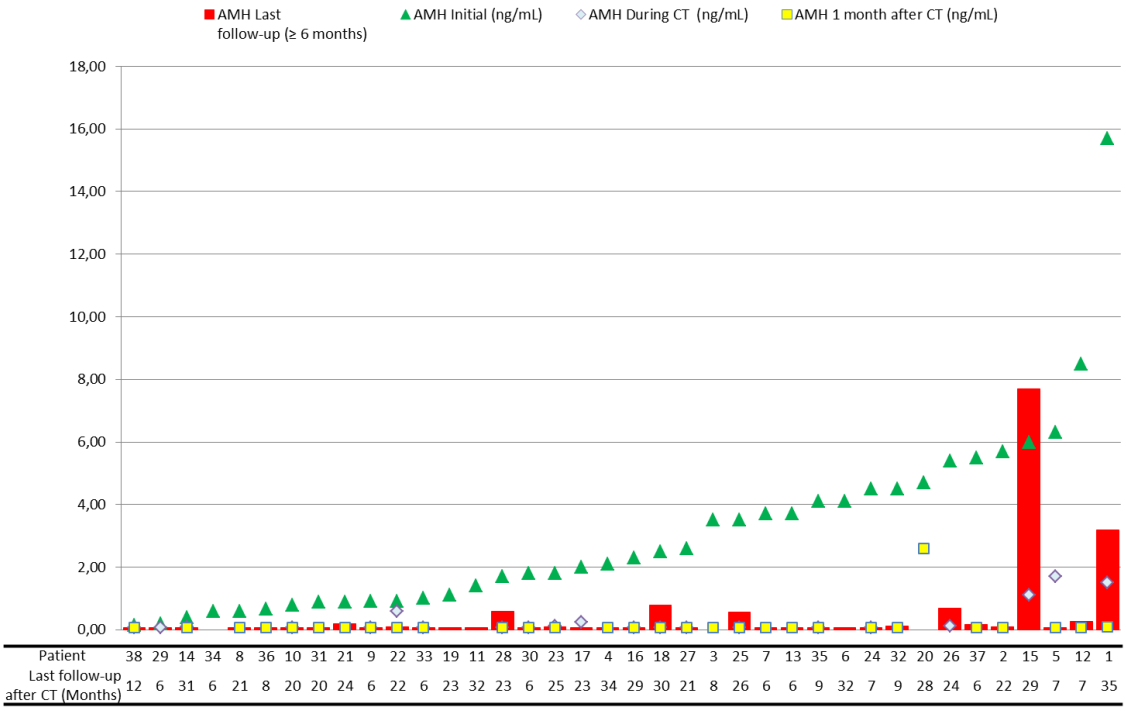
Legend: AMH - anti-Mullerian hormone; CT - chemotherapy; ŧ non-sequential taxane; ** Not assessed; ***Missed clinical appointment; § Relapse between the two last follow-ups; second line CT with T, TZM and PZM; [1] According to (Seifer, Baker et al. 2011).

Figures 4.7, 4.8 and 4.9 clearly show the persistent negative evolution of AMH levels from baseline until the last follow-up, in three different perspectives: patients are ordered according to time to last follow-up (Figure 4.7), baseline AMH levels (Figure 4.8) and age at recruitment (Figure 4.9). These graphics illustrate a tendency for higher AMH levels in patients with longer follow-up time, higher baseline AMH and younger at recruitment. These relations were confirmed by the subsequent significant correlations that were found (*vide* 3.3.1., 3.3.2 and 3.3.3). In addition, Figure 4.9 reinforces the fact that most patients presented lower than expected AMH levels, at the last follow-up.



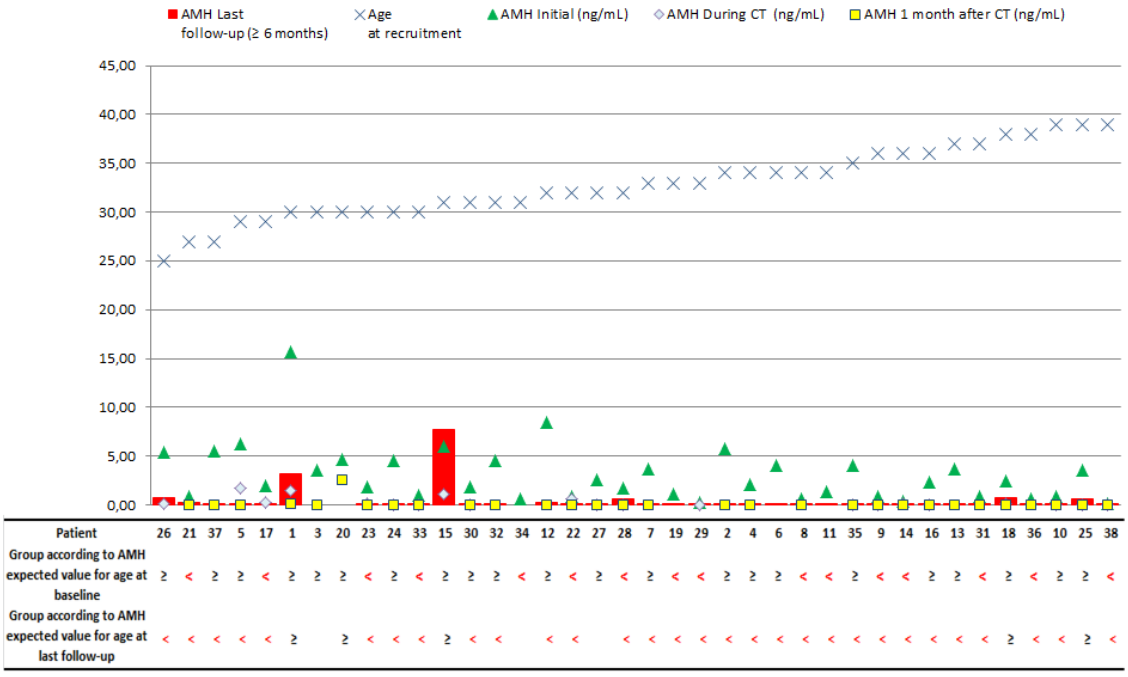
Legend: AMH - anti-Mullerian hormone; CT – chemotherapy.

Figure 4.7 Patients' AMH levels at the various time points of the study presented in ascending order with respect to time between the end of CT and the last follow-up.



Legend: AMH - anti-Mullerian hormone; CT – chemotherapy.

Figure 4.8 Patients' AMH levels at the various time points of the study presented in ascending order with respect to their baseline AMH levels.



Legend: AMH - anti-Mullerian hormone; CT – chemotherapy; ≥ Equal/above; < Below.

Figure 4.9 Patients' AMH levels at the various time points of the study and corresponding classification according to the expected value for age, presented in ascending order with respect to patient's age at recruitment.

Antral Follicle Count (AFC)

At baseline, 35 patients performed AFC and most presented normal counts considering the expected values for their age (16/35; 46%). The number of performed counts was reduced to 21, at the last available follow-up, most often because patients were under ovarian suppression with a GnRHa. Mean baseline AFC was of 10.6 ± 5.2 (median of 9), a number that progressively and significantly decreased to 2.2 ± 3.0 AF (median of 2), at the last follow-up (z= -3.9, p<0.001). The evolution of AFC in each patient, from baseline to the last follow-up, can be seen in Table 4.10. No patient recovered to their initial AFC. Patient 20 was the only who presented an AFC above the normal median value for her age, at her last follow-up (28 months after the end of CT).

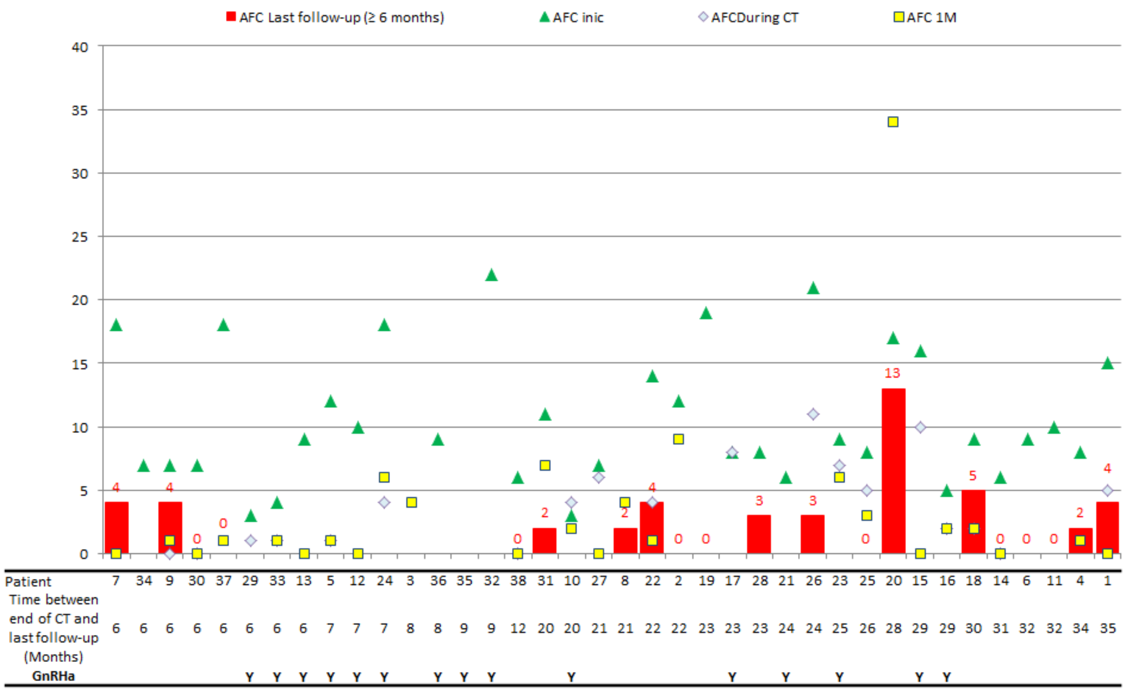
Table 4.10 Evolution of AFC, in each patient, from baseline until the last follow-up.

Patient	Age	AFC expected for age [1]	Group according to AFC expected for age at baseline	Baseline	During CT	1 month after CT	6 months after CT	Last follow-up	Age	AFC expected for age [1]	Group according to AFC expected for age at the last available follow-up	Time between end of CT and last follow-up (Months)
1	30	12,0	*Equal/above*	15	5	0	9	4	33	11,2	*Below*	35
2	34	10,9	*Equal/above*	12	ł	9	0	*	36	10,0	*Below*	22
3	30	12,0		**	***	4	**Pregnancy**		31	11,7		8
4	34	10,9	*Below*	8	***	1	0	2	37	9,6	*Below*	34
5	29	12,3	*Equal/above*	12	1	1	*		30	12,0		7
6	34	10,9	*Below*	9	***	***	0	**	37	9,6	*Below*	32
7	33	11,2	*Equal/above*	18	ł	0	4		34	10,9	*Below*	6
8	34	10,9		**	***	4	0	2	36	10,0	*Below*	21
9	36	10,0	*Below*	7	0	1	4		37	9,6	*Below*	6
10	39	8,5	*Below*	3	4	2	*	*	41	7,5		20
11	34	10,9	*Below*	10	***	***	0	*	37	9,6	*Below*	32
12	32	11,5	*Below*	10	***	0	*		33	11,2		7
13	37	9,6	*Below*	9	ł	0	*		38	9,1		6
14	36	10,0	*Below*	6	0	0	0	0	39	8,5	*Below*	31
15	31	11,7	*Equal/above*	16	10	0	*	§	34	10,9		29
16	36	10,0	*Below*	5	2	2	*	*	39	8,5		29
17	29	12,3	*Below*	8	8	***	***	*	31	11,7		23
18	38	9,1	*Equal/above*	9	2	2	5	**	41	7,5	*Below*	30
19	33	11,2	*Equal/above*	19	***	***	0	0	35	10,5	*Below*	23
20	30	12,0	*Equal/above*	17	ł	34	***	13	33	11,2	*Equal/above*	28
21	27	12,8	*Below*	6	ł	*	*	*	29	12,3		24
22	32	11,5	*Equal/above*	14	4	1	0	4	34	10,9	*Below*	22
23	30	12,0	*Below*	9	7	6	0	*	32	11,5	*Below*	25
24	30	12,0	*Equal/above*	18	4	6	**	*	31	11,7		7
25	39	8,5	*Equal/above*	8	5	3	4	0	41	7,5	*Below*	26
26	25	13,6	*Equal/above*	21	11	***	8	3	27	12,8	*Below*	24
27	32	11,5	*Below*	7	6	0	1	**Pregnancy**	33	11,2		21
28	32	11,5	*Below*	8	*	*	3	**	34	10,9	*Below*	23
29	33	11,2	*Below*	3	1	***	*		34	10,9		6
30	32	11,5	*Below*	7	0	**	0		32	11,5	*Below*	6
31	37	9,6	*Equal/above*	11	***	7	*	2	39	8,5	*Below*	20
32	31	11,7	*Equal/above*	22	***	*	***	*	32	11,5		9
33	30	12,0	*Below*	4	1	1	*		31	11,7		6
34	31	11,7	*Below*	7	ł	***	**Pregnancy**		31	11,7		6
35	35	10,5		**	***	*	*	*	36	10,0		9
36	38	9,1	*Equal/above*	9	***	*	*		39	8,5		8
37	27	12,8	*Equal/above*	18	***	1	0		28	12,5	*Below*	6
38	39	8,5	*Below*	6	0	0	0	0	40	8,0	*Below*	12

Legend: ł non-sequential taxane; * on goserelin; ** Not assessed; ***Missed clinical appointment; § Patient relapsed between the two last follow-ups and underwent second line CT with docetaxel, trastuzumab and pertuzumab; [1] According to (Almog, Shehata et al. 2011).

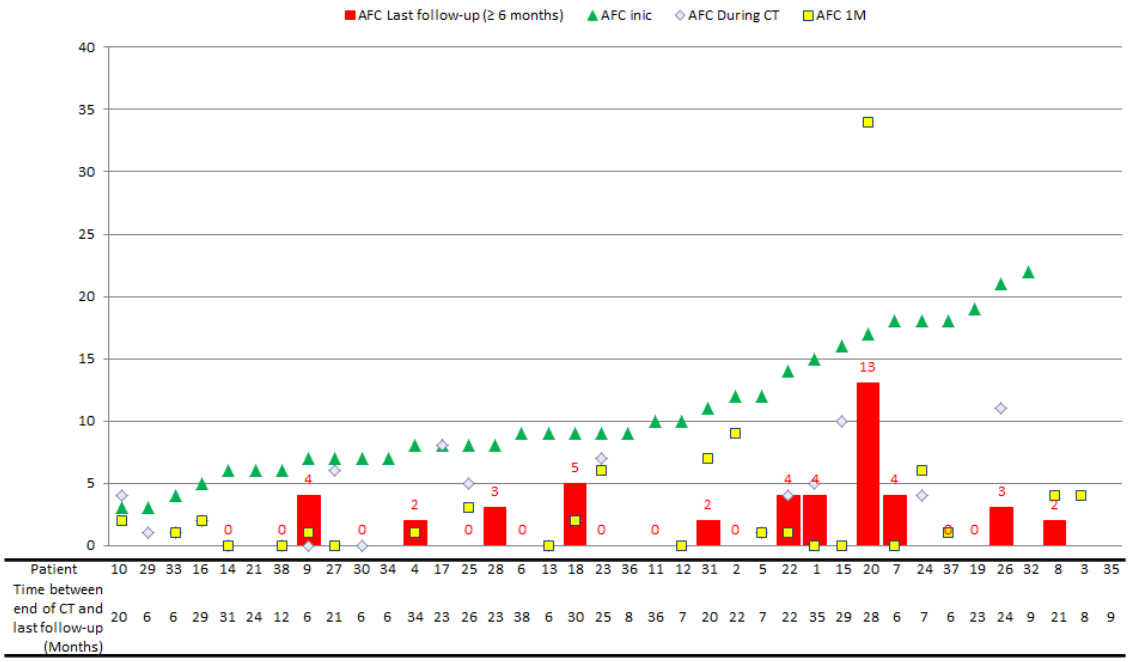
The evolution of AFC in relation to time to follow-up, baseline AFC and age at recruitment is represented in Figures 4.10 to 4.12. Data on patient's exposure to GnRHa at the last follow-up was included as AFC was not performed in this subset of patients under ovarian suppression.

A very slight tendency to a higher number of AF at the last follow-up in patients with longer follow-up (Figure 4.10) and those younger at recruitment (Figure 4.12) may be pointed, although the associations seem less significant than those found with AMH evolution. In fact, no correlations were found between these variables and AFC at the last follow-up (c.f. 3.2.1 and 3.3.3). Figure 4.11 highlights the transversally low number of AF at the last follow-up, as compared to the values that would be expected considering patients' age and irrespective of patients' baseline counts.



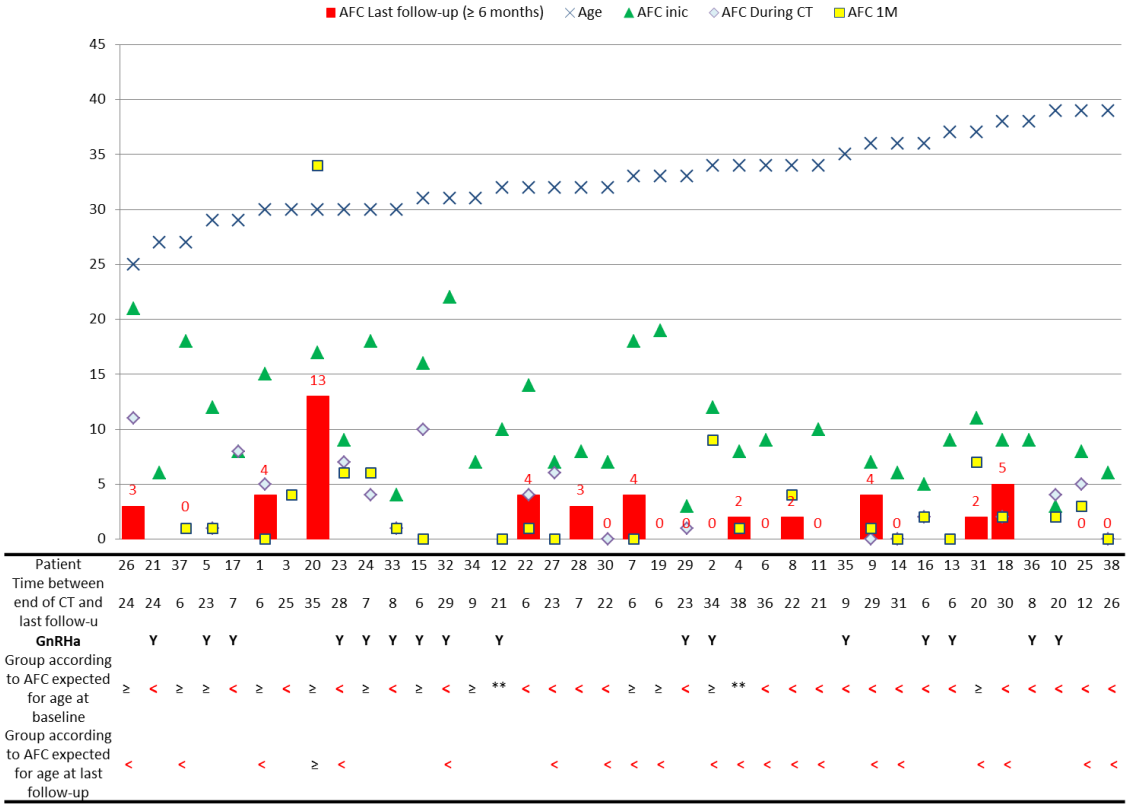
Legend: AFC - antral follicle count; CT – chemotherapy; GnRHa – gonadotropin-releasing hormone agonist.

Figure 4.10 Patients' AFC at the various time points of the study, presented in ascending order of time between the end of CT and the last follow-up.



Legend: AFC - antral follicle count; CT – chemotherapy; GnRHa – gonadotropin-releasing hormone agonist.

Figure 4.11 Patients' AFC at the various time points of the study and corresponding expected counts for age, presented in ascending order of the baseline AFC values.



Legend: AFC - antral follicle count; CT – chemotherapy; GnRHa – gonadotropin-releasing hormone agonist.

Figure 4.12 Patients' AFC at the various time points of the study, in ascending order of patient's age at recruitment.

Follicle-Stimulating Hormone

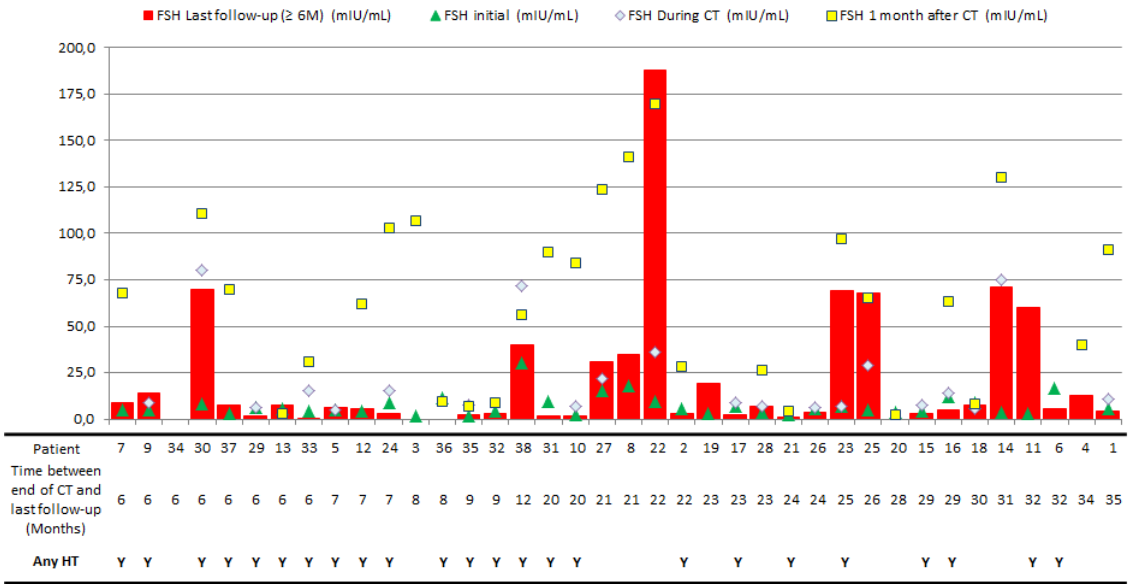
Thirty-six patients had their FSH levels assessed at baseline. Only one presented an FSH level >25 mIU/mL. Mean FSH levels at baseline were 7.1 ± 5.6 mIU/mL (median of 5.2 mIU/mL). Although a high variability was observed, these levels significantly increased at the follow-up 1 month after CT (n=28; z=-4.2, p<0.001). Considering each patient's FSH at last follow-up, a significant decrease was found comparatively to the first follow-up after CT (z= -3.8, p<0.001) and the distribution of FSH was no longer different as compared to baseline (z=-1.26; p=0.206). A detailed picture of the evolution of FSH levels throughout the study, in each patient, is presented in Table 4.11. One month after the end of CT, 20 patients presented FSH >25 mIU/mL. At the last follow-up, a minimum of six months after CT, eight participants (8/30; 27%) had levels consistent with menopausal status (>25 mIU/mL).

Table 4.11 Evolution of Follicle-Stimulating Hormone (FSH) levels in each patient, from baseline until the last follow-up.

Patient	Baseline (mIU/mL)	During CT (mIU/mL)	1 month after CT (mIU/mL)	6 months after CT (mIU/mL)	Last follow-up (mIU/mL)	Time between end of CT and last follow-up (Months)
1	5,8	10,7	91,0	6,4	4,4	35
2	5,3	ł	28,0	3,3	2,8	22
3	1,7	***	107,0	Pregnancy		8
4	**	***	40,0	37,0	13,0	34
5	4,9	5,2	**	6,4		7
6	16,9	***	***	4,5	5,6	32
7	5,2	ł	68,0	9,0		6
8	17,6	***	141,0	93,0	35,0	21
9	4,9	9,1	**	14,0		6
10	2,0	6,7	84,0	2,1	1,4	20
11	2,7	***	***	60,0	**	32
12	4,1	***	62,0	5,7		7
13	5,8	ł	2,9	7,8		6
14	3,7	75,0	130,0	147,0	71,0	31
15	4,5	7,4	**	2,9	§	29
16	12,0	14,0	63,0	4,9	**	29
17	6,9	9,0	***	***	2,0	23
18	9,0	5,4	8,2	3,3	7,6	30
19	2,8	***	***	7,3	19,0	23
20	3,8	ł	2,6	***	**	28
21	2,4	ł	4,0	1,3	**	24
22	9,5	36,0	170,0	188,0	**	22
23	6,9	6,7	97,0	69,0	**	25
24	8,8	15,0	103,0	2,7		7
25	5,0	29,0	65,0	68,0	**	26
26	5,7	6,1	***	3,5	**	24
27	15,0	22,0	124,0	31,0	Pregnancy	21
28	3,8	6,7	26,0	62,0	6,7	23
29	6,0	6,2	***	1,4		6
30	8,2	80,0	111,0	70,0		6
31	9,2	***	90,0	1,7	**	20
32	4,4	***	9,0	***	3,2	9
33	4,4	15,0	31,0	0,3		6
34	**	ł	***	Pregnancy		6
35	1,9	7,3	6,8	2,2	**	9
36	11,3	***	9,2	**		8
37	2,9	***	70,0	7,2		6
38	30,0	72,0	56,0	40,0	**	12

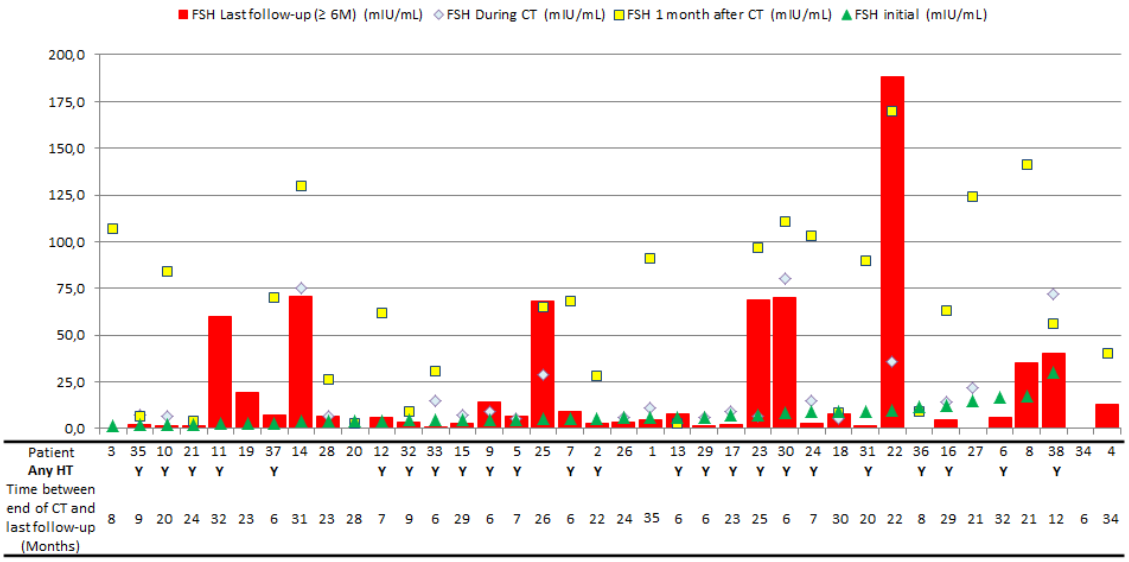
Legend: ł non-sequential taxane; * On GnRHa; ** Not assessed; ***Missed clinical appointment; § Relapse between the two last follow-ups and second line CT with docetaxel, trastuzumab and pertuzumab.

An overview of the evolution of FSH levels in relation with time, baseline levels and patients' age at recruitment can be seen in Figures 4.13 to 4.15. As compared to the previous results for AMH and AFC, FSH levels at the last follow-up seem less dependent on those variables. In fact, no significant correlations were found between FSH levels at the last follow-up and time to follow-up (ρ=0.035; p=0.881), baseline FSH (ρ=0.235; p=0.181) or patients' age (ρ=0.125; p=0.474). It is also noticeable that most patients presented lower FSH levels in the last follow-up as compared to the 1 month after CT follow-up. We have to keep in mind that many patients were still under HT at the last follow-up.



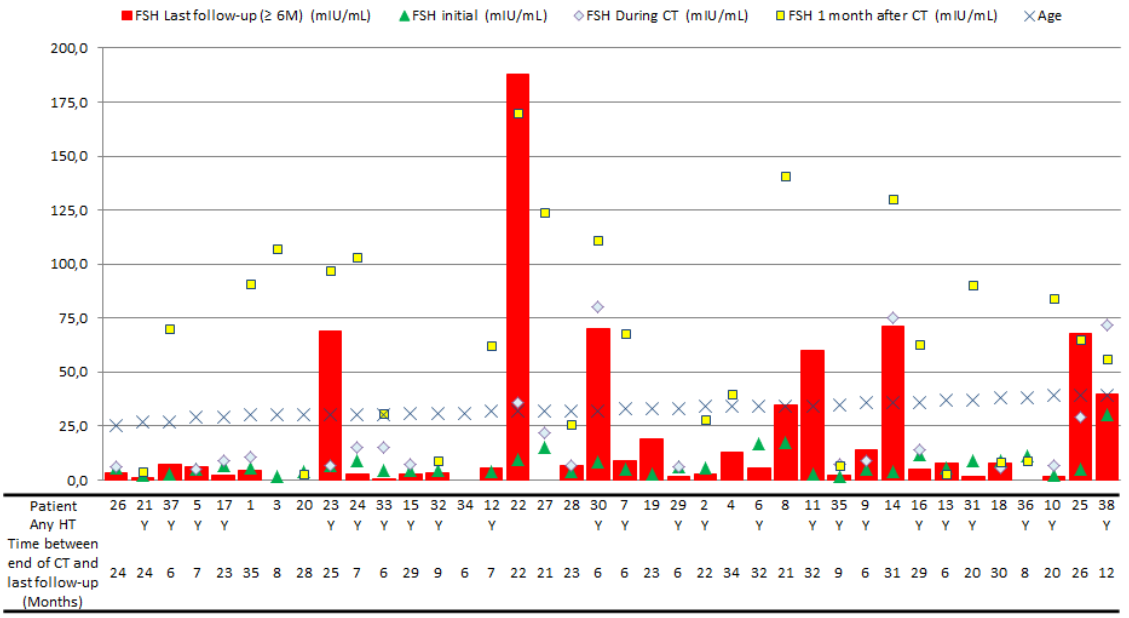
Legend: FSH - follicle-stimulating hormone; CT – chemotherapy; HT – hormonal therapy.

Figure 4.13 Patients' FSH levels at the various time points of the study, presented in ascending order of the time between the end of CT and their last follow-up.



Legend: FSH - follicle-stimulating hormone; CT – chemotherapy; HT – hormonal therapy.

Figure 4.14 Patients' FSH levels at the various time points of the study, presented in ascending order of their baseline FSH levels.



Legend: FSH - follicle-stimulating hormone; CT – chemotherapy; HT – hormonal therapy.

Figure 4.15 Patients' FSH levels at the various time points of the study, presented in ascending order of their age at recruitment.

Ovarian reserve markers at the last follow-up

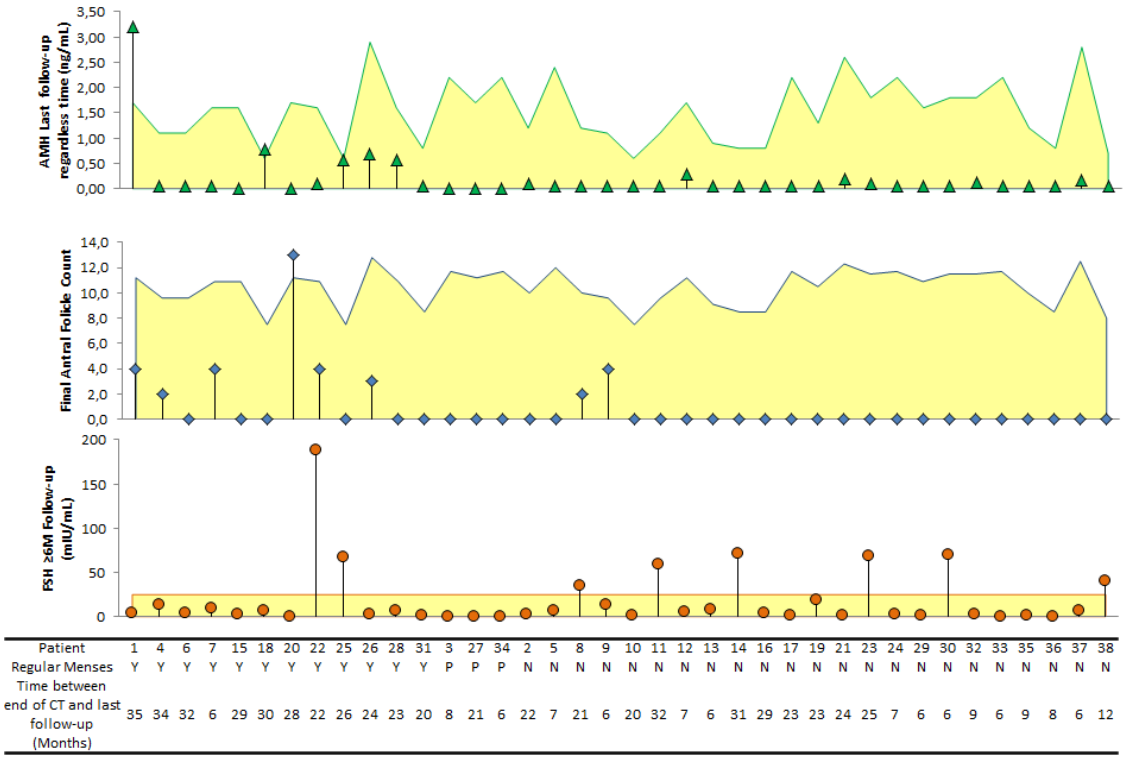
An overview of the levels of AMH, AFC and FSH at the last follow-up, in each patient, can be seen in Table 4.12. AMH and AFC were positively and significantly inter-correlated (n=21; ρ=0,429; p=0.05). Figure 4.16 gives a general insight on how the levels of OR markers compare with the corresponding levels expected according to patient's age (AMH, AFC) or the defined

cut-off value (FSH). It is clear from the observation of both Table 12 and Figure 16 that most patients presented lower than expected values of AMH (30/35; 86%) and AFC (20/21; 95%), at their last follow-up. As for FSH levels, the proportion was much less expressive (8/35; 23%). Figure 4.16 also highlights the fact that the levels of AMH and AFC were lower than expected in many of the patients who recovered regular menses.

Table 4.12 Levels of ovarian reserve markers (AMH, AFC and FSH) at each patient's last follow-up and corresponding categorization according to the corresponding expected values for age.

Patient	Age	AMH level expected for age (ng/mL)	AMH (ng/mL)	Group according to AMH level expected value for age	AFC expected for age	AFC	Group according to AFC expected for age	FSH (mIU/mL)
1	33	1,70	3,20	*Equal/above*	11,2	4	*Below*	4,4
2	36	1,20	0,10	*Below*	10,0	0	*Below*	2,8
3	31	2,20	Pregnancy		11,7	Pregnancy		Pregnancy
4	37	1,10	0,06	*Below*	9,6	2	*Below*	13,0
5	30	2,40	0,06	*Below*	12,0	*		6,4
6	37	1,10	0,06	*Below*	9,6	0	*Below*	5,6
7	34	1,60	0,06	*Below*	10,9	4	*Below*	9,0
8	36	1,20	0,06	*Below*	10,0	2	*Below*	35,0
9	37	1,10	0,06	*Below*	9,6	4	*Below*	14,0
10	41	0,60	0,06	*Below*	7,5	*		1,4
11	37	1,10	0,06	*Below*	9,6	0	*Below*	60,0
12	33	1,70	0,28	*Below*	11,2	*		5,7
13	38	0,90	0,06	*Below*	9,1	*		7,8
14	39	0,80	0,06	*Below*	8,5	0	*Below*	71,0
15	34	1,60	7,60	*Equal/above*	10,9	*		2,9
16	39	0,80	0,06	*Below*	8,5	*		4,9
17	31	2,20	0,06	*Below*	11,7	*		2,0
18	41	0,60	0,78	*Equal/above*	7,5	5	*Below*	7,6
19	35	1,30	0,06	*Below*	10,5	0	*Below*	19,0
20	33	1,70	2,60	*Equal/above*	11,2	13	*Equal/above*	2,6
21	29	2,60	0,19	*Below*	12,3	*		1,3
22	34	1,60	0,09	*Below*	10,9	4	*Below*	188,0
23	32	1,80	0,10	*Below*	11,5	0	*Below*	69,0
24	31	2,20	0,06	*Below*	11,7	*		2,7
25	41	0,60	0,57	*Equal/above*	7,5	0	*Below*	68,0
26	27	2,90	0,68	*Below*	12,8	3	*Below*	3,5
27	33	1,70	Pregnancy		11,2	Pregnancy		Pregnancy
28	34	1,60	0,58	*Below*	10,9	3	*Below*	6,7
29	34	1,60	0,06	*Below*	10,9	*		1,4
30	32	1,80	0,06	*Below*	11,5	0	*Below*	70,0
31	39	0,80	0,06	*Below*	8,5	2	*Below*	1,7
32	32	1,80	0,11	*Below*	11,5	*		3,2
33	31	2,20	0,06	*Below*	11,7	*		0,3
34	31	2,20	Pregnancy		11,7	Pregnancy		Pregnancy
35	36	1,20	0,06	*Below*	10,0	*		2,2
36	39	0,80	0,06	*Below*	8,5	*		9,2
37	28	2,80	0,18	*Below*	12,5	0	*Below*	7,2
38	40	0,70	0,06	*Below*	8,0	0	*Below*	40,0

Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; FSH – Follicle-stimulating Hormone; * Under ovarian suppression



Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; CT – chemotherapy; FSH – Follicle-stimulating Hormone; N – No; Y - Yes

Figure 4.16 Patients' levels of OR markers at the last follow-up, ordered according to menstruation status (Y/N), and their comparison with the corresponding expected levels for age (AMH, AFC) or the cut-off value for menopause (FSH>25 mIU/mL).

3.2.3. Secondary reproductive health outcomes

3.2.3.1. Recovery of ovarian function

At their last available follow-up, ten women had not recovered their ovarian function (10/19; 53%) (Table 4.13). Their ages varied between 30 and 39 years and nine of them had at least one OR marker below the level that would be expected for their age, at recruitment. Two of these patients had not preserved fertility before CT. Five of them reported amenorrhea, three irregular menses and all presented AMH levels below the LoQ and/or FSH levels consistent with ovarian failure. The two patients who recovered regular menses had their OR markers altered, particularly the levels of FSH. A total of nineteen patients (n=19) were not evaluated for recovery of ovarian function as they were under HT and presented premenopausal levels of FSH.

Table 4.13 Ovarian reserve markers, secondary reproductive health outcomes and treatments for the ten patients who had not recovered ovarian function at their last follow-up.

				Reproductive health outcomes								FP	Treatments		
				Primary outcomes					Secondary outcomes						
Patient	Age at baseline (Y)	Baseline OR markers according to age AMH/AFC	Age at last follow-up (Y)	AMH (ng/mL)	AFC	FSH 1M/6M (mIU/mL)	FSH ≥9M (mIU/mL)	Regular Menses	Recovery of ovarian function	Primary Ovarian Insufficiency (POI)	Other outcomes		TT	Any HT	GnRHa
6	34	Above/Below	37	0,06	0	4,5	5,6	N	N	N					
8	34	Below/**	36	0,06	2	93,0	35,0	N	N	Y	Oligomenorrhea				
11	34	Below/Below	37	0,06	0	60,0	**	N	N	Y					
14	36	Below/Below	39	0,06	0	147,0	71,0	N	N	Y	Oligomenorrhea	N			
19	33	Below/Above	35	0,06	0	7,3	19,0	N	N	N	Oligomenorrhea	N			
22	32	Below/Above	34	0,09	4	170,0	188,0	Y	N	N					
23	30	Below/Below	32	0,10	*	97,0	69,0	N	N	N.E.					
25	39	Above/Above	41	0,57	0	65,0	68,0	Y	N	N					
30	32	Above/Below	32	0,06	0	111,0	70,0	N	N	Y					
38	39	Below/Below	40	0,06	0	56,0	40,0	N	N	Y					

Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; FP – fertility preservation; FSH – Follicle-stimulating Hormone; GnRHa – gonadotropin-releasing hormone agonist; HT – hormonal therapy; OR – ovarian reserve; POI – premature ovarian insufficiency; TT – targeted therapy; Y – years; * Under ovarian suppression; ** Not assessed.

In contrast, nine women had recovered ovarian function at their last follow-up (9/19; 47%) (Table 4.14). They were aged 25 to 38 years at recruitment (27-41 years at their last follow-up) and most presented normal/high levels of OR at baseline. The oldest patient, who was 41 years old at the last follow-up, had a normal to high OR at baseline (AMH 2.5 ng/mL and 9 AF).

Table 4.14 Ovarian reserve markers, secondary reproductive health outcomes and treatments for the nine patients who had recovered ovarian function at their last follow-up.

				Reproductive health outcomes								Treatments		
				Primary outcomes					Secondary outcomes					
Patient	Age at baseline (Y)	Baseline OR markers according to age AMH/AFC	Age at last follow-up (Y)	AMH (ng/mL)	AFC	FSH 1M/6M (mIU/mL)	FSH ≥9M (mIU/mL)	Regular Menses	Recovery of ovarian function	Primary Ovarian Insufficiency (POI)	Other outcomes	TT	Any HT	GnRHa
1	30	Above/Above	33	3,20	4	6,4	4,4	Y	Y	N	Pregnancy			
3	30	Above/**	31	Pregnancy					Y	N	Pregnancy			
4	34	Above/Below	37	0,06	2	37,0	13,0	Y	Y	N				
18	38	Above/Above	41	0,78	5	3,3	7,6	Y	Y	N				
20	30	Above/Above	33	2,6	13	2,6	**	Y	Y	N				
26	25	Above/Above	27	0,68	3	***	3,5	Y	Y	N				
27	32	Above/Below	33	Pregnancy					Y	N	Pregnancy			
28	32	Below/Below	34	0,58	3	62	6,7	Y	Y	N				
34	31	Below/Below	31	Pregnancy					Y	N	Pregnancy			

Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; FP – fertility preservation; FSH – Follicle-stimulating Hormone; GnRHa – gonadotropin-releasing hormone agonist; HT – hormonal therapy; OR – ovarian reserve; POI – premature ovarian insufficiency; TT – targeted therapy; Y – years; * Under ovarian suppression; ** Not assesse; *** - missed clinical appointment.

Four of these women got pregnant at some point after CT (Table 4.15). Two of them were under therapy with trastuzumab at the time of pregnancy and reported spontaneous and medically-induced abortions. Pregnancy outcome was not known in the other two patients. Three of the woman who got pregnant had normal/high levels of OR markers at baseline. The only patient with low OR at baseline was treated to a taxane-only CT regimen, in opposition to the other three who were treated with an anthracycline-based and sequential taxane regimen.

None of the four pregnant women had hormone-positive tumours and, consequently, none received treatment with HT. After CT, two of these women presented AMH below the LoQ and three of them reached FSH levels well above the value of 25 mIU/mL.

Table 4.15 Detailed demographic, clinical and reproductive data for the four women who got pregnant after CT.

		Patient 1	Patient 3	Patient 27	Patient 34
Demographic and Clinical data	Age	30	30	32	31
	FP technique	Oocyte cryopreservation	Oocyte + embryo cryopreservation	Oocyte cryopreservation	_
	Tumour biology	HER2 positive	HER2 positive	Triple-negative	HER2 positive
	CT regimen	FEC → T	FEC → T	DD AC → weekly Paclitaxel	Weekly Paclitaxel
	CT Number of Cycles	3 → 3	3 → 3	4 → 12	12
	Targeted therapy	Trastuzumab	Trastuzumab	No	Trastuzumab
	Hormonal therapy	No	No	No	No
Baseline	Group according to expected value for age AMH/AFC	*Above/Above*	*Above/***	*Above/Below*	*Below/Below*
	AMH (ng/mL)	15,7	3,5	2,6	0,59
	AFC	15	**	7	7
	FSH (mIU/mL)	5,8	1,7	15,0	**
1 month after CT	AMH (ng/mL)	0,1	0,06	0,06	***
	AFC	0	4	0	***
	FSH (mIU/mL)	91,0	107,0	124,0	***
	Menses	No	No	No	***
6 months after CT	AMH (ng/mL)	1,7	***	0,06	_
	AFC	9	***	1	_
	FSH (mIU/mL)	6,4	***	31	_
	Menses	Yes	***	Yes	_
Last follow-up	AMH (ng/mL)	3,2	_	***	_
	AFC	4	_	***	_
	FSH (mIU/mL)	4,36	_	***	_
	Menses	Yes	_	***	_
	Time between end of CT and last follow-up (Months)	35	8	21	6
Pregnancy	Time of Pregnancy	8 months after the end of CT	8 months after the end of CT	14 months after the end of CT	3 months after the end of CT
	Pregnancy outcome	Spontaneous abortion	Medical abortion	_	_

Legend: AC – Adriamycin and cyclophosphamide; AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; DD – dose dense; FEC - fluorouracil, epirubicin and cyclophosphamide FP – fertility preservation; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone agonist; Her2 - HT – hormonal therapy; OR – ovarian reserve; T – docetaxel; * Under ovarian suppression; ** Not assessed; *** - missed clinical appointment.

3.2.3.2. Premature Ovarian Insufficiency

From the patients who did not recover ovarian function, a total of five (5/22; 23%) met the defined criteria for POI, an outcome that was only assessed in patients who were not under therapy with GnRHa. Patients with POI had ages between 32 and 39 years at recruitment (36-40 years at their last follow-up). Their levels of AMH remained below the LoQ and FSH was persistently high. One of them had not preserved her fertility before CT (Table 4.16).

Table 4.16 Detailed demographic, clinical and reproductive data for the five women who met criteria for Premature Ovarian Insufficiency.

		Patient 8	Patient 11	Patient 14	Patient 30	Patient 38
Demographic and Clinical data	Age	34	34	36	32	39
	FP technique	Oocyte cryopreservation	Oocyte cryopreservation	_	Oocyte cryopreservation	Oocyte cryopreservation
	Tumour biology	Triple-negative	Triple-positive	Triple-negative	Triple-positive	Triple-positive
	CT regimen	AC → T	FEC → T	FEC → T	FEC → T	FEC → weekly Paclitaxel
	CT Number of Cycles	4 → 4	3 → 3	3 → 3	3 → 3	4 → 12
	Targeted therapy	No	Trastuzumab	No	Trastuzumab	Trastuzumab
	Hormonal therapy	No	GnRHa	No	Tam	Tam
Baseline OR	Group according to expected value for age (AMH/AFC)	*Below/Below*	*Below/Below*	*Below/Below*	*Above/Below*	*Below/Below*
	AMH (ng/mL)	0,6	1,4	0,39	1,8	0,15
	AFC	_	10	6	7	6
	FSH (mIU/mL)	17,6	2,7	3,7	8,2	30
1 month after CT	AMH (ng/mL)	0,06	***	0,06	0,06	0,06
	AFC	4	***	0	_	0
	FSH (mIU/mL)	141	***	130	111	56
	Menses	No	***	No	No	No
6 months after CT	AMH (ng/mL)	0,06	0,06	0,06	0,06	0,06
	AFC	0	0	0	0	0
	FSH (mIU/mL)	93	60	147	70	40
	Menses	No	No	No	No	No
Last follow-up	AMH (ng/mL)	0,06	0,06	0,06	0,06	0,06
	AFC	2	0	0	0	0
	FSH (mIU/mL)	35	**	71	70	40
	Menses	Oligomenorrhea	No	Oligomenorrhea	No	No
	Time between end of CT and last follow-up (Months)	21	32	31	6	12

Legend: AC – Adriamycin and cyclophosphamide; AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; DD – dose dense; FEC - fluorouracil, epirubicin and cyclophosphamide FP – fertility preservation; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone agonist; Her2 - HT – hormonal therapy; OR – ovarian reserve; T – docetaxel; Tam – tamoxifen; * Under ovarian suppression; ** Not assessed; *** - missed clinical appointment.

A comparison of the characteristics and mean OR markers between the five patients with POI (n=5) and the four patients who got pregnant (n=4) can be seen in Table 4.17. Clearly, women identified with POI were older and presented a lower OR at baseline.

Table 4.17 Comparison of the characteristics of women who met criteria for POI and of women who got pregnant.

Characteristic	Patients with POI (n=5)	Patients who got pregnant (n=4)
Age at recruitment (years) Mean ± SD	35.0 ± 2.6	30.8 ± 1.0
Tumour receptor status	Triple negative tumour (n=2) Triple positive tumour (n=3)	Triple negative tumour (n=1) Her2-positive tumour (n=3)
AMH at baseline (ng/mL) Mean ± SD	0.9 ± 0.7	5.6 ± 6.8
AFC at baseline Mean ± SD (range)	7.2 ± 1.9 (6-10)	9.7 ± 4.6 (7-15)
FSH at baseline (mIU/mL) Mean ± SD	7.5 ± 6.8	12.4 ± 11.4
Group (expected AMH/AFC for age)	Low/Low (n=3); Low/** (n=1); Normal/Low (n=1)	Normal/Normal (n=1); Normal/** (n=1); Normal/Low (n=1); Low/Low (n=1)
Motherhood and FP	2 patients had children and one of them decided not to undergo FP; 3 patients were childless; all underwent FP	1 patient had children and decided not to undergo FP; 3 patients were childless; all underwent FP
Treatment type	CT only (n=2) CT, HT and trastuzumab (n=3)	CT only (n=1) CT and trastuzumab (n=3)
CT regimen	Anthracycline-based regimen and sequential taxane (n=5)	Anthracycline-based regimen and sequential taxane (n=3) Taxane-only regimen (n=1)
Other relevant characteristics (smoker status, weight, history of infertility)	Past smoker (n=2) Overweight (n=1) Personal history of infertility (n=1)	Past smoker (n=1) Overweight (n=1)

Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; FP – fertility preservation; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone agonist; Her2 - HT – hormonal therapy; OR – ovarian reserve; POI – Premature Ovarian Insufficiency; T – docetaxel; * Under ovarian suppression; ** Not assessed; *** - missed clinical appointment.

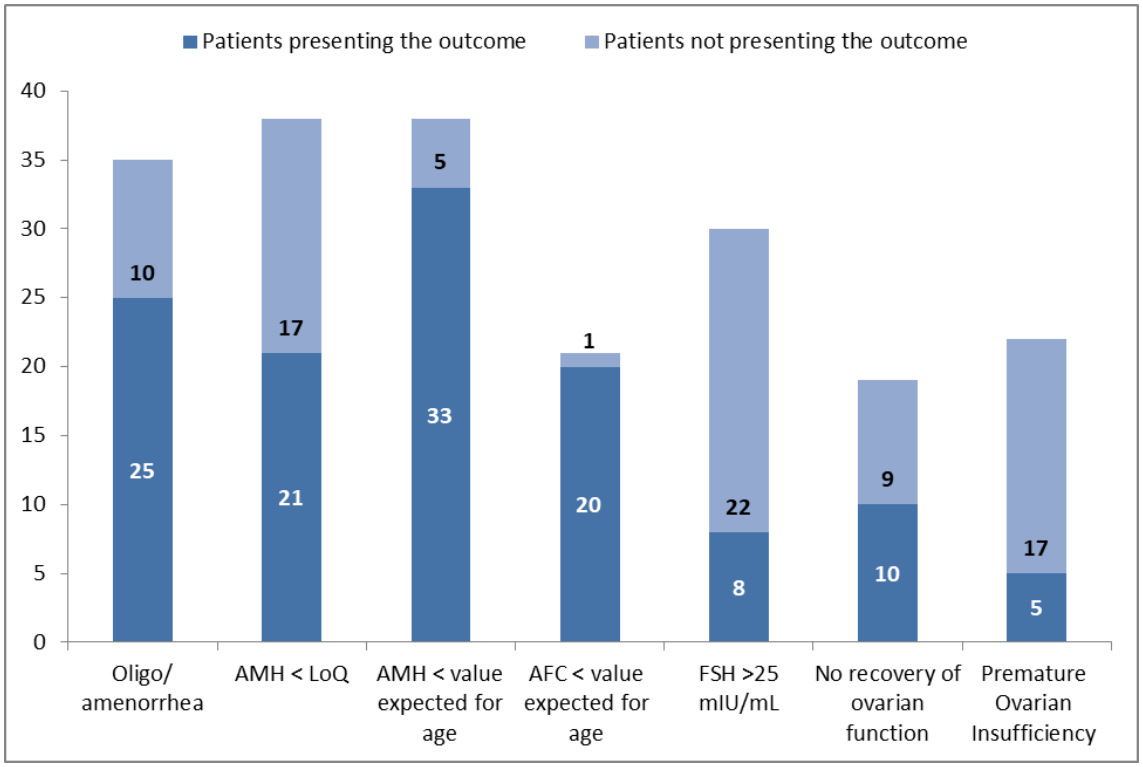
3.2.3.3. Overall adverse reproductive health outcomes

A summary of the various adverse reproductive health outcomes identified in this cohort of young BC women, at a mean of 18 months after CT, is presented in Table 4.18. In addition, Figure 4.17 puts in evidence the corresponding proportions, according to the total number of patients assessed for each outcome. A representation of the relationship between these secondary outcomes and the corresponding primary outcomes (occurrence of regular menses and levels of OR markers) at each patient's last follow-up, can be seen in Figure 4.18.

Table 4.18 Percentage of patients in which the several adverse reproductive health outcomes were identified, at the last follow-up.

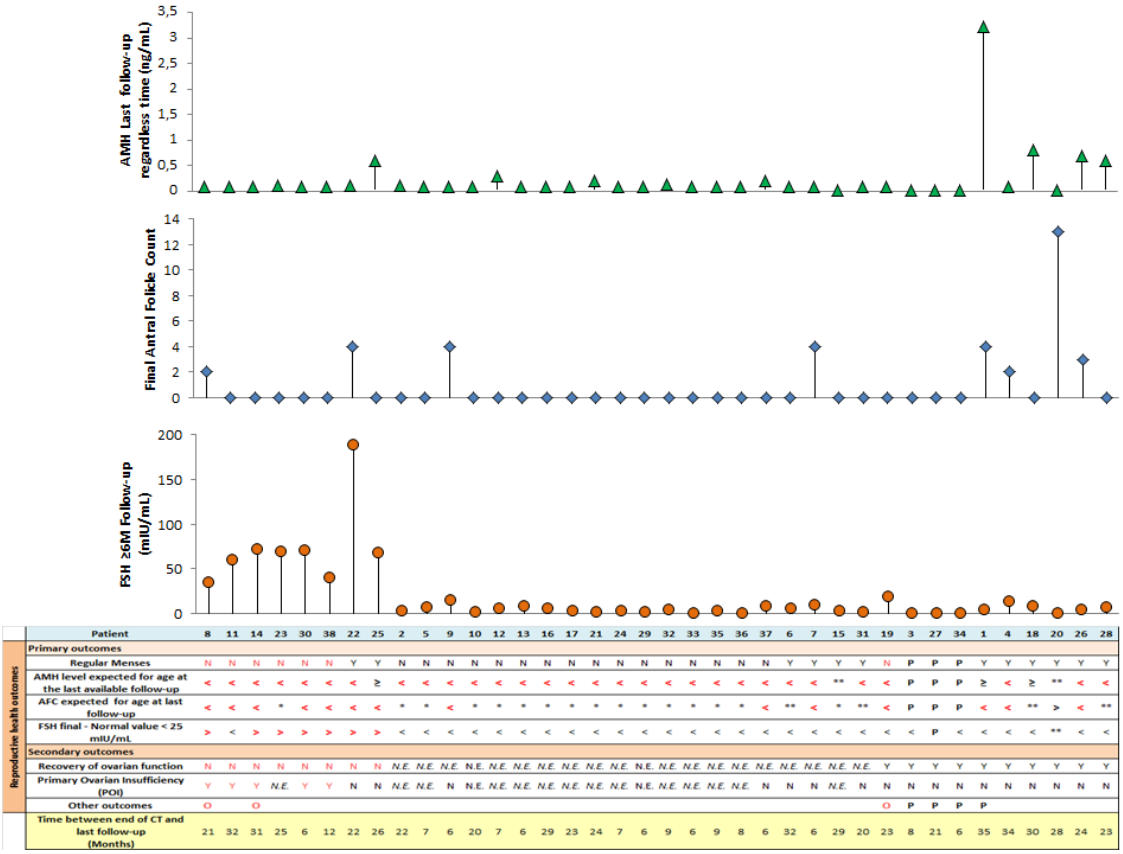
Adverse outcome	Oligo/ amenorrhea	AMH < LoQ	AMH < value expected for age	AFC < expected for age	FSH >25 mIU/mL	No recovery of ovarian function	Premature Ovarian Insufficiency
% of patients presenting the outcome	71%	55%	91%	95%	27%	53%	23%
(total n)	n=35	n=38	n=38	n=21	n=30	n=19	n=22

Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; FSH – Follicle-Stimulating Hormone; LoQ – Limit of Quantification



Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; FSH – Follicle-Stimulating Hormone; LoQ – Limit of Quantification

Figure 4.17 Number and proportion of patients presenting each adverse reproductive health outcome, at the last follow-up.



	Patient	8	11	14	23	30	38	22	25	2	5	9	10	12	13	16	17	21	24	29	32	33	35	36	37	6	7	15	31	19	3	27	34	1	4	18	20	26	28
Reproductive health outcomes	Primary outcomes																																						
	Regular Menses	N	N	N	N	N	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	Y	N	P	P	P	Y	Y	Y	Y	Y
	AMH level expected for age at the last available follow-up	<	<	<	<	<	<	<	≥	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	**	<	<	P	P	P	≥	<	≥	**	<	<
	AFC expected for age at last follow-up	<	<	<	*	<	<	<	<	*	*	<	*	*	*	*	*	*	*	*	*	*	*	*	*	<	**	<	*	**	<	P	P	P	<	<	**	>	<	**
	FSH final - Normal value < 25 mIU/mL	>	<	>	>	>	>	>	>	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	P	<	<	<	<	**	<	<	<
	Secondary outcomes																																						
	Recovery of ovarian function	N	N	N	N	N	N	N	N	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	Y	Y	Y	Y	Y	Y	Y	Y	Y
	Primary Ovarian Insufficiency (POI)	Y	Y	Y	N.E.	Y	Y	N	N	N.E.	N.E.	N	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N	N	N	N.E.	N	N	N	N	N	N	N	N	N	N
	Other outcomes	O		O																										O	P	P	P	P					
	Time between end of CT and last follow-up (Months)	21	32	31	25	6	12	22	26	22	7	6	20	7	6	29	23	24	7	6	9	6	9	8	6	32	6	29	20	23	8	21	6	35	34	30	28	24	23

Legend: AFC – Antral Follicle Count; AMH – Anti-Mullerian Hormone; CT – chemotherapy; FSH – Follicle-Stimulating Hormone;

Figure 4.18 Secondary reproductive health outcomes, menstrual status and corresponding levels of OR markers, at each patient's last follow-up.

3.3. Patient-related factors and adverse reproductive health outcomes

3.3.1. Age at recruitment

In alignment with the trend previously seen in Figure 4.9, age at recruitment was negatively correlated with the levels of AMH at the last follow-up (n=35; ρ=-0,377; p=0,026), i.e. younger patients presented significantly higher AMH levels. Moreover, AMH levels at the last follow-up were significantly higher in the group of younger patients (n= 16; age <33 years) as compared to those aged 33 or more years (n=19) and this difference was significant (U=70.5; p=0.002). In opposition, no significant correlation of patients' age with final AFC was found (n=21; ρ=-0,183; p=0,427).

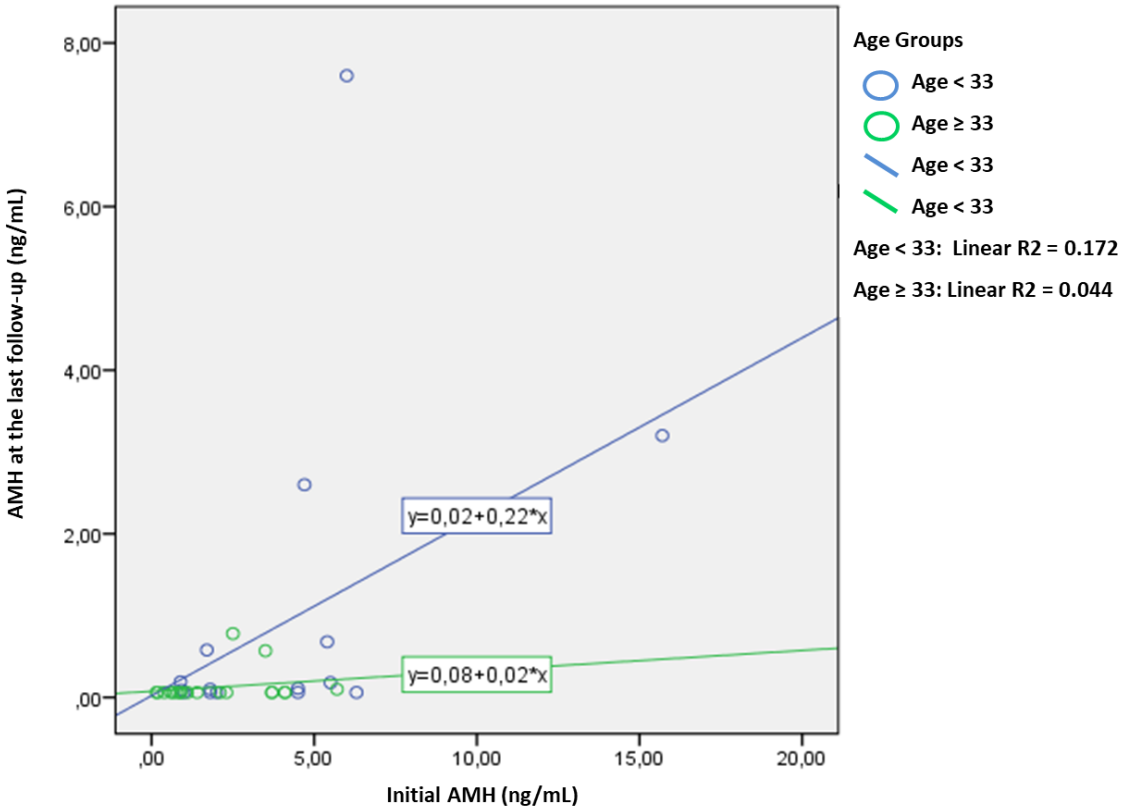
In this cohort of women with BC, age at recruitment was significantly lower in patients who recovered ovarian function as compared to those who did not recover (U=20; p=0,038) (Table 4.19). However, ages of women in which POI was identified or not, women reporting or not regular menses and of those with/without FSH > 25 mIU/mL at the last follow-up were not significantly different.

Table 4.19 Mean and median age at recruitment in patients who recovered and in those who did not recover ovarian function.

	Age at recruitment (years) Mean ± SD (Median)
Patients who recovered ovarian function (n=9)	31.3 ± 3.5 (31)
Patients who did not recover ovarian function (n=10)	34.3 ± 2.9 (34)

3.3.2. Baseline ovarian reserve

A significant positive correlation between AMH levels at baseline and at the last follow-up was found (n=35; ρ=0,517; p=0,001) a fact that confirms the previous analysis of Figure 4.8. Although statistical significance was not reached, a subgroup analysis found that this correlation was stronger in the subgroup of younger patients (age <33 years; n=19; ρ=0,414; p=0,111) as compared to the older patients (age ≥33 years; n=19; ρ=0,209; p=0,390). The corresponding scatterplots for these two correlations can be seen in Figure 4.19.



Legend: AMH – Anti-Mullerian Hormone

Figure 4.19 Scatterplots for correlations between baseline and final AMH levels in the age subgroups according to the cohort's median age (33 years).

On the contrary, no significant correlation was found between baseline and final AFC (n=20; *ρ*=0,275; p=0,241). Levels of AMH at the last follow-up were also higher in the subgroup of patients with a baseline AMH above the median value for the cohort (2.2 ng/mL), as compared to those with baseline AMH below this median value.

Baseline AMH levels also influenced the likelihood of recovering ovarian function, experiencing POI or reaching postmenopausal FSH levels: baseline AMH levels were significantly higher in the subgroup of women who recovered ovarian function (n=19; U=19.5; p=0.037) and significantly lower in patients with POI or menopausal FSH levels at the last follow-up (n=22; U=12, p=0.017 and n=19; U=15, p=0.022, respectively) (Table 4.20). After excluding patients under ovarian suppression, differences in baseline AMH were not significant in women who were menstruating as compared to those reporting amenorrhea or oligomenorrhea at the last follow-up. Significant differences in AFC at baseline were only found between the groups of patients with/without POI at the last follow-up (U=9.5; p=0.032).

Table 4.20 Mean and median AMH levels in the groups of patients who recovered or did not recover ovarian function, identified or not with POI and with or without FSH>25 mIU/mL.

Groups of patients	**Baseline AMH** (ng/mL) Mean ± SD (Median)
Patients who recovered ovarian function (n=9)	4.31 ± 4.52 (2.60)
Patients who did not recover ovarian function (n=10)	1.58 ± 1.30 (1.25)
Patients with POI (n=5)	0.87 ± 0.70 (0.60)
Patients without POI (n=17)	3.49 ± 3.53 (2.60)
Patients with FSH >25 mIU/mL (n=8)	1.32 ± 1.08 (1.16)
Patients with FSH ≤ 25 mIU/mL (n=27)	3.14 ± 3.04 (2.10)

Legend: AMH – Anti-Mullerian Hormone; FSH – follicle-stimulating hormone.

3.4. Treatment-related factors and adverse reproductive health outcomes

3.4.1. Chemotherapy regimens

No significant differences were found in OR levels at the last follow-up when comparing the two main groups of CT regimens (anthracycline-based and sequential taxane *versus* taxane-based) nor between more specific subgroups (FEC-taxane, EC/AC-taxane, taxane without anthracycline, taxane-only and others) (Table 4.21). However, in the group of patients treated with sequential taxane, levels of AMH were significantly higher before exposure to taxane (during CT) than at the subsequent follow-up, 1 month after CT (n=17; z=-2.2; p=0.028).

In relation to the length of chemotherapy, no significant correlations were found between duration of exposure in weeks and the levels of AMH or AFC at the last follow-up.

Table 4.21 Mean and median AMH levels at the last follow-up in the five subgroups of CT regimens.

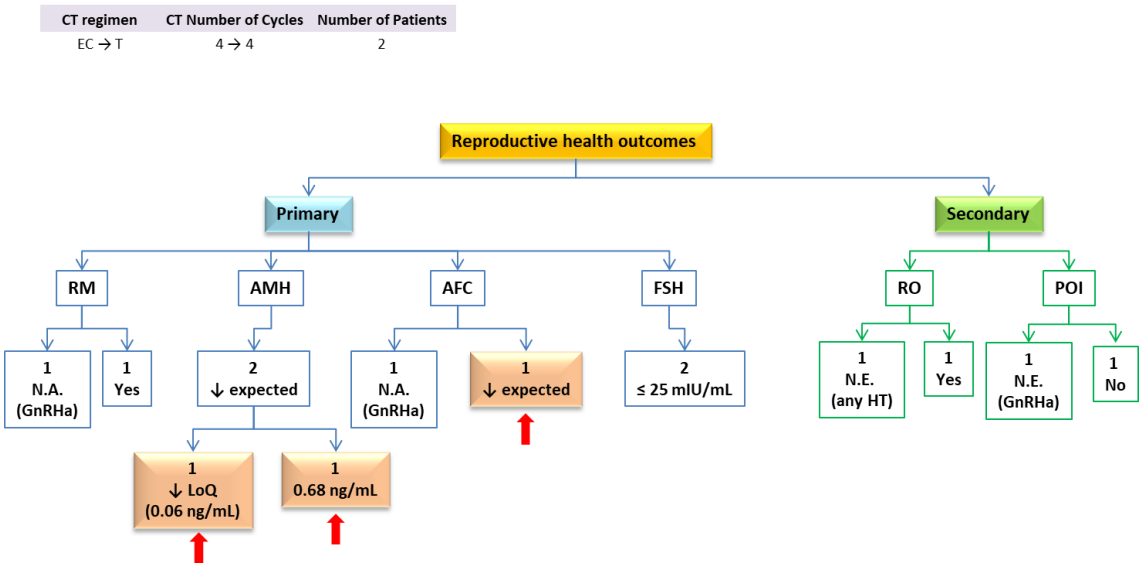
CT regimen subgroup	n	AMH Mean ± SD (ng/mL)	AMH Median (ng/mL)
FEC - taxane	23	0.62 ± 1.66	0.06
EC/AC-taxane	7	0.15 ± 0.23	0.06
Taxane only	1	2.60	2.60
Taxane without anthracycline	3	0.12 ± 0.07	0.10
Other (T-TE)	1	0.06	0.06
Total	35	0.52 ± 1.40	0.06

Legend: AC – Adriamycin and cyclophosphamide; AMH – anti-Mullerian hormone; CT – Chemotherapy; EC - epirubicin and cyclophosphamide; FEC - Fluorouracil, epirubicin and cyclophosphamide; SD – standard deviation; T – docetaxel; TE – docetaxel and epirubicin.

The adverse reproductive health outcomes that were observed after exposure to each specific CT regimen are presented in detail in Figures 4.20 to 4.31. The only regimen in which no negative outcome was identified was the *paclitaxel-only* regimen. In this group (n=2), one patient got pregnant and the other recovered regular menses and normal expected OR levels (Figure 4.27).

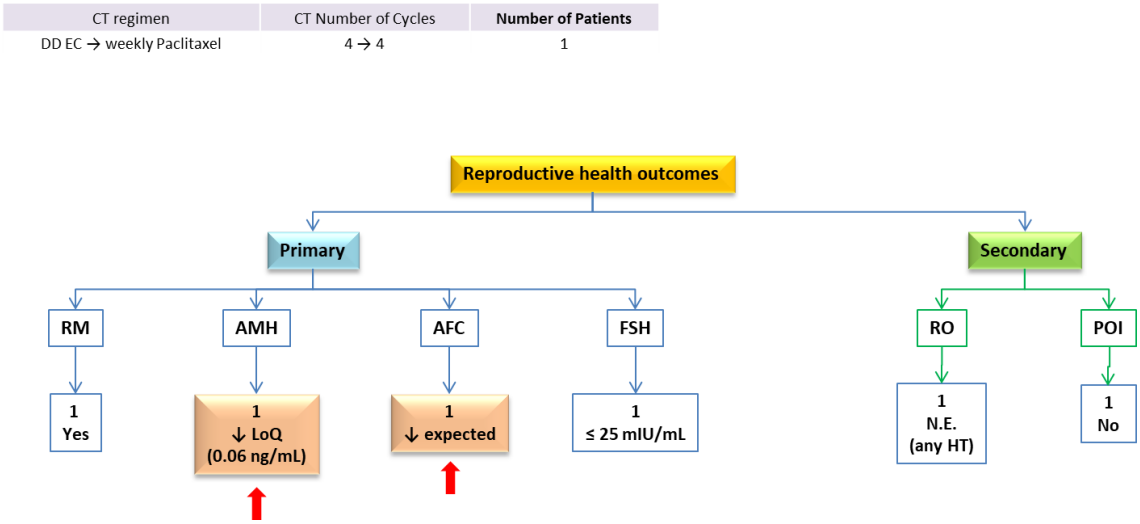
In the group of patients exposed to dose-dense (DD) regimens (*DD EC/DD AC – paclitaxel*), one got pregnant and the other recovered regular menses although her AMH levels and AFC remained low. Ovarian function recovery was not evaluated in these two patients because of exposure to HT (Figures 4.21 and 4.22). The patient with the "regular" *AC-paclitaxel* regimen met the criteria for ovarian function recovery but she did not recover to the expected levels of AMH and AFC (Figure 4.23). All patients who did not recover ovarian function had been treated

with triple-drug CT regimens (alkylating agent, anthracycline and taxane), namely *3 FEC – 3 T* (n=6), *4 AC - 4T* (n=3) and *FEC – weekly paclitaxel* (n=1) (Figures 4.24, 4.30 and 4.31). Among the five women who met criteria for POI, most (n=3) had been exposed to the *3 FEC – 3 T* regimen (Figure 4.31).



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; EC – epirubicin and cyclophosphamide; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone; HT – hormonal therapy; LoQ – limit of quantification; N.A. – not applicable; N.E. - not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function; T - docetaxel.

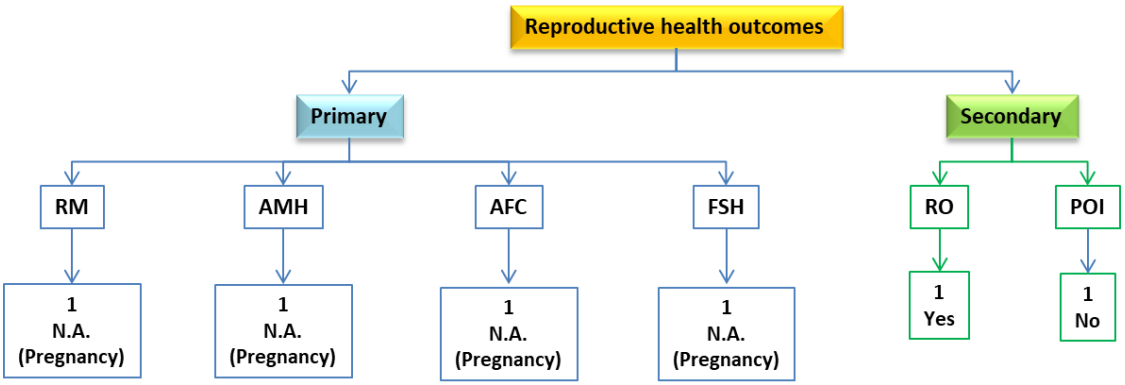
Figure 4.20 Reproductive health outcomes in patients treated with the EC – T chemotherapy regimen (n=2).



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; DD – dose dense; EC – epirubicin and cyclophosphamide; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone; HT – hormonal therapy; LoQ – limit of quantification; N.A. – not applicable; N.E. - not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function.

Figure 4.21 Reproductive health outcomes in patients treated with the dose-dense (DD) EC – weekly paclitaxel chemotherapy regimen (n=1).

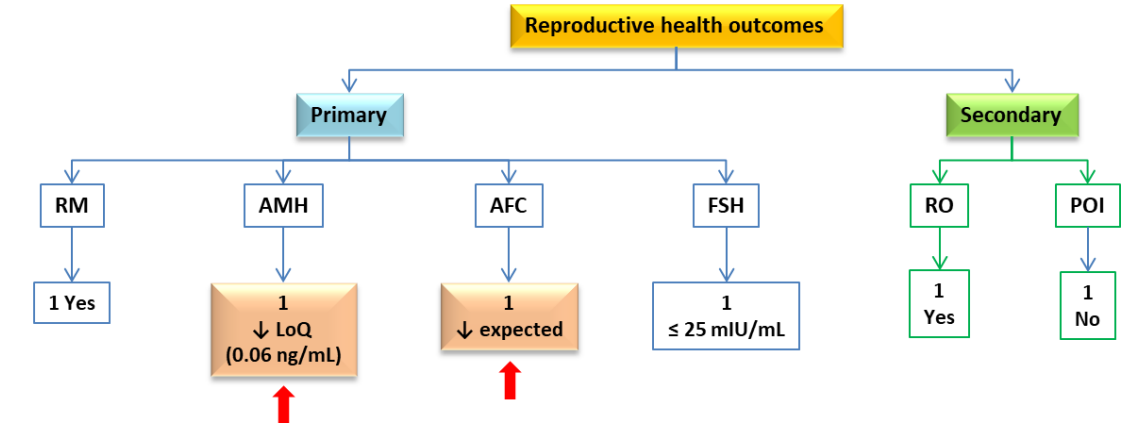
CT regimen	CT Number of Cycles	Number of Patients
DD AC → weekly Paclitaxel	4 → 12	1



Legend: AC – adriamycin and cyclophosphamide; AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; DD – dose dense; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone; HT – hormonal therapy; LoQ – limit of quantification; N.A. – not applicable; N.E. - not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function.

Figure 4.22 Reproductive health outcomes in patients treated with the dose-dense (DD) AC – weekly paclitaxel chemotherapy regimen (n=1).

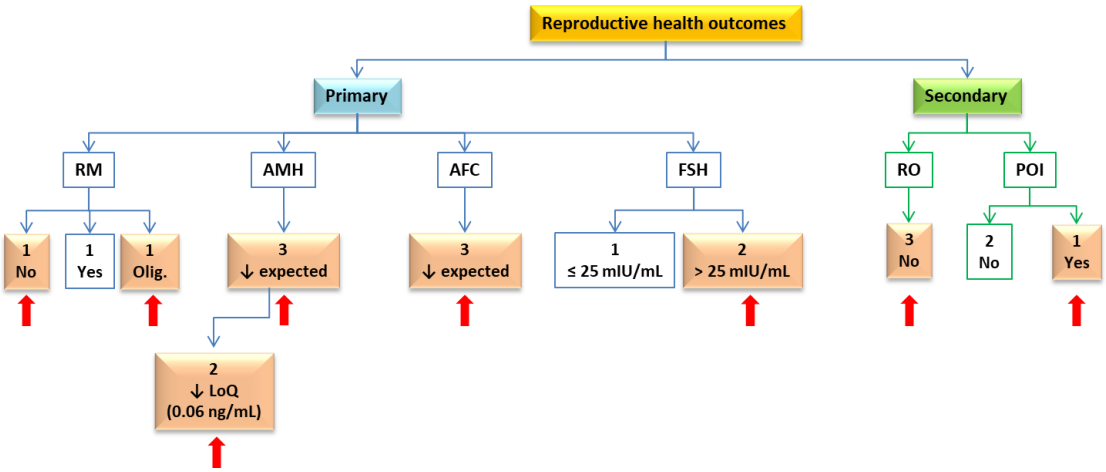
CT regimen	CT Number of Cycles	Number of Patients
AC → weekly Paclitaxel	4 → 12	1



Legend: AC – adriamycin and cyclophosphamide; AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; DD – dose dense; FSH – follicle-stimulating hormone; LoQ – limit of quantification; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function.

Figure 4.23 Reproductive health outcomes in patients treated with the AC – weekly paclitaxel chemotherapy regimen (n=1).

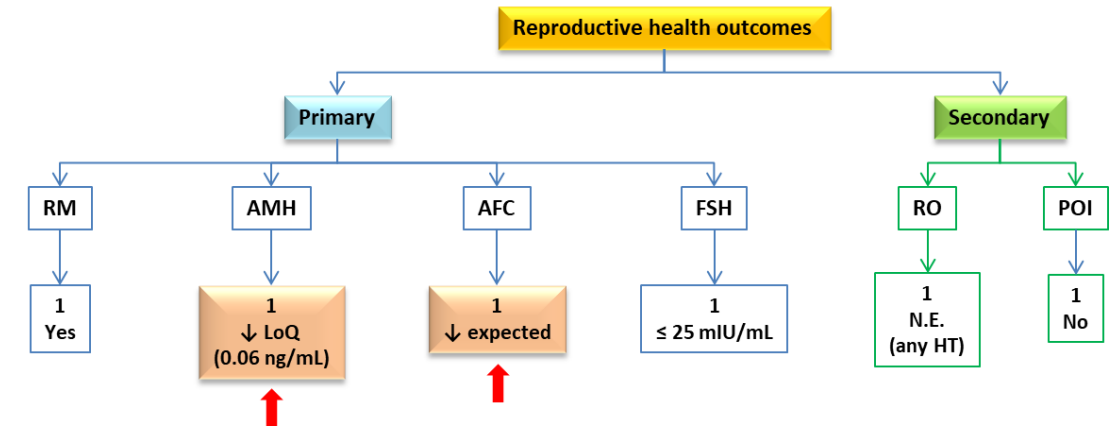
CT regimen	CT Number of Cycles	Number of Patients
AC → T	4 → 4	3



Legend: AC – adriamycin and cyclophosphamide; AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; DD – dose dense; FSH – follicle-stimulating hormone; LoQ – limit of quantification; Olig – oligomenorrhea; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function; T - docetaxel.

Figure 4.24 Reproductive health outcomes in patients treated with the AC – T chemotherapy regimen (n=3).

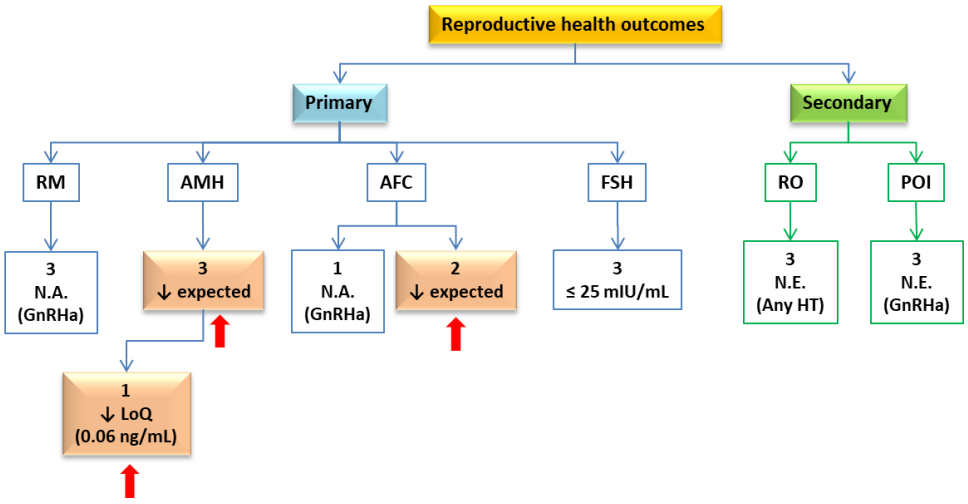
CT regimen	CT Number of Cycles	Number of Patients
T → TE	4 → 4	1



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; FSH – follicle-stimulating hormone; LoQ – limit of quantification; N.E. – not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function; T – docetaxel; TE – docetaxel and epirubicin.

Figure 4.25 Reproductive health outcomes in patients treated with the T - TE chemotherapy regimen (n=1).

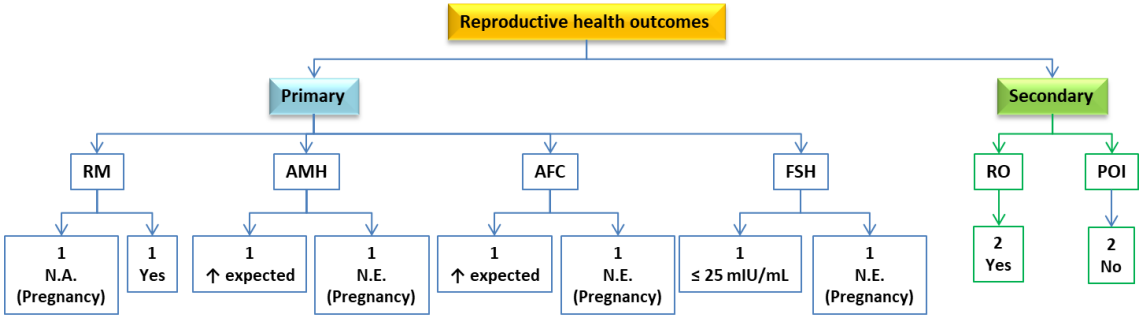
CT regimen	CT Number of Cycles	Number of Patients
TC	4	3



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone; LoQ – limit of quantification; N.A. – not applicable; N.E. – not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function; TC – docetaxel and cyclophosphamide.

Figure 4.26 Reproductive health outcomes in patients treated with the T - TE chemotherapy regimen (n=3).

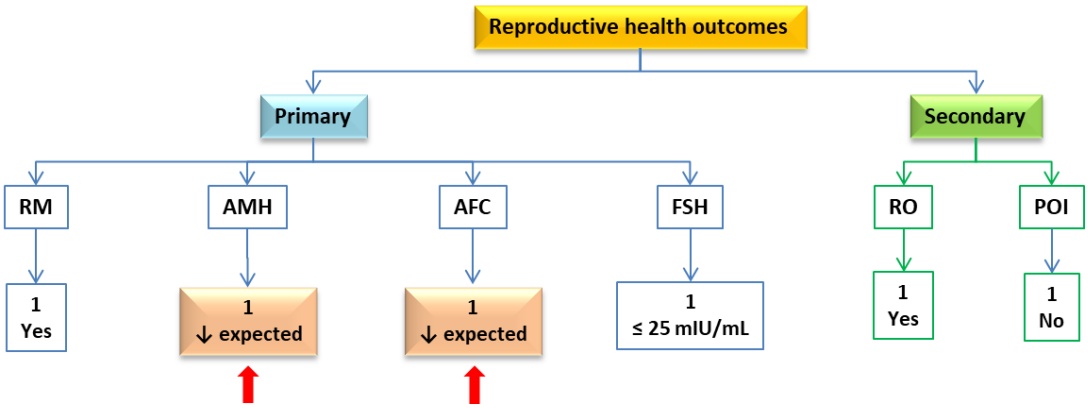
CT regimen	CT Number of Cycles	Number of Patients
Weekly Paclitaxel	12	2



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; FSH – follicle-stimulating hormone; N.A. – not applicable; N.E. – not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function.

Figure 4.27 Reproductive health outcomes in patients treated with the paclitaxel-only chemotherapy regimen (n=2).

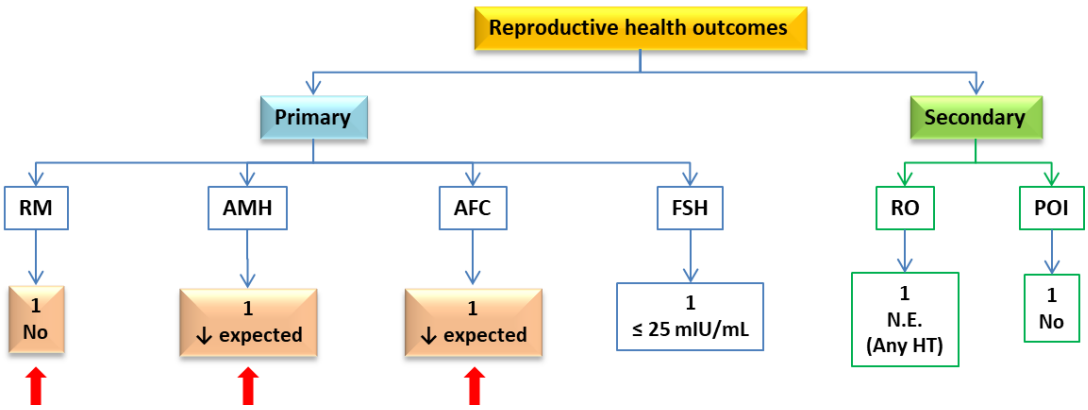
CT regimen	CT Number of Cycles	Number of Patients
FEC → T → adjuvant capecitabine	4 → 3 → 2	1



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-relasing hormone; LoQ – limit of quantification; N.A. – not applicable; N.E. – not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function; TC – docetaxel and cyclophosphamide.

Figure 4.28 Reproductive health outcomes in patients treated with the FEC – T - capecitabine chemotherapy regimen (n=1).

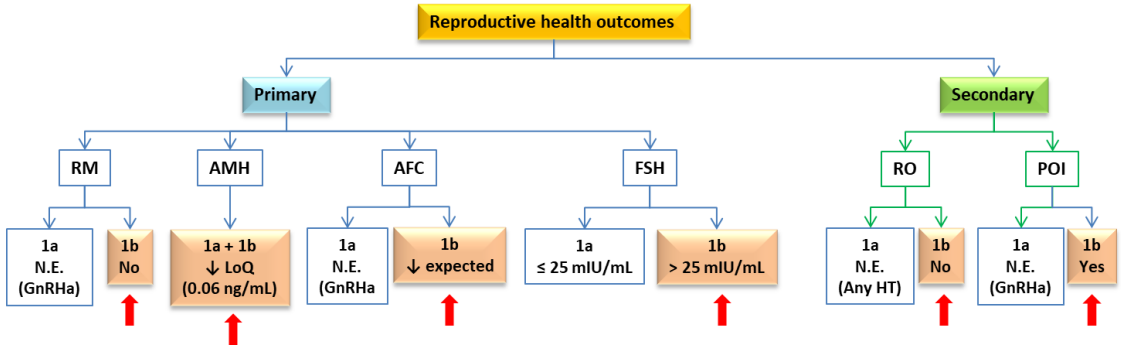
CT regimen	CT Number of Cycles	Number of Patients
FEC → T → weekly Paclitaxel	3 → 1 → 3	1



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; FEC – fluorouracil, epirubicin and cyclophosphamide; FSH – follicle-stimulating hormone; HT – hormonal therapy; N.A. – not applicable; N.E. - not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function; T - docetaxel.

Figure 4.29 Reproductive health outcomes in patients treated with the FEC – T – weekly paclitaxel chemotherapy regimen (n=1).

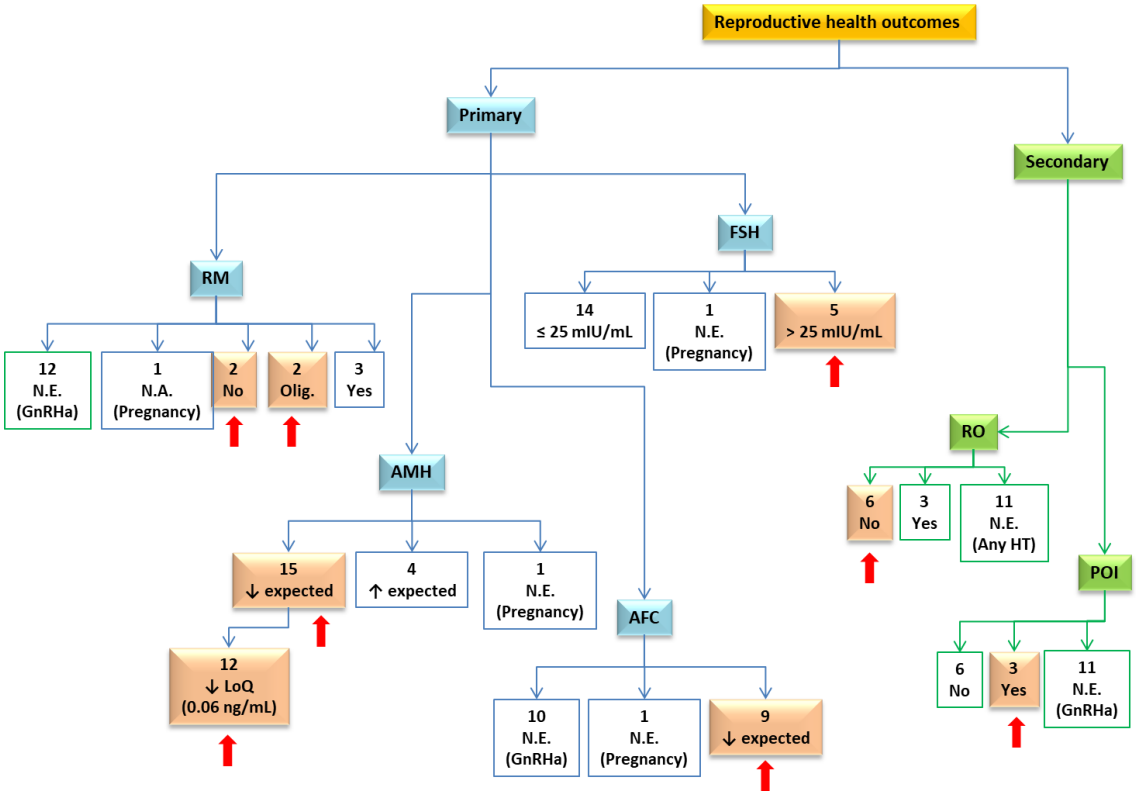
CT regimen	CT Number of Cycles	Number of Patients
FEC → weekly Paclitaxel	4 → 5	1a
FEC → weekly Paclitaxel	4 → 12	1b



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; FEC – fluorouracil, epirubicin and cyclophosphamide; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone; HT – hormonal therapy; LoQ – limit of quantification; N.A. – not applicable; N.E. - not evaluated; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function; T - docetaxel.

Figure 4.30 Reproductive health outcomes in patients treated with the FEC – weekly paclitaxel chemotherapy regimen (n=2).

CT regimen	CT Number of Cycles	Number of Patients
FEC → T	3 → 3	19
FEC → T	4 → 4	1



Legend: AFC – antral follicle count; AMH – anti-Mullerian hormone; CT –chemotherapy; FEC – fluorouracil, epirubicin and cyclophosphamide; FSH – follicle-stimulating hormone; GnRHa – gonadotropin-releasing hormone; HT – hormonal therapy; LoQ – limit of quantification; N.A. – not applicable; N.E. - not evaluated; Olig. – Oligomenorrhea; POI – premature ovarian insufficiency; RM – regular menses; RO – recovery of ovarian function; T – docetaxel.

Figure 4.31 Reproductive health outcomes in patients treated with the FEC – T chemotherapy regimen (n=20).

3.4.2. Treatment combinations, exposure to trastuzumab and to hormonal therapy

AMH levels at the last follow-up were also compared in the four different treatment combination groups: treatment with CT only, CT + HT, CT + TT and CT + HT + TT (Table 4.22). AMH was significantly higher in the group treated with CT + TT as compared to the groups exposed to CT only (U=2; p=0.046) and to CT+ HT (U=0; p=0.002). In accordance with these results, we also found that AMH levels at the last follow-up were higher (U=84.5; p=0.036) in patients treated with trastuzumab in comparison with those who were not (Table 4.23). Yet, no significant differences in mean age, baseline AMH levels or time to follow-up were found between the same groups.

Table 4.22 Mean and median AMH levels at the last follow-up in the four treatment combination groups.

Treatment group	**N**	**AMH** Mean ± SD (ng/mL)	**AMH** Median (ng/mL)
CT only	7	0.23 ± 0.28	0.06
CT + HT	16	0.08 ± 0.04	0.06
CT + TT	3	1.52 ± 1.46	0.78
CT + HT + TT	9	1.21 ± 2.54	0.06
Total	35	0.52 ± 1.40	0.06

Legend: AMH – anti-Mullerian hormone; CT – Chemotherapy; HT – hormonal therapy; TT – targeted therapy

Patients under treatment with any type of HT at the last follow-up exhibited significantly lower AMH (U=88; p=0.05) and FSH (U=83; p=0.056), although these groups were not different regarding their baseline age or AMH levels. When comparing the same OR markers in patients exposed, or not, to GnRHa at the last follow-up, no significant differences in AMH levels were found, although FSH remained lower in those under ovarian suppression (U=57; p=0.002) (Table 4.23).

Table 4.23 Mean and median AMH levels at the last follow-up in patients exposed or not to trastuzumab, any hormonal therapy and GnRH agonist.

Treatment group	N	AMH Mean ± SD (ng/mL)	AMH Median (ng/mL)
Patients exposed to TT	12	1.28 ± 2.26	0.19
Patients not exposed to TT	23	0.12 ± 0.16	0.06
Patients exposed to HT	23	0.41 ± 1.57	0.06
Patients not exposed to HT	12	0.73 ± 1.06	0.33
Patients exposed to GnRHa	16	0.56 ± 1.88	0.06
Patients not exposed to GnRHa	19	0.49 ± 0.89	0.06

Legend: AMH – anti-Mullerian hormone; GnRHa – gonadotropin-releasing hormone agonist; HT – hormonal therapy; SD –standard deviation; TT – targeted therapy

3.5. Time to last follow after CT and adverse reproductive health outcomes

The levels of AMH at the last follow-up were significantly and positively correlated with the time passed since the end of CT (n=35; ρ=0,366; p=0,031), i.e. we found higher AMH in patients with longer time to follow-up. This result is in line with the previously mentioned recovery in AMH levels in 12 of the participants. In contrast, no correlations were found between time and AFC or FSH levels at last follow-up.

4. Discussion

Adverse reproductive health outcomes

It is of concern that more than half of the young premenopausal BC patients in this cohort did not recover ovarian function (10/19; 53%), at a mean of 18 months after the end of CT. Other studies have shown that ovarian function recovery (return of menstrual cycle and/or premenopausal hormone levels) may occur up to 2 years after the end of CT in women with BC (Sukumvanich, Case et al. 2010, Hamy, Porcher et al. 2014, Jacobson, Mertens et al. 2016), so we anticipate that most women in this cohort have now a very low, if any, chance of recovery.

Additionally, we have identified POI in five women, a number that corresponds to 23% (5/22) of those who could be evaluated for this outcome. In women with established POI, no interventions have shown to increase ovarian activity and natural conception rates. Furthermore, a diagnosis of POI has a significant negative impact on the psychological wellbeing and quality of life of these women as they may also experience genitourinary symptoms and present a reduced bone mineral density and an increased risk of cardiovascular

disease (Webber, Davies et al. 2016). We have to keep in mind that a considerable number of patients in our study were not evaluated for recovery (n=19) or POI (n=16) due to exposure to HT, so it is likely that the incidence of these adverse outcomes would increase with a longer follow-up. A similar prevalence of POI in young BC patients was observed for the control group of the OPTION trial (6/30; 20%) (Leonard, Adamson et al. 2017). However, in a retrospective study conducted in patients with HR-positive tumours, only 8.7% of the patients with age under 40 (n=23) had chemotherapy-induced ovarian function failure (Vriens, De Bie et al. 2017). A possible explanation is the fact that most of these patients were under HT with tamoxifen, which may have caused a reduction in FSH levels. Our results are also in accordance with the subsequent data analysis of the OPTION trial by Anderson and colleagues (Anderson, Mansi et al. 2017), that concluded that women who developed POI had lower pre-treatment AMH concentrations than those who did not.

At the last follow-up, other adverse reproductive health outcomes are noteworthy: i) only one woman recovered her baseline AMH levels, ii) only five presented AMH levels considered normal according to age and iii) serum AMH was below the LoQ in 60% of the participants (21/35). Moreover, ten patients presented low OR and/or postmenopausal hormone levels despite the recovery of regular menses. These results of low and persistent OR markers after CT exposure in women with BC are in line with several other published studies (Su 2010, Peigne and Decanter 2014, Dezellus, Barriere et al. 2017, Freour, Barriere et al. 2017, Trapp, Steidl et al. 2017). Also similarly to our results, other studies have reported AMH levels remaining below the age-expected values in BC patients, even with 3 to 5 years of follow-up (Perdrix, Saint-Ghislain et al. 2017) and several other authors reported undetectable AMH levels after the end of CT in patients who had already recovered spontaneous menses (Peigne and Decanter 2014). Infertility or early menopause has occurred even in women who resumed menses after treatment (Letourneau, Ebbel et al. 2012). In many women with BC, the need to continue HT for several years will further narrow their reproductive window.

Despite the manifestation of significant adverse reproductive health effects in this cohort, four women became pregnant during the course of the study (4/38; 11%). In three of them, the normal to high OR levels at baseline may have contributed to a less pronounced effect in their fertility, as indicated by several other studies (Peigne and Decanter 2014). However, the fourth patient had a baseline AMH level that was lower than the age-expected value. In addition, two of the pregnant women presented AMH levels below the LoQ at their last follow-up. Previous studies also reported pregnancy in survivors with low levels of OR (Hamy, Porcher et al. 2016, Dezellus, Barriere et al. 2017). It is worth recalling that the pregnant woman with low baseline OR was treated with the paclitaxel-trastuzumab regimen. Women who became pregnant were

younger and had higher OR markers at baseline, as compared to women who met criteria for POI at the last follow-up (Table 16). Statistical analyses were not conducted considering the small size of the groups. Not surprisingly, all pregnant women were diagnosed with HR-negative tumours and, consequently, were not under treatment with any form of HT.

Patient-related factors

It is widely accepted that older age is one of the most important risk factors for CT-induced amenorrhea and/or low OR and commonly used cut-offs range from 35 to 40 years (Abusief, Missmer et al. 2010, Yu, Douglas et al. 2010, Su, Haunschild et al. 2014, Anderson, Mansi et al. 2017, Dezellus, Barriere et al. 2017). In our very young cohort of patients (median age of 33 years; range 25-39) that may best represent the group of BC patients who engage in fertility counselling, age was negatively correlated with AMH levels at the last follow-up and this correlation was stronger in younger patients. Furthermore, patients who recovered ovarian function were younger. Age at recruitment was also noticeably different in the groups of women who got pregnant (n=4) and women who underwent POI (n=5).

In our cohort, the baseline levels of AMH significantly influenced both the recovery of ovarian function and the occurrence of POI. Additionally, AMH levels at baseline and at the last follow-up were positively correlated. These results reinforce the usefulness of this hormone as a predictor of ovarian function after CT and are in line with several studies where pre-CT level of AMH was associated with the occurrence of amenorrhea (Anderson and Wallace 2013) or with post-CT AMH levels (Dezellus, Barriere et al. 2017, Trapp, Steidl et al. 2017). Our results also confirm AMH as the most sensitive marker of ovarian damage in BC patients exposed to CT.

Treatment-related factors

In our study, CT regimens were not totally homogeneous but exposure to a three drug regimen composed of an anthracycline, cyclophosphamide and a taxane was an important common feature to the majority of the participants. Anthracyclines and cyclophosphamide are widely recognized as associated with moderate to high risk of CT-induced amenorrhea. Taxanes are currently included in modern CT regimens for BC as they have proved to reduce recurrence and increase survival. However, the addition of these agents to anthracycline-based regimens has been associated with an increased negative impact of CT on fertility in several published clinical studies (Berliere, Dalenc et al. 2008, Abusief, Missmer et al. 2010, Perdrix, Saint-Ghislain et al. 2017) and meta-analysis (Zhao, Liu et al. 2014), despite a few studies reporting opposite results (Perez-Fidalgo, Rosello et al. 2010, Sukumvanich, Case et al. 2010). In our cohort, we found significant differences in the levels of AMH before and after the

administration of the taxane, so the additional exposure to these agents may have contributed for the significant adverse reproductive health outcomes, despite the young age of patients at recruitment. Our study failed to associate the post-CT levels of AMH with the type and duration of CT regimen, probably because of the low number of patients in some of those groups. Nevertheless, other prospective (Dezellus, Barriere et al. 2017) and retrospective (Hamy, Porcher et al. 2014) studies in BC patients were also unsuccessful in proving this type of association.

One of the possible ways to overcome the negative effects of CT treatments in fertility is to select less gonadotoxic CT regimens. Most CT regimens currently used for BC include the above mentioned combination of three agents, which significantly increases the risk of CT-induced amenorrhea (Zhao, Liu et al. 2014). In view of these and other published results (Ruddy, Guo et al. 2015) we may assume that less complex CT regimens or those that do not include alkylating agents (such as the weekly paclitaxel regimen) may be an alternative to consider in young women with a BC diagnosis who value the possibility of future offspring. This regimen is currently recommended, in association with trastuzumab, for low risk Her_2-positive tumours (Coates, Winer et al. 2015). In our study, two women were treated with this CT regimen: one became pregnant during the study and the other patient was one of the few who recovered menstrual function and presented normal AMH levels at the last follow-up. Despite the very small sample, these results are in accordance with those from the APT trial (Ruddy, Guo et al. 2015), where the weekly paclitaxel regimen caused a less pronounced gonadotoxic effect. To test this theory, it would be important to conduct prospective controlled studies assessing the reproductive outcomes of premenopausal women exposed to this specific regimen.

An interesting and somehow unexpected result of our investigation was the significant higher AMH levels in the 12 patients treated with trastuzumab, as compared to patients who were not. Moreover, patients in the CT + TT treatment subgroup (n=3) also presented higher AMH at the last follow-up, as compared to the CT only (n=7) and CT and HT (n=16) subgroups. Trastuzumab is a monoclonal antibody that targets HER2-expressing tumour cells and pre-clinical reproductive studies in cynomolgus monkeys, using doses up to 25 times the weekly human maintenance dose of 2 mg/kg, showed no evidence of impaired fertility (Lorenzi, Simonelli et al. 2016). Our results also concur for the lack of gonadotoxicity that was already pointed by other clinical studies (Abusief, Missmer et al. 2010, Meng, Tian et al. 2013) and add further data to the hypothesis of trastuzumab as a protector of ovarian vasculature from CT-induced damage (Ben-Aharon, Granot et al. 2015). In this study, the authors observed a trend toward reduced vascular toxicity, as demonstrated by a milder decrease in ovarian blood flow, in patients treated with trastuzumab (n=7) compared with those treated with CT only. The

relevance of our observation is also supported by the results of a recently published cross-sectional analysis where exposure to trastuzumab was associated with increased AMH in BC survivors with normal menstrual cycles (n=25) (Morarji, McArdle et al. 2017). Moreover, this finding corroborates the previously mentioned outcomes of the adjuvant paclitaxel-trastuzumab trial (Ruddy, Guo et al. 2015). Despite these encouraging results, further clinical investigations are warranted, with larger samples, to confirm the direct effect of trastuzumab on ovarian function and to clarify its potential mechanism of action.

In our study, we found evidence of the influence of HT in the levels of OR markers: patients under therapy with tamoxifen, aromatase inhibitor and/or GnRHa at the last follow-up exhibited significantly lower levels of AMH and FSH, despite no differences in their baseline ages and AMH levels were found. However, when the isolated effect of ovarian suppression with GnRHa was investigated, only FSH levels remained different. Previous studies have also reported that patients under treatment with tamoxifen and/or a GnRHa may experience reduced FSH levels (Su, Maas et al. 2013, Su, Haunschild et al. 2014) (Hamy, Porcher et al. 2016). Regarding the influence of GnRHa on AMH, some authors believe they have no direct effect on OR due to the absence of FSH, LH, or GnRH receptors in primordial follicles (Oktay and Bedoschi 2016) but others have reported reduced AMH levels in patients under ovarian suppression (Anderson, Themmen et al. 2006, Trapp, Steidl et al. 2017) so further investigation is needed to confirm AMH as a reliable marker in this setting. Until then, diagnosis of ovarian insufficiency in BC patients under HT must rely not only on FSH but also on oestradiol levels and clinical symptoms of oestrogen deficiency, in accordance with recent recommendations (van Dorp, Mulder et al. 2016, Vriens, De Bie et al. 2017). Furthermore, clinicians have to keep in mind that low FSH levels in BC patients under HT may be misleading, so oestradiol levels should also be assessed in those presenting premenopausal FSH (Vriens, De Bie et al. 2017). This issue is of even greater concern considering that confirmation of menopausal status in BC patients with HR-positive tumours is needed for the adequate selection of HT (Torino, Barnabei et al. 2014).

Limitations

This small cohort study may have lacked statistical power to detect differences between treatment combinations and different CT regimens, for instance between patients exposed or not to cyclophosphamide or anthracyclines. Moreover, all women were treated with taxanes, most often sequentially to an anthracycline-based protocol, a fact that restrained the analysis of the specific effects of those agents in the reproductive markers. Furthermore, our results might have been influenced by the low baseline OR levels seen in 16 participants, in

comparison with the corresponding age-expected values. Nevertheless, the median AMH level of 2.2 ng/mL was similar to levels reported in other studies that included younger BC patients (Hamy, Porcher et al. 2014). Previously published studies have not found significant differences between AMH levels in women with BC and healthy controls (Lutchman Singh, Muttukrishna et al. 2007, Yu, Douglas et al. 2010, Su, Flatt et al. 2013), except for a group of older patients (age ≥37 years) (Su, Flatt et al. 2013). In our study, initial AMH levels may have been influenced by hormonal contraception and/or smoking. It is reported that these two exposures may significantly reduce the levels of this OR marker (Dolleman, Verschuren et al. 2013, Johnson, Sammel et al. 2014, Birch Petersen, Hvidman et al. 2015) although the effects of hormonal contraceptives are reported to be reversible after 2 months of discontinuation (van den Berg, van Dulmen-den Broeder et al. 2010). In addition, seven of those women had been diagnosed with triple-negative tumours. Up to 20% of triple negative BC patients harbour germline *BRCA* mutations and some published results suggest that *BRCA1/2* mutation carriers may present lower AMH levels (Phillips, Collins et al. 2016) or even experience earlier onset of menopause (Lin, Beattie et al. 2013), as compared to non-carriers. Our results reinforce the need for pre-treatment fertility counselling of BC patients with triple-negative tumours, *BRCA* mutations or smokers, due to their increased chance of CT-induced loss of ovarian reserve and POI occurrence.

Other major limitation is the fact that a very significant number of participants (n= 20) was still under the influence of HT at the last follow-up, which restricted the assessment of the outcomes *ovarian function recovery* and *POI*. Nevertheless, this limitation also occurs in clinical practice: many BC patients remain in treatment with HT for several years and the identification of reliable markers to assess their menopausal status is still an unsolved issue (Amir, Freedman et al. 2010, Krekow, Hellerstedt et al. 2016). The hormonal and ultrasound evaluations performed during and after CT were scheduled irrespective of patients' menstrual cycle, in order to overlap with other hospital appointments and facilitate patient's participation. This can constitute a limitation of the study methodology as AMH and AFC are not prone to significant variation but the levels of FSH fluctuate during the menstrual cycle.

5. Conclusions

Our study in young women with BC revealed significant effects of CT on several reproductive outcomes and confirmed their strong relation with patient's age and baseline level of AMH. We have confirmed AMH as the most sensitive marker of OR in young premenopausal women with BC and its effectiveness as a predictor of ovarian function recovery and occurrence of POI. However, we did not find any relation between AMH levels and the occurrence of pregnancy.

Despite the noteworthy effects, our results also point to the possibility of a lower gonadotoxicity when patients are treated with less complex CT regimens or when treatment includes targeted therapy with trastuzumab. Also, this investigation highlights the lack of reliable OR markers in women with BC under treatment with HT and the consequent risk of undetected ovarian failure in this population.

Overall, and despite the mentioned limitations, our results strongly emphasize the relevance of pre-treatment counselling regarding infertility risks and fertility preservation for all premenopausal BC patients, with a special emphasis on older patients or those with low pre-chemotherapy OR. Moreover, they support the need for a systematic evaluation of ovarian function in BC survivors using a variety of clinical and hormonal markers, in order to ensure an adequate counselling. Nevertheless, many questions remain and additional research is needed to confirm the lower gonadotoxicity of CT regimens like the paclitaxel-trastuzumab regimen, the possibility of a protective effect with exposure to trastuzumab and the usefulness of measuring AMH in patients with BC under HT and/or ovarian suppression.

References

Abusief, M. E., S. A. Missmer, E. S. Ginsburg, J. C. Weeks and A. H. Partridge (2010). "The effects of paclitaxel, dose density, and trastuzumab on treatment-related amenorrhea in premenopausal women with breast cancer." Cancer **116**(4): 791-798.

Almog, B., F. Shehata, E. Shalom-Paz, S. L. Tan and T. Tulandi (2011). "Age-related normogram for antral follicle count: McGill reference guide." Fertil Steril **95**(2): 663-666.

Amir, E., O. Freedman, L. Allen, T. Colgan and M. Clemons (2010). "Defining ovarian failure in amenorrheic young breast cancer patients." Breast **19**(6): 545-548.

Anampa, J., D. Makower and J. A. Sparano (2015). "Progress in adjuvant chemotherapy for breast cancer: an overview." BMC Med **13**: 195.

Anders, C. K., R. Johnson, J. Litton, M. Phillips and A. Bleyer (2009). "Breast cancer before age 40 years." Semin Oncol **36**(3): 237-249.

Anderson, R. A., J. Mansi, R. E. Coleman, D. J. A. Adamson and R. C. F. Leonard (2017). "The utility of anti-Mullerian hormone in the diagnosis and prediction of loss of ovarian function following chemotherapy for early breast cancer." Eur J Cancer **87**: 58-64.

Anderson, R. A., A. P. Themmen, A. Al-Qahtani, N. P. Groome and D. A. Cameron (2006). "The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer." Hum Reprod **21**(10): 2583-2592.

Anderson, R. A. and W. H. Wallace (2013). "Antimullerian hormone, the assessment of the ovarian reserve, and the reproductive outcome of the young patient with cancer." Fertil Steril **99**(6): 1469-1475.

Assi, H. A., K. E. Khoury, H. Dbouk, L. E. Khalil, T. H. Mouhieddine and N. S. El Saghir (2013). "Epidemiology and prognosis of breast cancer in young women." J Thorac Dis **5 Suppl 1**: S2-8.

Ben-Aharon, I., T. Granot, I. Meizner, N. Hasky, A. Tobar, S. Rizel, R. Yerushalmi, A. Ben-Haroush, B. Fisch and S. M. Stemmer (2015). "Long-Term Follow-Up of Chemotherapy-Induced Ovarian Failure in Young Breast Cancer Patients: The Role of Vascular Toxicity." Oncologist **20**(9): 985-991.

Berliere, M., F. Dalenc, N. Malingret, A. Vindevogel, P. Piette, H. Roche, J. Donnez, M. Symann, J. Kerger and J. P. Machiels (2008). "Incidence of reversible amenorrhea in women with breast cancer undergoing adjuvant anthracycline-based chemotherapy with or without docetaxel." BMC Cancer **8**: 56.

Birch Petersen, K., H. W. Hvidman, J. L. Forman, A. Pinborg, E. C. Larsen, K. T. Macklon, R. Sylvest and A. N. Andersen (2015). "Ovarian reserve assessment in users of oral contraception seeking fertility advice on their reproductive lifespan." Hum Reprod **30**(10): 2364-2375.

Broekmans, F. J., D. de Ziegler, C. M. Howles, A. Gougeon, G. Trew and F. Olivennes (2010). "The antral follicle count: practical recommendations for better standardization." Fertil Steril **94**(3): 1044-1051.

Coates, A. S., E. P. Winer, A. Goldhirsch, R. D. Gelber, M. Gnant, M. Piccart-Gebhart, B. Thurlimann and H. J. Senn (2015). "Tailoring therapies--improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015." Ann Oncol **26**(8): 1533-1546.

Dewailly, D., C. Y. Andersen, A. Balen, F. Broekmans, N. Dilaver, R. Fanchin, G. Griesinger, T. W. Kelsey, A. La Marca, C. Lambalk, H. Mason, S. M. Nelson, J. A. Visser, W. H. Wallace and R. A. Anderson (2014). "The physiology and clinical utility of anti-Mullerian hormone in women." Hum Reprod Update **20**(3): 370-385.

Dezellus, A., P. Barriere, M. Campone, C. Lemanski, L. Vanlemmens, L. Mignot, T. Delozier, C. Levy, C. Bendavid, M. Debled, T. Bachelot, C. Jouannaud, C. Loustalot, M. A. Mouret-Reynier, A. Gallais-Umbert, D. Masson and T. Freour (2017). "Prospective evaluation of serum anti-Mullerian hormone dynamics in 250 women of reproductive age treated with chemotherapy for breast cancer." Eur J Cancer **79**: 72-80.

Dolleman, M., W. M. Verschuren, M. J. Eijkemans, M. E. Dolle, E. H. Jansen, F. J. Broekmans and Y. T. van der Schouw (2013). "Reproductive and lifestyle determinants of anti-Mullerian hormone in a large population-based study." J Clin Endocrinol Metab **98**(5): 2106-2115.

Freour, T., P. Barriere and D. Masson (2017). "Anti-mullerian hormone levels and evolution in women of reproductive age with breast cancer treated with chemotherapy." Eur J Cancer **74**: 1-8.

Hagen, C. P., K. Sorensen, R. A. Anderson and A. Juul (2012). "Serum levels of antimullerian hormone in early maturing girls before, during, and after suppression with GnRH agonist." Fertil Steril **98**(5): 1326-1330.

Hamy, A. S., R. Porcher, C. Cuvier, S. Giacchetti, M. H. Schlageter, C. Coussieu, H. Gronier, J. P. Feugeas, N. Adoui, J. M. Lacorte, C. Poirot, M. Habdous and M. Espie (2014). "Ovarian reserve in breast cancer: assessment with anti-Mullerian hormone." Reprod Biomed Online **29**(5): 573-580.

Hamy, A. S., R. Porcher, S. Eskenazi, C. Cuvier, S. Giacchetti, F. Coussy, H. Hocini, B. Tournant, F. Perret, S. Bonfils, P. Charveriat, J. M. Lacorte and M. Espie (2016). "Anti-Mullerian hormone in breast cancer patients treated with chemotherapy: a retrospective evaluation of subsequent pregnancies." Reprod Biomed Online **32**(3): 299-307.

International Agency for Research on Cancer. (2012). "Breast Cancer Fact Sheet: Estimated Incidence, Mortality and Prevalence Worldwide in 2012 " GLOBOCAN 2012 Retrieved January 2018, from http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

Jacobson, M. H., A. C. Mertens, J. B. Spencer, A. K. Manatunga and P. P. Howards (2016). "Menses resumption after cancer treatment-induced amenorrhea occurs early or not at all." Fertil Steril **105**(3): 765-772.e764.

Johnson, L. N., M. D. Sammel, K. E. Dillon, L. Lechtenberg, A. Schanne and C. R. Gracia (2014). "Antimullerian hormone and antral follicle count are lower in female cancer survivors and healthy women taking hormonal contraception." Fertil Steril **102**(3): 774-781 e773.

Krekow, L. K., B. A. Hellerstedt, R. P. Collea, S. Papish, S. M. Diggikar, R. Resta, S. J. Vukelja, F. A. Holmes, P. K. Reddy, L. Asmar, Y. Wang, P. S. Fox, S. R. Peck and J. O'Shaughnessy (2016). "Incidence and Predictive Factors for Recovery of Ovarian Function in Amenorrheic Women in Their 40s Treated With Letrozole." J Clin Oncol **34**(14): 1594-1600.

Kumar, P. and A. Sharma (2014). "Gonadotropin-releasing hormone analogs: Understanding advantages and limitations." J Hum Reprod Sci **7**(3): 170-174.

Leonard, R. C. F., D. J. A. Adamson, G. Bertelli, J. Mansi, A. Yellowlees, J. Dunlop, G. A. Thomas, R. E. Coleman and R. A. Anderson (2017). "GnRH agonist for protection against ovarian toxicity during chemotherapy for early breast cancer: the Anglo Celtic Group OPTION trial." Ann Oncol **28**(8): 1811-1816.

Letourneau, J. M., E. E. Ebbel, P. P. Katz, K. H. Oktay, C. E. McCulloch, W. Z. Ai, A. J. Chien, M. E. Melisko, M. I. Cedars and M. P. Rosen (2012). "Acute ovarian failure underestimates age-specific reproductive impairment for young women undergoing chemotherapy for cancer." Cancer **118**(7): 1933-1939.

Lin, W. T., M. Beattie, L. M. Chen, K. Oktay, S. L. Crawford, E. B. Gold, M. Cedars and M. Rosen (2013). "Comparison of age at natural menopause in BRCA1/2 mutation carriers with a non-clinic-based sample of women in northern California." Cancer **119**(9): 1652-1659.

Lorenzi, E., M. Simonelli and A. Santoro (2016). "Infertility risk and teratogenicity of molecularly targeted anticancer therapy: A challenging issue." Crit Rev Oncol Hematol **107**: 1-13.

Lutchman Singh, K., S. Muttukrishna, R. C. Stein, H. H. McGarrigle, A. Patel, B. Parikh, N. P. Groome, M. C. Davies and R. Chatterjee (2007). "Predictors of ovarian reserve in young women with breast cancer." Br J Cancer **96**(12): 1808-1816.

Meng, K., W. Tian, M. Zhou, H. Chen and Y. Deng (2013). "Impact of chemotherapy-induced amenorrhea in breast cancer patients: the evaluation of ovarian function by menstrual history and hormonal levels." World J Surg Oncol **11**: 101.

Morarji, K., O. McArdle, K. Hui, G. Gingras-Hill, S. Ahmed, E. M. Greenblatt, E. Warner, S. Sridhar, A. M. F. Ali, A. Azad and D. C. Hodgson (2017). "Ovarian function after chemotherapy in young breast cancer survivors." Curr Oncol **24**(6): e494-e502.

Nelson, S. M. (2013). "Biomarkers of ovarian response: current and future applications." Fertil Steril **99**(4): 963-969.

OECD - Social Policy Division - Directorate of Employment, L. a. S. A. (2015). SF 2.3: Age of mothers at childbirth and age-specific fertility. OECD Family Database.

Oktay, K. and G. Bedoschi (2016). "Appraising the Biological Evidence for and Against the Utility of GnRHa for Preservation of Fertility in Patients With Cancer." J Clin Oncol **34**(22): 2563-2565.

Partridge, A. H., K. J. Ruddy, S. Gelber, L. Schapira, M. Abusief, M. Meyer and E. Ginsburg (2010). "Ovarian reserve in women who remain premenopausal after chemotherapy for early stage breast cancer." Fertil Steril **94**(2): 638-644.

Peigne, M. and C. Decanter (2014). "Serum AMH level as a marker of acute and long-term effects of chemotherapy on the ovarian follicular content: a systematic review." Reprod Biol Endocrinol **12**: 26.

Perdrix, A., M. Saint-Ghislain, M. Degremont, M. David, Z. Khaznadar, A. Loeb, M. Leheurteur, F. Di Fiore and F. Clatot (2017). "Influence of adjuvant chemotherapy on anti-Mullerian hormone in women below 35 years treated for early breast cancer." Reprod Biomed Online **35**(4): 468-474.

Perez-Fidalgo, J. A., S. Rosello, E. Garcia-Garre, E. Jorda, P. Martin-Martorell, B. Bermejo, I. Chirivella, C. Guzman and A. Lluch (2010). "Incidence of chemotherapy-induced amenorrhea in hormone-sensitive breast cancer patients: the impact of addition of taxanes to anthracycline-based regimens." Breast Cancer Res Treat **120**(1): 245-251.

Phillips, K. A., I. M. Collins, R. L. Milne, S. A. McLachlan, M. Friedlander, M. Hickey, C. Stern, J. L. Hopper, R. Fisher, G. Kannemeyer, S. Picken, C. D. Smith, T. W. Kelsey and R. A. Anderson (2016). "Anti-Mullerian hormone serum concentrations of women with germline BRCA1 or BRCA2 mutations." Hum Reprod **31**(5): 1126-1132.

Ribnikar, D., J. M. Ribeiro, D. Pinto, B. Sousa, A. C. Pinto, E. Gomes, E. C. Moser, M. J. Cardoso and F. Cardoso (2015). "Breast cancer under age 40: a different approach." Curr Treat Options Oncol **16**(4): 16.

Rossi, E., A. Morabito, F. Di Rella, G. Esposito, A. Gravina, V. Labonia, G. Landi, F. Nuzzo, C. Pacilio, E. De Maio, M. Di Maio, M. C. Piccirillo, G. De Feo, G. D'Aiuto, G. Botti, P. Chiodini, C. Gallo, F. Perrone and A. de Matteis (2009). "Endocrine effects of adjuvant letrozole compared with tamoxifen in hormone-responsive postmenopausal patients with early breast cancer: the HOBOE trial." J Clin Oncol **27**(19): 3192-3197.

Ruddy, K. J., H. Guo, W. Barry, C. T. Dang, D. A. Yardley, B. Moy, P. K. Marcom, K. S. Albain, H. S. Rugo, M. J. Ellis, I. Shapira, A. C. Wolff, L. A. Carey, B. A. Overmoyer, C. Hudis, I. E. Krop, H. J. Burstein, E. P. Winer, A. H. Partridge and S. M. Tolaney (2015). "Chemotherapy-related amenorrhea after adjuvant paclitaxel-trastuzumab (APT trial)." Breast Cancer Res Treat **151**(3): 589-596.

Ruddy, K. J. and A. H. Partridge (2012). "Fertility (male and female) and menopause." J Clin Oncol **30**(30): 3705-3711.

Seifer, D. B., V. L. Baker and B. Leader (2011). "Age-specific serum anti-Mullerian hormone values for 17,120 women presenting to fertility centers within the United States." Fertil Steril **95**(2): 747-750.

Shandley, L. M., J. B. Spencer, A. Fothergill, A. C. Mertens, A. Manatunga, E. Paplomata and P. P. Howards (2017). "Impact of tamoxifen therapy on fertility in breast cancer survivors." Fertil Steril **107**(1): 243-252 e245.

Su, H. C., C. Haunschild, K. Chung, S. Komrokian, S. Boles, M. D. Sammel and A. DeMichele (2014). "Prechemotherapy antimullerian hormone, age, and body size predict timing of return of ovarian function in young breast cancer patients." Cancer **120**(23): 3691-3698.

Su, H. I. (2010). "Measuring ovarian function in young cancer survivors." Minerva Endocrinol **35**(4): 259-270.

Su, H. I., K. Chung, M. D. Sammel, C. R. Gracia and A. DeMichele (2011). "Antral follicle count provides additive information to hormone measures for determining ovarian function in breast cancer survivors." Fertil Steril **95**(5): 1857-1859.

Su, H. I., S. W. Flatt, L. Natarajan, A. DeMichele and A. Z. Steiner (2013). "Impact of breast cancer on anti-mullerian hormone levels in young women." Breast Cancer Res Treat **137**(2): 571-577.

Su, H. I., K. Maas, P. M. Sluss, R. J. Chang, J. E. Hall and H. Joffe (2013). "The impact of depot GnRH agonist on AMH levels in healthy reproductive-aged women." J Clin Endocrinol Metab **98**(12): E1961-1966.

Sukumvanich, P., L. D. Case, K. Van Zee, S. E. Singletary, E. D. Paskett, J. A. Petrek, E. Naftalis and M. J. Naughton (2010). "Incidence and time course of bleeding after long-term amenorrhea after breast cancer treatment: a prospective study." Cancer **116**(13): 3102-3111.

Torino, F., A. Barnabei, L. De Vecchis, V. Sini, F. Schittulli, P. Marchetti and S. M. Corsello (2014). "Chemotherapy-induced ovarian toxicity in patients affected by endocrine-responsive early breast cancer." Crit Rev Oncol Hematol **89**(1): 27-42.

Trapp, E., J. Steidl, B. Rack, M. S. Kupka, U. Andergassen, J. Juckstock, A. Kurt, T. Vilsmaier, A. de Gregorio, N. de Gregorio, M. Tzschaschel, C. Lato, A. Polasik, H. Tesch, A. Schneeweiss, M. W. Beckmann, P. A. Fasching, W. Janni and V. Muller (2017). "Anti-Mullerian hormone (AMH) levels in premenopausal breast cancer patients treated with taxane-based adjuvant chemotherapy - A translational research project of the SUCCESS A study." Breast **35**: 130-135.

UK Cancer Research. (2013, 11 May 2016). "Breast cancer survival statistics by age." Retrieved January 2018, from http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/survival#heading-One.

van den Berg, M. H., E. van Dulmen-den Broeder, A. Overbeek, J. W. Twisk, R. Schats, F. E. van Leeuwen, G. J. Kaspers and C. B. Lambalk (2010). "Comparison of ovarian function markers in users of hormonal contraceptives during the hormone-free interval and subsequent natural early follicular phases." Hum Reprod **25**(6): 1520-1527.

van Dorp, W., R. L. Mulder, L. C. Kremer, M. M. Hudson, M. M. van den Heuvel-Eibrink, M. H. van den Berg, J. M. Levine, E. van Dulmen-den Broeder, N. di Iorgi, A. Albanese, S. H. Armenian, S. Bhatia, L. S. Constine, A. Corrias, R. Deans, U. Dirksen, C. R. Gracia, L. Hjorth, L. Kroon, C. B. Lambalk, W. Landier, G. Levitt, A. Leiper, L. Meacham, A. Mussa, S. J. Neggers, K. C. Oeffinger, A. Revelli, H. M. van Santen, R. Skinner, A. Toogood, W. H. Wallace and R. Haupt (2016). "Recommendations for Premature Ovarian Insufficiency Surveillance for Female Survivors of Childhood, Adolescent, and Young Adult Cancer: A Report From the International Late Effects of Childhood Cancer Guideline Harmonization Group in Collaboration With the PanCareSurFup Consortium." J Clin Oncol **34**(28): 3440-3450.

Vriens, I. J., A. J. De Bie, M. J. Aarts, M. de Boer, I. E. van Hellemond, J. H. Roijen, R. J. van Golde, A. C. Voogd and V. C. Tjan-Heijnen (2017). "The correlation of age with chemotherapy-induced ovarian function failure in breast cancer patients." Oncotarget **8**(7): 11372-11379.

Webber, L., M. Davies, R. Anderson, J. Bartlett, D. Braat, B. Cartwright, R. Cifkova, S. de Muinck Keizer-Schrama, E. Hogervorst, F. Janse, L. Liao, V. Vlaisavljevic, C. Zillikens and N. Vermeulen (2016). "ESHRE Guideline: management of women with premature ovarian insufficiency." Hum Reprod **31**(5): 926-937.

Yu, B., N. Douglas, M. J. Ferin, G. S. Nakhuda, K. Crew, R. A. Lobo and D. L. Hershman (2010). "Changes in markers of ovarian reserve and endocrine function in young women with breast cancer undergoing adjuvant chemotherapy." Cancer **116**(9): 2099-2105.

Zhao, J., J. Liu, K. Chen, S. Li, Y. Wang, Y. Yang, H. Deng, W. Jia, N. Rao, Q. Liu and F. Su (2014). "What lies behind chemotherapy-induced amenorrhea for breast cancer patients: a meta-analysis." Breast Cancer Res Treat **145**(1): 113-128.

GENERAL DISCUSSION

General discussion

In the cancer setting, there is a clear need for a patient-centred approach and shared decision making, i.e. decisions must be shared by doctor and patient and informed by the best evidence (Towle and Godolphin 1999). The first main goal of this research was to **contribute for shared decisions in the context of fertility preservation**. The systematic method we have used for the provision of information, based on the identification of information needs and the most relevant and up-to-date recommendations, the involvement of all the relevant stakeholders in the context of cancer care and quality evaluation using international standards, revealed itself to be a sound approach for the development of evidence-based, relevant and useful information resources. Cancer patients and survivors had an active participation by sharing their information needs and preferences and by appraising and providing suggestions for improvement in the stage of pre-testing of the information resources. In addition, the collaboration of professionals working in cancer care, human reproduction and primary care was essential to improve the contents and the organization of the information resources. Another factor that was determinant for success was the diligent cooperation of the most important national Portuguese cancer patients' organization (LPCC), through financial support and active involvement in the publication and dissemination stages. Finally, the contributions of several Portuguese professional and scientific societies in the fields of reproductive medicine, oncology and pharmaceutical sciences were, and still are, of crucial importance to guarantee an effective dissemination and clinical implementation of the developed information resources.

The results of this comprehensive information program are totally innovative in Portugal: the developed resources provide broad information contents both on infertility risks and fertility preservation options in male and female patients and target, at the same time, cancer patients and health professionals. The inclusion of these contents in the website of the Centre for Fertility Preservation (CFP) for a more convenient and easy access has broadened their availability even more.

Some difficulties have arisen along this path. We found the first and major barrier when we tried to pursue our aim of identifying the information needs of Portuguese cancer patients and oncologists. This step was deemed fundamental for the development of information contents and formats that would be adjusted to those specific needs, anticipating that they could be distinct from those internationally reported. At the time this objective was established, in the

beginning of 2013, no information was available regarding Portuguese oncologists' knowledge or practices concerning this subject or the particular needs of Portuguese cancer patients or survivors. As previously mentioned, the limited number of responses to both questionnaires may have compromised the generalizability of our results. In what concerns the participation of clinicians, a similar number of responses (n=35) were obtained in a more recent survey sent by email to 339 Portuguese oncologists, intending to assess their knowledge about FP techniques and patient referral practices (Martins and Guimarães 2016). In accordance, another recently published study that was conducted by the CFP's team showed that Portuguese oncologists report lack of time with patients as the most important barrier to FP discussions (Melo, Fonseca et al. 2018). These investigators also found that the lack of communication skills and patient-related factors, such as bad prognosis or being single, were related to a lower frequency of informing their patients about infertility risks and FP. Since questionnaires were delivered to patients by their cancer care clinicians, reasons for low participation by patients may be similar. Cancer care professionals may be mainly focused on the immediate success of cancer treatment and less attentive to long-term issues such as the quality of life in survivorship. Treatments for cancer may result in long-term issues including those impacting the physical, emotional, spiritual or social domains (Duska and Dizon 2014). At present, an increasing focus among patients, clinicians, researchers, and politicians regarding cancer survivors' health and well-being is observed. For instance, by searching Medline for references mentioning "cancer survivorship", "survivors" or "survival", a very significant increase in research is seen in the last decades, with the number of references rising from around 100 publications in 1997, to nearly 600 in 2007 and to over 2,000 publications in 2017.

We believe that the information resources are currently contributing to facilitate **decision-making** in the context of FP, helping oncologists to overcome the widely reported barrier of lack of knowledge and information (Logan, Perz et al. 2018) and leading patients to more informed clinical judgments, especially in the context of female FP, where all options present *pros* and *cons* and patients have to include their preferences in the decision process. The developed information resources also contribute for a **more accurate infertility risk assessment**, which is the first and fundamental step for engaging in discussions about FP: they include information on risks associated with specific cancer treatments and about patient and treatment factors that may influence that risk; in the comprehensive resource for oncologists, a selection of tools to support risk estimation was also incorporated.

We are certain that the present research work has also given valuable inputs to the **journey of oncofertility in Portugal**, by means of the above mentioned information program and through active cooperation to establish national clinical guidance concerning FP and several other information and education initiatives. In Portugal, the mean age of the mother at birth of first child has significantly increased in the last decades, from 25 years (1960) to 30.3 years (2016) (PORDATA 2016). Consequently, many young female patients with BC will be childless at the time of their diagnosis and will have to deal with reproductive concerns and engage in family planning decisions. Fortunately, most Portuguese public institutions already provide access to FP techniques and/or other fertility options. With the contributions from this investigation and the efforts of the CFP's team and of many other professionals and institutions, Portuguese patients and oncologists are now more aware of the infertility risks associated with cancer treatments. Therefore, opportunities to overcome these risks are available to all young cancer patients as long as referral by their oncologists is made in a timely and efficient manner.

An evidence of the positive contributions for the acknowledgement of oncofertility in Portugal is the evolution of the CFP's casuistic, from 2012 through 2016. In this time period, the number of cancer patients coming to the CFP for fertility preservation counselling has seen a tenfold increase: only eleven patients were consulted in 2012 and over 100 were consulted in 2016. In consequence, an increasing number of FP techniques have been performed and the number of oocyte and ovarian tissue cryopreservations has reached a total of 86, in the year of 2016. Moreover, the mentioned results of an email survey sent to Portuguese oncologists, at a time where the information booklet for oncologists had already been published, indicate some advance in their awareness and their daily practices concerning this topic (Martins and Guimarães 2016). Almost all participants (97%) believed that more attention should be given to fertility preservation and 52.4% mentioned addressing the subject with their patients. However, the reported knowledge on the FP techniques was in general low and the techniques of testicular and ovarian tissue cryopreservation were relatively unknown procedures. Almost half of the inquired oncologists reported never having referred a patient to a reproductive medicine department or specialist. Patient prognosis, tumour stage and fears related to the costs of FP techniques, along with legal or ethical issues, were amongst the most reported reasons not to discuss the subject of FP with patients. A previous survey that was conducted in 2012, in a sample of 30 clinicians from the haematology, bone tumours, gynaecology and surgery departments of the Coimbra Hospital and University Centre, revealed a generalized lack of information concerning the possibility of FP in female cancer patients (unpublished data).

The developed information resources are being distributed to cancer care professionals with the cooperation of the Portuguese oncology and reproductive medicine professional societies. They are also reaching cancer patients all over the country through the campaigns and events of the LPCC and with the help of Portuguese pharmacists, through the cooperation of the Portuguese Pharmaceutical Society (SRC-OF). It is now important to continue these efforts of dissemination continuously update the information contents and further evaluate the impact of information in clinical context, by means of relevant measures such as acceptability, knowledge, decision conflict or self-efficacy. In parallel, other barriers that are preventing oncologists from discussing infertility risks and FP with their patients, such as communication skills, must be properly addressed.

Our second main goal was to contribute for a **more accurate infertility risk assessment** in young female patients with breast cancer**.** To pursue this goal, two parallel, yet complementary, methods of investigation were used.

The first method was to carry out a systematic review and meta-analysis of published studies identifying patient, treatment or disease-related factors associated with the recovery of ovarian function after CT, in premenopausal women with breast cancer. In this review the aim was to confirm the existence of one or more factors that would help to predict, in each specific cancer patient, the chance of recovering ovarian function. Our review was the first of its kind: at that time, published meta-analyses had only assessed the administration of GnRH agonists as a possible factor associated with ovarian function recovery, and even so with conflicting results. Other novelty was the definition of ovarian function recovery used, not limited to menses recovery, but including additional and more reliable outcomes of recovery such as the increase in OR markers to premenopausal levels and the occurrence of pregnancy or live birth. Some of our conclusions were limited by the poor quality of some studies, the lack of uniform definitions and the predominant use of amenorrhea as marker of ovarian function in clinical trials. Nevertheless, in the meta-analysis that was performed, younger patients (≤40 years) were more likely to recover menses. The administration of GnRH agonist for ovarian protection was also a factor for recovery although substantial heterogeneity between studies was a major limitation. On the contrary, the addition of taxanes to the CT regimen proved to negatively impact recovery.

The second method consisted in a clinical observational study recruiting young BC patients consulted in the CFP of CHUC, EPE for decision-making regarding FP. The prospective design was chosen to: 1) avoid bias associated with retrospective analysis, and 2) to guarantee the temporal association between the exposure (CT) and the outcomes.

One of the novel aspects of this study was the young age of the recruited patients (all were less than 40 and median age was 33 years) which best represent the subgroup of patients with BC that value future fertility and will appoint to fertility counselling. Other innovative feature was the chosen surrogate fertility markers. We have reported not only menstrual status but also OR markers which are, at present, recognized as more reliable. At the time the study was initiated, most of the published research had used the presence of amenorrhea as the surrogate marker for ovarian failure and infertility. Additionally, we wanted to gather information for a better understanding of the impact of modern, and complex, treatment combinations for BC on those OR markers. Information was still limited regarding the effects of treatments such as the taxanes, targeted therapy (TT) and the various possible combinations of CT, hormonal therapy (HT) and TT on female fertility.

An interesting point is the fact that a number of results from the clinical study and from the systematic review are in accordance and support each other, namely:

I. **Younger age** was a significant factor for ovarian function recovery both in the meta-analysis and in the clinical study, although the cut-off was different (40 years in the meta-analysis and 30 years of median age in the clinical study). Age at recruitment was inversely correlated with OR after exposure to CT, in the prospective study. Age also influenced the strength of correlation between OR levels before and after CT, which was stronger in younger (<33 years) patients.

II. **The addition of taxanes** to the anthracycline-based CT regimens was identified as a negative factor for ovarian function recovery in the meta-analysis, a result that may explain the significant adverse reproductive health outcomes identified in the clinical study, where all the participants were exposed to docetaxel or paclitaxel, most often in addition to an anthracycline-based CT regimen. Our clinical investigation has also found significant differences in the levels of OR markers before and after taxane administration. The various results confirm the negative impact of the sequential addition of taxanes to the standard CT regimens of FEC, FAC or AC on the fertility of BC patients. Still, it remains unclear if, and how much of, this negative impact comes from the taxanes' specific gonadotoxic effects, is due to an added or synergistic effect with other cytotoxic agents or is the result of a more prolonged exposure to CT. Nevertheless, women exposed to less complex CT regimens that include taxanes, such as the weekly paclitaxel regimen in association with trastuzumab, had favourable reproductive health outcomes, though our sample was too small to draw any firm conclusions. The absence of recognized gonadotoxic agents such as cyclophosphamide

or doxorubicin in this regimen may explain these results. Up till now, the effects of the paclitaxel-trastuzumab regimen on female fertility had only been reported in a single clinical study (Ruddy, Guo et al. 2015), probably due to its narrower therapeutic indications as compared to standard combination regimens. At a mean of 51 months after CT, the rate of amenorrheic women (28%) appeared lower than those seen historically with standard alkylator-based BC regimens. Concerning the reproductive toxicity of taxanes, results of pre-clinical studies are conflicting. In female Wistar rats, exposure to paclitaxel caused a decrease of antral follicles, but not of primordial or pre-antral follicles and the authors suggest that the ovarian toxicity of this agent is mild and transient (Tarumi, Suzuki et al. 2009). However, contrasting results were published by *Lopes and colleagues* after a pre-clinical study evaluating ovarian toxicity of docetaxel in mice, where they found a decrease in the number of primary follicles through the induction of apoptosis of granulosa cells (Lopes, Smith et al. 2014). In addition, we have come to a very innovative result that may further add to this theory of a lower impact on female fertility when patients are treated with the paclitaxel-trastuzumab regimen: in our cohort, women exposed to trastuzumab had significant higher OR after CT, irrespective of their age, baseline ovarian reserve or the time passed since the end of CT. As previously discussed, this ground-breaking outcome has only been subtly reported in two other clinical studies (Ben-Aharon, Granot et al. 2015, Morarji, McArdle et al. 2017), as their samples were equally small and differences were not always significant. It is imperative to further test the hypothesis of a protective effect on fertility from exposure to trastuzumab and to understand the mechanisms subjacent to this apparent protective effect. These promising results may: 1) support the choice of less gonadotoxic treatments for BC and 2) contribute to identify possible targets for future development of approaches for fertility protection.

Overall, both investigations indicate that modern complex treatment combinations for BC expose young premenopausal women to a moderate to high risk of gonadotoxicity. Although younger age and normal-to-high OR before exposure to CT were confirmed as protective factors, many young women with BC will not recover to their age-expected levels of OR and some will be at risk for premature ovarian failure. Further information on the gonadotoxicity associated with specific treatments and CT regimens for BC was gathered: our work revealed strong evidence for the addition of taxanes to standard anthracycline/alkylating agent-based CT regimens to decrease the odds of recovering ovarian function and to increase the risk of lower OR; and limited yet cutting-edge results indicate a possible less toxic effect of some CT regimens, such as the taxane-only CT regimen and/or to a protective effect of the TT agent

trastuzumab on female fertility, as demonstrated by the higher OR after CT. More investigations are needed to confirm whether these treatment approaches may be a valuable option for young women who prioritize future fertility.

Our prospective study has also emphasized another clinically significant issue: the lack of reliable markers of OR in patients exposed to some form of HT. Interpretation of OR markers in patients with HR-positive BC under the influence of tamoxifen, an aromatase inhibitor and/or a GnRH agonist is a difficult task and may impair a reliable assessment of their ovarian function and menopausal status. Twenty-four participants had HR-positive tumours and, from those, sixteen were still under some form of HT at the last follow-up. Their levels of FSH and AMH at the last follow-up were significantly changed and the assessment of a number of secondary outcomes was not possible.

The small sample included may be pointed as a strong limitation of the clinical study but, in fact, we have included all eligible patients that came for a pre-chemotherapy FP consultation at the CFP and even extended the recruitment period to almost two and a half years. In the pre-established research protocol we had estimated that a minimum of 72 participants would be required in order to detect differences corresponding to one third of the standard deviation in serum levels of pre and post-CT AMH, with a power of 80% and a type I error of 0.05. This was a very conservative estimative as the differences in AMH levels were known to be much more significant. The observed losses to follow up (7 patients) are a recognized limitation of prospective studies which may sometimes introduce bias but, in our study, we see no reason to believe that women who dropout would present different reproductive health outcomes. Although the participants were highly motivated to engage in the research, dropouts occurred mainly due to the additional effort needed to comply with the follow-up appointments at the CFP. In fact, three of the participants made a single post-CT evaluation and only half have attended to all scheduled appointments. Our small sample size may have prevented us from detecting differences, for example between the various groups of CT regimens.

Currently, 60% of cancer patients are expected to survive 5 years or more from the diagnosis time point. As the number of cancer survivors increase, a shift in the paradigm of cancer care is happening slow but steadily: cancer patients perceive fertility preservation counselling as critical in the process of cancer care, regardless of their age or parity. Furthermore, the opportunity to engage in FP improves their coping with the diagnosis, reduces long-term regret and dissatisfaction concerning fertility and improves their physical quality of life.

The problem addressed by this work remains current and relevant. The interest in issues related to fertility preservation has been rising at a global level: in the last years, numerous international organizations, including scientific societies and other professional or clinical organizations, have published (or updated) a number of clinical, technical and ethical recommendations on this subject (Martinez and International Society for Fertility Preservation–ESHRE–ASRM Expert Working Group 2017, Oktay, Harvey et al. 2018, Schuring, Fehm et al. 2018, von Wolff, Germeyer et al. 2018). The results presented in this thesis are an additional contribution for this worldwide and meaningful concern about the future fertility of cancer patients.

References

Ben-Aharon, I., T. Granot, I. Meizner, N. Hasky, A. Tobar, S. Rizel, R. Yerushalmi, A. Ben-Haroush, B. Fisch and S. M. Stemmer (2015). "Long-Term Follow-Up of Chemotherapy-Induced Ovarian Failure in Young Breast Cancer Patients: The Role of Vascular Toxicity." Oncologist **20**(9): 985-991.

Duska, L. R. and D. S. Dizon (2014). "Improving quality of life in female cancer survivors: current status and future questions." Future Oncol **10**(6): 1015-1026.

Logan, S., J. Perz, J. Ussher, M. Peate and A. Anazodo (2018). "Clinician provision of oncofertility support in cancer patients of a reproductive age: A systematic review." Psychooncology **27**(3): 748-756.

Lopes, F., R. Smith, R. A. Anderson and N. Spears (2014). "Docetaxel induces moderate ovarian toxicity in mice, primarily affecting granulosa cells of early growing follicles." Mol Hum Reprod **20**(10): 948-959.

Martinez, F. and International Society for Fertility Preservation–ESHRE–ASRM Expert Working Group (2017). "Update on fertility preservation from the Barcelona International Society for Fertility Preservation-ESHRE-ASRM 2015 expert meeting: indications, results and future perspectives." Hum Reprod **32**(9): 1802-1811.

Martins, M. and J. M. Guimarães (2016). "Fertility Preservation in Cancer Patients Undergoing Gonadotoxic Treatment [*Preservação da fertilidade em doentes oncológicos submetidos a terapêutica gonadotóxica*]." Portuguese Journal of Oncology [*Revista Portuguesa de Oncologia*] **2**(1): 30-50.

Melo, C., A. Fonseca, C. Silva, T. Almeida-Santos and M. C. Canavarro (2018). "Portuguese oncologists' practices regarding female fertility preservation: Which barriers most relate to these practices?" Eur J Cancer Care (Engl) **27**(2): e12812.

Morarji, K., O. McArdle, K. Hui, G. Gingras-Hill, S. Ahmed, E. M. Greenblatt, E. Warner, S. Sridhar, A. M. F. Ali, A. Azad and D. C. Hodgson (2017). "Ovarian function after chemotherapy in young breast cancer survivors." Curr Oncol **24**(6): e494-e502.

Oktay, K., B. E. Harvey, A. H. Partridge, G. P. Quinn, J. Reinecke, H. S. Taylor, W. H. Wallace, E. T. Wang and A. W. Loren (2018). "Fertility Preservation in Patients With Cancer: ASCO Clinical Practice Guideline Update." J Clin Oncol: Jco2018781914.

PORDATA. (2016, 2017-05-08). "Mean age of the mother at birth of first child in Portugal." Retrieved January 2018, from https://www.pordata.pt/Portugal/Idade+m%C3%A9dia+da+m%C3%A3e+ao+nascimento+do+primeiro+filho-805.

Ruddy, K. J., H. Guo, W. Barry, C. T. Dang, D. A. Yardley, B. Moy, P. K. Marcom, K. S. Albain, H. S. Rugo, M. J. Ellis, I. Shapira, A. C. Wolff, L. A. Carey, B. A. Overmoyer, C. Hudis, I. E. Krop, H. J. Burstein, E. P. Winer, A. H. Partridge and S. M. Tolaney (2015). "Chemotherapy-related amenorrhea after adjuvant paclitaxel-trastuzumab (APT trial)." Breast Cancer Res Treat **151**(3): 589-596.

Schuring, A. N., T. Fehm, K. Behringer, M. Goeckenjan, P. Wimberger, M. Henes, J. Henes, M. F. Fey and M. von Wolff (2018). "Practical recommendations for fertility preservation in women by the FertiPROTEKT network. Part I: Indications for fertility preservation." Arch Gynecol Obstet **297**(1): 241-255.

Tarumi, W., N. Suzuki, N. Takahashi, Y. Kobayashi, K. Kiguchi, K. Sato and B. Ishizuka (2009). "Ovarian toxicity of paclitaxel and effect on fertility in the rat." J Obstet Gynaecol Res **35**(3): 414-420.

Towle, A. and W. Godolphin (1999). "Framework for teaching and learning informed shared decision making." Bmj **319**(7212): 766-771.

von Wolff, M., A. Germeyer, J. Liebenthron, M. Korell and F. Nawroth (2018). "Practical recommendations for fertility preservation in women by the FertiPROTEKT network. Part II: fertility preservation techniques." Arch Gynecol Obstet **297**(1): 257-267.

CONCLUSIONS

Conclusions

This thesis has addressed several of the reported barriers and needs for an **adequate FP decision-making process**: lack of awareness, difficulties in the assessment of the risk of infertility in each patient and lack of knowledge about the available options for FP.

The specific goal of developing evidence-based, relevant and useful information resources concerning infertility risks and fertility preservation options in cancer patients was, in our view, fully accomplished. The information resources were developed through a systematic approach, based on reported information needs and high quality standards, and all of them were successfully published and are currently available to Portuguese health professionals and cancer patients in many institutions of primary, cancer and reproductive healthcare. In addition, this research has decisively contributed to the significant advances in oncofertility that have been happening in our country in the last years.

The results here presented will certainly enable **a more accurate assessment of the risk of infertility** in the specific context of female breast cancer. Besides reinforcing previously published results about relevant markers and predictors, this work highlighted some difficulties that remain and has brought to light a few innovative hypotheses.

Data from the observational study confirm AMH as the most relevant ovarian reserve marker in this setting. Both this study and the systematic review conducted during this research have contributed to identify patient-related and treatment-related variables that may be used as predictors of post-treatment ovarian reserve levels and other reproductive health outcomes. This association was either positive, for lower age, higher AMH and exposure to trastuzumab, or negative, for taxane and hormonal therapy exposure. While younger age and higher pre-treatment levels of OR are already acknowledged as predictors of ovarian function recovery in premenopausal women with BC, the present results add to the existent data on the negative effect of the addition of taxanes and the influence of HT on the levels of FSH and AMH. They also bring to light an interesting and novel hypothesis: trastuzumab may be a fertility protective factor.

The results gathered from both investigations will also **support more informed decisions concerning fertility preservation** in each premenopausal woman with a breast cancer diagnosis.

We believe that the overall results of this research represent very significant contributions to a multitude of aspects related with oncofertility, both at the national and international levels. Each and every research work that was conducted has given important contributions both for shared decisions concerning fertility preservation and for a more easy and accurate assessment of the risk of infertility in each cancer patient, especially in the case of young pre-menopausal patients with BC. Information needs and preferences were addressed and a vast amount of information to support risk assessment was produced. By looking at the results as a whole, we see they are synergistic and reinforce each other in many ways.

The research results herein published have a high and immediate applicability in clinical practice and therefore contribute, both at present and in the near future, to a more facilitated and conscious journey on oncofertility.

ANNEXES

Annex 1.1 Study approval from the Ethics Committee of CHUC, EPE

Centro Hospitalar e Universitário de Coimbra, E.P.E.

COMISSÃO DE ÉTICA PARA A SAÚDE

Visto / À U.I.D.
para difusão
16/04/2013
Prof. Doutor José Pedro Figueiredo
Director Clínico
C.H.U.C. - EPE

Exmo. Senhor:
Director Clínico do
CHUC, E.P.E.

N/Ref.ª	Of. Nº	Data
CES	0069	08.04.2013

ASSUNTO: [CHUC-001-13] – *Estudo Observacional "Preservação da fertilidade em Oncologia: produção de materiais de comunicação para profissionais e doentes."* - Mestre Cristina Miranda da Silva - Farmacêutica dos Serviços Farmacêuticos do CHUC e Doutoranda da Faculdade de Farmácia da Universidade de Coimbra (estudo a ser realizado no Serviço de Reprodução Humana dos HUC-CHUC).

Cumpre-me informar Vossa Ex.ª que a Comissão de Ética para a Saúde do CHUC, EPE, reunida em 05 de Abril de 2013, com a presença da maioria dos seus membros, após análise do projecto mencionado em epígrafe e ouvido o relator, emitiu **parecer favorável** à sua realização. Deliberação aprovada por unanimidade.

O estudo tem como objectivos: 1) Pesquisar, junto de médicos oncologistas, os conhecimentos, atitudes e prática face à possibilidade de Preservação da Fertilidade dos doentes oncológicos em idade reprodutiva; 2) Pesquisar, junto de indivíduos com história de doença oncológica, o conhecimento, na altura do diagnóstico, sobre os diversos aspectos relacionados com eventuais alterações na fertilidade e possibilidades de Preservação da Fertilidade; 3) Produzir materiais de comunicação que permitam informar e educar, quer os profissionais de saúde quer os doentes, sobre a temática da Preservação da Fertilidade nos doentes oncológicos.

Esta investigação pretende contribuir para melhorar a comunicação de informação apropriada aos doentes oncológicos, antes do tratamento, relativa aos riscos de infertilidade e às opções disponíveis. Este conhecimento será fundamental para que possam decidir de forma informada quando confrontados com os possíveis prejuízos para a sua fertilidade.
O estudo terá uma 1.ª fase com identificação das principais lacunas de informação, através da aplicação de questionários a oncologistas e a indivíduos com história de doença oncológica e uma 2.ª fase com desenvolvimento de materiais de comunicação, direccionados às necessidades de Oncologistas e doentes.

Av. Bissaya Barreto / Pct.ª Prof. Mota Pinto
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Centro Hospitalar e Universitário de Coimbra, E.P.E.

COMISSÃO DE ÉTICA PARA A SAÚDE

Os indivíduos que preencham os critérios de inclusão serão informados sobre o estudo e seus objectivos, através dos médicos assistentes, nas consultas de *follow-up* da sua doença oncológica. Os interessados em participar assinarão um consentimento informado e ser-lhes-á entregue o questionário, que deverão preencher e remeter à investigadora via correio postal ou entregar numa consulta posterior.

Mais se informa que a CES do CHUC deve ser semestralmente actualizada em relação ao desenvolvimento dos estudos favoravelmente analisados e informada da data da conclusão dos mesmos, que deverá ser acompanhada de relatório final.

Com os melhores cumprimentos,

P' A COMISSÃO DE ÉTICA PARA A SAÚDE
DO CHUC, E.P.E.

H.U.C. C.H.U.C. E.P.E. Comissão de Ética para a Saúde

Prof. Doutor José Joaquim Sousa Barros
Presidente da CES do HUC-CHUC

A CES do HUC-CHUC: Prof. Doutor José Joaquim Sousa Barros; Prof.ª Doutora Maria Fátima Pinto Saraiva Martins; Dr. Mário Rui Almeida Branco; Enf.º Adélio Tinoco Mendes; Padre José António Afonso Pais; Prof. Doutor Carlos Alberto Fontes Ribeiro; Dra. Alexandra Vilela; Padre José António Afonso Pais
A CES do HSC-CHUC: Dra. Cláudia Santos; Dra. Conceição Pascoal; Dr. Paulo Figueiredo; Enf.ª Fernanda Pereira.
A CES do HG-CHUC: Dra. Alice Torcato; Dr. José Alves Grilo Gonçalves; Enf.º Fernando Mateus; Dra. Maria Helena Gomes; Dr. José António Pinheiro; Dra. Margarida Cunha Martins

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Annex 1.2 Authorization from the Administration Board of CHUC, EPE

Centro Hospitalar e Universitário de Coimbra, E.P.E.
Unidade de Inovação e Desenvolvimento
Centro de Ensaios Clínicos

AUTORIZAÇÃO DE PROJECTO DE INVESTIGAÇÃO

CHUC	001	13

NOME DO PROJECTO:

Preservação da Fertilidade em Oncologia: produção de materiais de comunicação para profissionais e doentes

INVESTIGADOR PRINCIPAL:

Mestre Cristina Miranda da Silva

Tendo por base o parecer da Comissão de Ética, é autorizada a realização, no Centro Hospitalar e Universitário de Coimbra, do Projecto de Investigação supracitado.

DATA: 30 / 5 / 13

Presidente do Conselho de Administração

Dr. José Martins Nunes
Presidente do Conselho de Administração
C.H.U.C. - EPE

(Dr. José Martins Nunes)

Annex 1.3 Declaration of authorization from the Gynaecology Unit of CHUC, EPE

SERVIÇO DE GINECOLOGIA
CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA, EPE (CHUC-EPE)

(Diretora: Professora Doutora Isabel Torgal)

Declaração

Declara-se, para os devidos efeitos, que o Serviço de Ginecologia do Centro Hospitalar e Universitário Coimbra, CHUC-EPE, concretamente a consulta de Doença da Mama (Drª Sónia Cerqueira), acolherá e proporcionará as condições institucionais necessárias à realização do projeto de investigação *Preservação da fertilidade em Oncologia: Processo de tomada de decisão e Necessidades de informação de doentes e oncologistas.*

O projeto de investigação a ser desenvolvido afigura-se-nos com qualidade científica e interesse para esta Instituição, motivo pelo qual passo e assino esta declaração.

Coimbra, 20 de Fevereiro de 2013

HOSPITAIS DA UNIVERSIDADE DE COIMBRA
SERVIÇO DE GINECOLOGIA
Direção de Serviço

Professora Doutora Isabel Torgal
Diretora do Serviço de Ginecologia

Annex 1.4 Declaration of authorization from the Haematology Unit of CHUC, EPE

SERVIÇO DE HEMATOLOGIA CLÍNICA
CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA, EPE (CHUC-EPE)

(Diretora: Dra. Adriana Teixeira)

Declaração

Declara-se para os devidos efeitos que o Serviço de Hematologia Clínica do Centro Hospitalar e Universitário Coimbra, CHUC-EPE, acolherá e proporcionará as condições institucionais necessárias à realização do projeto de investigação *Preservação da fertilidade em Oncologia: Processo de tomada de decisão e Necessidades de informação de doentes e oncologistas.*

O projeto de investigação a ser desenvolvido afigura-se-nos com qualidade científica e interesse para esta Instituição, motivo pelo qual passo e assino esta declaração.

Coimbra, 19 de Fevereiro de 2013

Dra. Adriana Teixeira
Diretora do Serviço de Hematologia Clínica

Annex 1.5 Declaration of authorization from the Oncology Unit of CHUC, EPE

SERVIÇO DE ONCOLOGIA
CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA, EPE

(Diretora: Dra. Anabela Sá)

Declaração

Declara-se para os devidos efeitos que o Serviço de Oncologia do Centro Hospitalar e Universitário Coimbra, CHUC-EPE, acolherá e proporcionará as condições institucionais necessárias à realização do projeto de investigação *Preservação da fertilidade em Oncologia: Processo de tomada de decisão e necessidades de informação de doentes e oncologistas.*

O projeto de investigação a ser desenvolvido afigura-se-nos com qualidade científica e interesse para esta Instituição, motivo pelo qual passo e assino esta declaração.

Coimbra, 30 de Janeiro de 2013

Dra. Anabela Sá
Diretora do Serviço de Oncologia

Annex 1.6 Questionnaire directed to cancer survivors (excerpt)

Preservação da fertilidade em Oncologia:
Processo de tomada de decisão e necessidades de informação de doentes e oncologistas.

Introdução: Dados os avanços médicos, a preservação da fertilidade em homens e mulheres antes do início dos tratamentos da doença oncológica é cada vez mais uma possibilidade. Em Portugal, a preservação da fertilidade masculina realiza-se desde o início da década de 90; relativamente às mulheres, as técnicas de preservação da fertilidade avançaram com caráter experimental em 2008, mas só recentemente foram criadas as condições para a sua implementação na prática clínica de rotina.

Objetivo Geral: Pretendemos estudar o processo de tomada de decisão de doentes oncológicos relativa à preservação da fertilidade, determinando como os fatores individuais e os do sistema de saúde influenciam esta decisão, e compreender a importância que a parentalidade assume na sobrevivência. Deste modo, tencionamos contribuir para uma melhor comunicação entre oncologistas e doentes e promover uma decisão informada em relação à preservação da fertilidade.

Papel dos Participantes: A sua colaboração nesta investigação é voluntária e consiste no preenchimento de instrumentos de autorresposta sobre diferentes áreas da sua vida (saúde física; aspetos emocionais; relações sociais, familiares; ...).

Todos os questionários serão identificados por um código e os dados serão tratados apenas coletivamente, de forma a garantir, em todos os momentos, o anonimato dos participantes.

Em qualquer momento e por qualquer motivo (inclusive se sentir a sua privacidade invadida) pode desistir de colaborar nesta investigação.

A sua decisão sobre colaborar, ou não, com esta investigação não irá prejudicar o atendimento clínico que lhe é disponibilizado ou a relação com o seu médico.

Papel dos Investigadores: Os investigadores comprometem-se a:

a) Garantir total confidencialidade sobre os dados que forem fornecidos pelos participantes;
b) Utilizar os dados fornecidos pelos participantes somente para fins de investigação (os resultados têm unicamente valor coletivo).

Consentimento Informado

Eu, _____, declaro ter consciência dos objetivos e procedimentos da investigação *Preservação da fertilidade em Oncologia: Processo de tomada de decisão e necessidades de informação de doentes e oncologistas,* bem como do meu papel enquanto participante neste estudo.

_____, _____ de _____ de 201_.
Nome do participante: _____
Assinatura: _____
Nome do investigador: _____

Equipa de investigação:
Professora Doutora Ana Cristina Rama
Professora Doutora Ana Teresa Almeida Santos
Professora Doutora Maria Cristina Canavarro
Drª Cláudia Melo (claudiasmelosilva@gmail.com)
Drª Cristina Silva (tina.silva@sapo.pt)

ESTA FOLHA INFORMATIVA É PARA SI. NÃO NECESSITA DE A DEVOLVER JUNTO DOS QUESTIONÁRIOS.

PRESERVAÇÃO DA FERTILIDADE EM ONCOLOGIA:
PROCESSO DE TOMADA DE DECISÃO E NECESSIDADES DE INFORMAÇÃO DE DOENTES E ONCOLOGISTAS

Dados os avanços médicos, a preservação da fertilidade em homens e mulheres antes do início dos tratamentos da doença oncológica é cada vez mais uma possibilidade. Em Portugal, a preservação da fertilidade masculina realiza-se desde o início da década de 90; relativamente às mulheres, as técnicas de preservação da fertilidade avançaram com caráter experimental em 2008, mas só recentemente foram criadas as condições para a sua implementação na prática clínica de rotina.

Pretendemos estudar o processo de tomada de decisão de doentes oncológicos relativa à preservação da fertilidade, determinando como os fatores individuais e os do sistema de saúde influenciam esta decisão, e compreender a importância que a parentalidade assume na sobrevivência. Deste modo, tencionamos contribuir para uma melhor comunicação entre oncologistas e doentes e promover uma decisão informada em relação à preservação da fertilidade.

Se pretender receber uma breve síntese dos resultados, à medida que forem estando disponíveis, pode deixar-nos o seu contacto de email, na ficha de dados. De notar que **os resultados apresentados serão sempre globais**, e nunca sobre análise individual de casos.

Caso queira contactar-nos por algum motivo (para esclarecimento de dúvidas ou outro), deixamos os nossos contactos:

Drª Cláudia Melo Tlm: 913588808 Email: claudiasmelosilva@gmail.com

Drª Cristina Silva Tlm: 918842294 Email: tina.silva@sapo.pt

PRESERVAÇÃO DA FERTILIDADE EM ONCOLOGIA:

PROCESSO DE TOMADA DE DECISÃO E NECESSIDADES DE INFORMAÇÃO DE DOENTES E ONCOLOGISTAS

O presente protocolo de avaliação demora, aproximadamente, 15 minutos a ser preenchido e é constituído por quatro partes.

A primeira é composta por perguntas relativas aos seus dados sociodemográficos, ao seu estado de saúde e história reprodutiva. A segunda parte engloba perguntas em relação à informação que recebeu, antes de iniciar os seus tratamentos, sobre a possibilidade de ter filhos após recuperação da doença oncológica, assim como sobre as suas motivações para ser/não ser pai (mãe). A terceira parte, avalia até que ponto determinados problemas o(a) incomodaram na última semana. A quarta, e última parte, avalia atitudes face à preservação da fertilidade, bem como a informação que recebeu sobre esta possibilidade, antes de iniciar os tratamentos da doença oncológica, e o processo de tomada de decisão que experienciou.

Agradecemos a sua colaboração, que é essencial para a persecução dos objetivos desta investigação.

FICHA DE DADOS

1 2 3 4

Antes de avançar, indique:

A data de preenchimento do presente questionário: ___ /___ /_____

O seu endereço de email, se pretender receber uma síntese dos resultados: _____

De seguida, ser-lhe-ão colocadas algumas perguntas relativas a dados sociodemográficos, à sua doença oncológica e à sua história reprodutiva.

Leia com atenção e responda de forma clara e breve ou assinale com uma cruz (X) a(s) opção (opções) que melhor corresponde(m) à sua situação.

Dados Sociodemográficos

1. **Sexo:** Masculino ☐ Feminino ☐
2. **Que idade tem atualmente?** _____ Anos.
3. **Que idade tinha na altura do diagnóstico da doença oncológica?** _____ Anos.
4. **Quais são as suas habilitações literárias?**
   Até ao 4.º ano (antigo ensino primário) ☐ 2.º ou 3.º ciclo (do 5.º ao 9.º ano) ☐
   Ensino secundário (do 10.º ao 12.º ano) ☐ Ensino superior ☐
5. **Quantos anos de escolaridade tem?** (ex. se tivesse a licenciatura, teria 17 anos de escolaridade) ___ Anos.
6. **Qual é a sua profissão?** _____
7. **Está empregado(a)?** Não ☐ Sim ☐
   6.1. Se não está empregado(a), isso deve-se a aspetos relacionados com a sua doença oncológica?
   Não ☐ Sim ☐
8. **Qual é a sua religião?** Não tenho ☐ Católica ☐ Outra ☐ Qual? _____
9. **Qual é o seu estado civil atual?**
   Solteiro(a) ☐ União de facto ☐ Casado(a) ☐ Divorciado(a) ☐ Viúvo(a) ☐
10. **Tem, neste momento, uma relação amorosa?** Não ☐ Sim ☐
    9.1. Se sim, há quanto tempo? _____ Anos; _____ Meses.
11. **Qual era o seu estado civil na altura do diagnóstico da doença oncológica?**
    Solteiro(a) ☐ União de facto ☐ Casado(a) ☐ Divorciado(a) ☐ Viúvo(a) ☐
12. **Tinha, na altura do diagnóstico da doença oncológica, uma relação amorosa?** Não ☐ Sim ☐
    11.1. Se sim, há quanto tempo? _____ Anos; _____ Meses.

Dados Clínicos

1. **Que doença oncológica lhe foi diagnosticada?** _____
2. **Quando soube do seu diagnóstico** (mês/ano)**?** ____ / _____
3. **Em que estadio de evolução da doença oncológica se encontrava?** _____ ☐ Não sei
4. **Que tratamento(s) da doença oncológica realizou e em que datas o(s) iniciou e terminou** (mês/ano)**?**

Cirurgia	☐	Data: ____ /_____
Quimioterapia	☐	Iniciou em ____ /_____; Terminou em ____ /_____
Radioterapia	☐	Iniciou em ____ /_____; Terminou em ____ /_____
Terapêutica hormonal	☐	Iniciou em ____ /_____; Terminou em ____ /_____
Outro. Qual? _____	☐	Iniciou em ____ /_____; Terminou em ____ /_____

5. **Atualmente tem algum outro problema de saúde relevante?** Não☐ Sim☐
   5.1. Se sim, qual (quais)? _____

Dados Reprodutivos

1. **Quantos filhos tem?** _____
   1.1. Tem filhos biológicos? Não☐ Sim☐
      1.1.1. Se sim, algum nascido depois dos tratamentos da doença oncológica? Não☐ Sim☐
   1.2. Tem filhos adotivos? Não☐ Sim☐
2. **Planeia ter filhos no futuro?** Não☐ Sim☐ Não sei / Estou indeciso(a)☐
3. **Os tratamentos da doença oncológica interferiram na sua função reprodutiva?**
   Não☐ Sim☐ Não sei / Não tenho a certeza☐
4. **Antes de iniciar o(s) tratamentos(s) da doença oncológica preservou a sua fertilidade?**
   Não☐ Sim☐
   4.1. Se não preservou, que razão ou razões atribui (assinale 1 ou mais opções)**?**
      Não podia, por razões médicas ☐
      Não podia, por outras razões ☐
      Não quis ☐
      Não tinha conhecimento dessa possibilidade ☐
   4.2. Se preservou, qual (quais) foi (foram) a(s) técnica(s) usada(s)?
      Preservação de sémen ☐
      Preservação de embriões ☐
      Preservação de ovócitos ☐
      Preservação de tecido ovárico ☐
   4.3. Se preservou, já recorreu ao material preservado (sémen, ovócitos, embriões e/ou tecido ovárico)**?**
      Não☐ Sim☐
      4.3.1. Se recorreu, teve sucesso / resultou daí algum filho? Não☐ Sim☐
      4.3.2. Se não recorreu, que razão ou razões atribui? (assinale 1 ou mais opções)
         Ainda não passou tempo suficiente desde o fim dos tratamentos da doença oncológica ☐
         Vou tentar primeiro ter filhos de forma "natural" ☐
         Não preciso do material preservado para ter filhos ☐
         Já não posso ter filhos, por razões médicas ☐
         Já não quero ter filhos ☐

ATITUDES FACE À PARENTALIDADE NA SOBREVIVÊNCIA

1 2 3 4

Informação sobre Parentalidade na Sobrevivência

De seguida, ser-lhe-ão colocadas algumas perguntas relativas à informação que obteve, ou não, **antes do início dos tratamentos da doença oncológica,** sobre a possibilidade de ter filhos após a sua recuperação. Leia com atenção e assinale com uma cruz (X) a opção que mais se identifica consigo.

4. Como classificaria a importância da discussão dos seguintes tópicos com doentes oncológicos antes do início dos tratamentos da doença oncológica?

	Nada Importante	Pouco Importante	Moderadamente Importante	Muito Importante	Extremamente Importante
Risco de surgirem efeitos na fertilidade futura					
Risco da transmissão genética da doença oncológica					
Risco dos filhos virem a ter uma doença oncológica					
Risco do feto desenvolver malformações devido aos tratamentos da doença oncológica					
Possibilidade de ter filhos biológicos após a recuperação da doença oncológica					
Tipo de efeitos do(s) tratamento(s) da doença oncológica na função reprodutora e fertilidade					
Duração dos efeitos do(s) tratamento(s) na função reprodutora e fertilidade					
No caso da mulher, risco de recorrência da doença oncológica devido a uma gravidez					
No caso da mulher, risco de ter menopausa precoce					

5. Como classificaria a utilidade das seguintes estratégias/materiais para informar os doentes oncológicos sobre a possibilidade de ter filhos após a recuperação da doença oncológica?

	Nada Útil	Pouco Útil	Moderadamente Útil	Muito Útil	Extremamente Útil
Consulta com médico especialista em reprodução humana					
Folhetos informativos					
Vídeos informativos					
CD-ROM					
Informação na internet					
Grupos de apoio					
Outras estratégias/materiais. Quais? _____					

ATITUDES FACE À PRESERVAÇÃO DA FERTILIDADE

1 2 3 4

Informação sobre Preservação da Fertilidade

De seguida, ser-lhe-ão colocadas algumas perguntas relativas à informação que obteve, ou não, em relação à preservação da fertilidade, **antes do início dos tratamentos da doença oncológica**. Leia com atenção e assinale com uma cruz (X) a opção que mais se identifica consigo.

Se é homem:

Prossiga o preenchimento do questionário na pergunta 1, nesta página.

Se é mulher:

Em que ano lhe foi diagnosticada a sua doença oncológica?

- **Se foi antes de 2008,** avance de imediato para a pergunta 4, na página seguinte.
- **Se foi em 2008 ou depois,** prossiga o preenchimento do questionário na pergunta 1, nesta página.

4. Como classificaria a importância da discussão dos seguintes tópicos com doentes oncológicos antes do início dos tratamentos da doença oncológica?

	Nada Importante	Pouco Importante	Moderadamente Importante	Muito Importante	Extremamente Importante
Objetivos da preservação da fertilidade					
Opções de preservação da fertilidade antes e durante os tratamentos da doença oncológica					
Técnicas de preservação da fertilidade existentes					
Vantagens das técnicas de preservação da fertilidade					
Desvantagens das técnicas de preservação da fertilidade					
Taxas de sucesso das técnicas de preservação da fertilidade					
Interferência das técnicas de preservação da fertilidade no tratamento da doença oncológica					
Custos económicos da preservação da fertilidade					
Disponibilidade de especialistas na área da preservação da fertilidade					
Por quanto tempo podem ficar preservados os ovócitos, tecido ovárico, sémen ou embriões					

5. Como classificaria a utilidade das seguintes estratégias/materiais para informar os doentes oncológicos sobre a preservação da fertilidade?

	Nada Útil	Pouco Útil	Moderadamente Útil	Muito Útil	Extremamente Útil
Consulta com médico especialista em reprodução humana					
Folhetos informativos					
Vídeos informativos					
CD-ROM					
Informação na internet					
Grupos de apoio					
Outras estratégias/materiais. Quais? _____					

Annex 1.7 Questionnaire directed to cancer care clinicians

PRESERVAÇÃO DA FERTILIDADE EM DOENTES ONCOLÓGICOS

Data do preenchimento: ____ /____ /_____

1. **Dados sociodemográficos:**
   Sexo: Masculino ☐ Feminino ☐
   Idade: _____
   Especialidade: _____

2. **Em média, quantos doentes oncológicos em idade reprodutiva tem, em consulta, num ano?**
   Homens: _____
   Mulheres: _____

3. **Com que frequência informa os seus doentes sobre o possível impacto dos tratamentos oncológicos na fertilidade?**
   Sempre ☐ Muitas vezes ☐ Algumas vezes ☐ Poucas vezes ☐ Nunca ☐
   3.1. **Quando informa, que tipo de estratégias/materiais informativos utiliza (exemplo: comunicação verbal, folheto informativo, internet)?** _____

4. **Com que frequência questiona os seus doentes sobre as suas intenções de manter a função reprodutiva?**
   Sempre ☐ Muitas vezes ☐ Algumas vezes ☐ Poucas vezes ☐ Nunca ☐

5. **Com que frequência informa os doentes em risco de infertilidade sobre a possibilidade de preservarem a mesma?**
   Sempre ☐ Muitas vezes ☐ Algumas vezes ☐ Poucas vezes ☐ Nunca ☐
   5.1. **Quando informa, que tipo de estratégias/materiais informativos utiliza (exemplo: consulta, folheto informativo, internet)?** _____

6. **Quantos doentes se lembra de já ter indicado para preservação da fertilidade?**
   Homens: _____
   Mulheres: _____
   6.1. **Destes, quantos se lembra de já ter encaminhado para o médico especialista em reprodução humana?** _____

7. **Em que medida se identifica com cada uma das seguintes afirmações?**

	Não me Identifico Nada	Identifico-me Pouco	Identifico-me em Parte	Identifico-me Muito	Identifico-me Totalmente
Encaminho os doentes oncológicos em risco de infertilidade para um médico especialista em reprodução humana.					
Os doentes oncológicos não têm interesse na questão da fertilidade pois estão perante uma doença grave, por isso não abordo o assunto.					
As taxas de sucesso das técnicas de preservação da fertilidade são tão baixas que não vale a pena encaminhar os doentes oncológicos para médico especialista em reprodução humana.					
Tenho pouco tempo disponível para abordar a possibilidade de infertilidade em consulta.					
Só quando o doente é casado é que informo sobre a possibilidade de ficar infértil após os tratamentos oncológicos.					
Tenho formação adequada sobre o impacto dos tratamentos oncológicos na fertilidade para dar esta informação.					
Não abordo a questão da fertilidade com os doentes oncológicos dado o risco de doença oncológica e/ou malformações na descendência.					
Discuto o possível impacto dos tratamentos oncológicos na fertilidade com todos os doentes oncológicos que acompanho.					
Quando o(a) doente tem um prognóstico reservado não converso com ele(a) sobre a possibilidade de ficar infértil após os tratamentos oncológicos.					

	Não me Identifico Nada	Identifico-me Pouco	Identifico-me em Parte	Identifico-me Muito	Identifico-me Totalmente
Tenho materiais informativos para dar aos doentes sobre o impacto dos tratamentos oncológicos na fertilidade.					
Não abordo os riscos dos tratamentos oncológicos na fertilidade com doentes oncológicos menores de 18 anos.					
Quando o(a) doente tem de iniciar o tratamento oncológico rapidamente não informo sobre a possibilidade de infertilidade após os tratamentos.					
Só informo o(a) doente oncológico sobre os riscos dos tratamentos oncológicos na fertilidade quando é o(a) doente a tocar no assunto.					
Sinto-me confortável para conversar com os doentes sobre a possibilidade dos tratamentos oncológicos terem impacto na sua fertilidade.					
Quando o(a) doente já é pai (mãe) não informo sobre a possibilidade de ficar infértil após os tratamentos oncológicos.					
Muitas técnicas de preservação da fertilidade são ainda experimentais, pelo que ainda não devo encaminhar os doentes oncológicos para o médico especialista em reprodução humana.					
Não converso com os doentes sobre a possibilidade dos tratamentos oncológicos afetarem a fertilidade, pois não sei para onde os encaminhar.					
Quando o(a) doente é homossexual não abordo a possibilidade de ficarem inférteis após os tratamentos oncológicos.					
Não conheço estruturas e/ou especialistas na área da reprodução humana para poder encaminhar os doentes.					
Abordo sempre a possibilidade de infertilidade com os doentes, mesmo com aqueles que têm elevadas probabilidades de permanecerem férteis após os tratamentos oncológicos.					

8. Para a sua prática clínica, como classificaria a importância de receber mais informação sobre cada um dos seguintes tópicos?

	Nada Importante	Pouco Importante	Razoavelmente Importante	Muito Importante	Extremamente Importante
Fatores que influenciam o risco de infertilidade relacionado com os tratamentos oncológicos					
Tipos de tratamentos oncológicos associados a um maior risco de infertilidade					
Risco de doença oncológica ou malformações nos descendentes de sobreviventes de doença oncológica					
Técnicas de preservação da fertilidade existentes					
Indicações das várias opções de preservação da fertilidade					
Interferência das técnicas de preservação da fertilidade na doença oncológica					
Possibilidades e limitações das técnicas de preservação da fertilidade					
Taxa de sucesso das várias técnicas de preservação da fertilidade					
Interferência das técnicas de preservação da fertilidade no início do tratamento da doença oncológica					
Custos económicos da preservação da fertilidade					
Disponibilidade de estruturas e/ou especialistas na área da reprodução humana e preservação da fertilidade					
Por quanto tempo podem ficar preservados os ovócitos, esperma ou embriões					

Agradecemos a sua colaboração, que é essencial para a persecução dos objetivos desta investigação.

Annex 4.1 Authorization for personal data processing from the Portuguese National Commission on Data Protection (CNPD)

COMISSÃO NACIONAL
DE PROTECÇÃO DE DADOS

AUTORIZAÇÃO N.º 6958 /2014

I. Do Pedido

Cristina Miranda da Silva notificou à Comissão Nacional de Protecção de Dados (CNPD) um tratamento de dados pessoais com a finalidade de elaborar um estudo observacional designado *Efeitos da terapia da antineoplásica na fertilidade de mulheres com cancro da mama: contribuição de novos marcadores na identificação do risco.*

Este é um estudo observacional, longitudinal, prospetivo.

A população em estudo é constituída por mulheres em idade reprodutiva com diagnóstico de cancro da mama inicial (não metastizado) e indicação para terapêutica sistémica com antineoplásicos.

Prevê-se a participação, no mínimo, de 72 doentes.

Serão incluídas mulheres com idade compreendida entre os 18 e os 40 anos, com diagnóstico de cancro da mama inicial (não metastizado) e indicação para tratamento com terapêutica sistémica (neo) adjuvante que inclua antineoplásicos.

O recrutamento decorrerá durante 12 a 18 meses, a partir do momento em que se inicie o estudo. As doentes que cumpram os critérios de inclusão serão identificadas pelo médico oncologista assistente, antes de iniciar tratamentos, que informará sobre o estudo e respetivos objetivos. As doentes que aceitem participar serão referenciadas, pelo médico oncologista, para uma primeira Consulta de Preservação da Fertilidade no Serviço de Reprodução Humana do CHUC, EPE, que deverá acontecer antes de iniciarem terapêutica antineoplásica.

No dia da consulta as doentes assinarão consentimento informado e serão entrevistadas pela investigadora. Realizarão ainda colheita sanguínea.

Rua de São Bento, 148-3º • 1200-821 LISBOA
Tel: 213 928 400 Fax: 213 976 832
www.cnpd.pt

21 393 00 39
LINHA PRIVACIDADE
Dias úteis das 10 às 13 h

COMISSÃO NACIONAL
DE PROTECÇÃO DE DADOS

A participação no estudo implica a recolha de dados demográficos e clínicos, assim como a recolha de resultados laboratoriais.

No "caderno de recolha de dados" não há identificação nominal do titular, sendo aposto um código de participante. A chave desta codificação só pode ser conhecida da equipa de investigadores.

Os destinatários serão ainda informados sobre a natureza facultativa da sua participação e garantida confidencialidade no tratamento.

II. Da Análise

A CNPD já se pronunciou na sua Deliberação n.º 227/2007 sobre o enquadramento legal, os fundamentos de legitimidade, os princípios orientadores para o correcto cumprimento da Lei n.º 67/98, de 26 de outubro (Lei de Proteção de Dados – LPD), bem como as condições gerais aplicáveis ao tratamento de dados pessoais para esta finalidade.

No caso em apreço, a notificação enquadra-se no âmbito tipificado por aquela Deliberação.

A informação tratada é recolhida de forma lícita (cfr. alínea a) do n.º 1 do artigo 5.º da LPD), para finalidades determinadas, explícitas e legítimas (cfr. alínea b) do mesmo artigo) e não é excessiva.

O fundamento de legitimidade é o consentimento expresso do titular dos dados.

III. Da Conclusão

Assim, nos termos das disposições conjugadas do n.º 2 do artigo 7.º, n.º1 do artigo 27.º, al. a) do n.º 1 do artigo 28.º e artigo 30.º da Lei de Protecção de Dados, com as condições e limites fixados na referida Deliberação n.º 227/2007, que se dão aqui por

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LINHA PRIVACIDADE
Dias úteis das 10 às 13 h

COMISSÃO NACIONAL
DE PROTECÇÃO DE DADOS

reproduzidos e que fundamentam esta decisão, autoriza-se o tratamento de dados supra referido, para a elaboração do presente estudo.

Termos do tratamento
Responsável pelo tratamento: Cristina Miranda da Silva
Finalidade: Estudo observacional designado *Efeitos da terapia da antineoplásica na fertilidade de mulheres com cancro da mama: contribuição de novos marcadores na identificação do risco.*
Categoria de Dados pessoais tratados: código do participante, dados demográficos (idade, estado civil, habilitações e profissão),peso, altura, data de diagnóstico da doença oncológica, características do tumor (biologia, histologia, grau e *estadio*), hábitos de consumo de tabaco, álcool e outras drogas, história pessoal ou familiar de infertilidade, história cirúrgica, problemas de saúde, história farmacoterapêutica, uso de contracetivos hormonais tipo, data e dose dos tratamentos da doença oncológica níveis de marcadores de fertilidade.
Entidades a quem podem ser comunicados: Não há.
Formas de exercício do direito de acesso e retificação: Junto do médico assistente.
Interconexões de tratamentos: Não há.
Transferências de dados para países terceiros: Não há.
Prazo de conservação: A chave de codificação dos dados do titular deve ser destruída um mês após o fim do estudo.

Dos termos e condições fixados na Deliberação n.º 227/ 2007 e na presente Autorização decorrem obrigações que o responsável deve cumprir. Deve, igualmente, dar conhecimento dessas condições a todos os intervenientes no circuito de informação.

Lisboa, 29 de julho de 2014

Filipa Calvão (Presidente)

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Annex 4.2 Study approval from the Ethics Committee from CHUC, EPE

Comissão de Ética para a Saúde

CHUC
CENTRO HOSPITALAR
E UNIVERSITÁRIO
DE COIMBRA

Visto / À U.I.D.
para difusão
Prof. Doutor José Pedro Figueiredo
Director Clínico
C.H.U.C. - EPE

Exmo. Senhor
Prof. Doutor José Pedro Figueiredo
Digmº Director Clínico do
CHUC, EPE

S/Refª	S/Comunicação	N/Ref. – Ofício n.º	Data
		CES/091	17.06.2014

Assunto: [CHUC-020-14] – *Estudo Observacional "Efeitos de terapêutica antineoplásica na fertilidade de mulheres com cancro da mama: contribuição de novos marcadores na identificação do risco."* - Mestre Cristina Miranda da Silva - Farmacêutica e a realizar o Doutoramento em Ciências Farmacêuticas pela Faculdade de Farmácia da Universidade de Coimbra (estudo a ser realizado no Serviço de Reprodução Humana do CHUC).

Cumpre-me informar Vossa Ex.ª de que a Comissão de Ética para a Saúde do Centro Hospitalar e Universitário de Coimbra, reunida em 13 de Junho de 2014, com a presença da maioria dos seus membros, após análise do projecto mencionado em epígrafe e ouvido o relator, emitiu **parecer favorável** à sua realização. Deliberação aprovada por unanimidade.

Fundamentação:

O estudo tem como objectivo avaliar o efeito de diferentes esquemas de terapêutica antineoplásica na reserva ovárica de mulheres com cancro da mama.

Em relação à metodologia: Antes, durante e após a terapêutica antineoplásica: Consulta do processo clínico, entrevista (se necessário), ecografia endovaginal e colheita de sangue para doseamentos da hormona antimulleriana (é referido que estes exames são rotina no centro de fertilidade).

As mulheres são da consulta e não há visitas adicionais aos CHUC, uma vez que a entrevista e a colheita de sangue é feita quando a doente vem às consultas habituais programadas. Não há riscos

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Praceta Prof. Mota Pinto,
3000-075 Coimbra - Portugal
Telefone: +351 239 400 400

Contacto:
Telefone: 239 400 408 Telefax: 239 405 646
E-mail: secetica@huc.min-saude.pt

1

Comissão de Ética para a Saúde

CHUC
CENTRO HOSPITALAR
E UNIVERSITÁRIO
DE COIMBRA

acrescidos para a mulher, a qual é informada de todos os procedimentos, podendo desistir a qualquer momento sem qualquer prejuízo.

Mais se informa que a CES do CHUC deve ser semestralmente actualizada em relação ao desenvolvimento dos estudos favoravelmente analisados e informada da data da conclusão dos mesmos, que deverá ser acompanhada de relatório final.

Com os melhores cumprimentos.

P´A COMISSÃO DE ÉTICA PARA A SAÚDE
DO CHUC, E.P.E.

Prof. Doutor José Joaquim Sousa Barros
Presidente da CES do CHUC

A CES do CHUC: Prof. Doutor José Joaquim Sousa Barros; Prof.ª Doutora Maria Fátima Pinto Saraiva Martins; Dr. Mário Rui Almeida Branco; Enf.º Adélio Tinoco Mendes; Prof. Doutor Carlos Alberto Fontes Ribeiro; Padre José António Afonso Pais; Dr. José António Feio; Dr. José Alves Grilo Gonçalves; Enf.º Fernando Mateus; Dr. José António Pinheiro; Dra. Cláudia Santos; Dr. Paulo Figueiredo.

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E-mail: secetica@huc.min-saude.pt

2

Annex 4.3 Authorization from the Administration Board of CHUC, EPE

CHUC
CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA

Centro Hospitalar e Universitário de Coimbra, E.P.E.
Unidade de Inovação e Desenvolvimento
Centro de Ensaios Clínicos

AUTORIZAÇÃO DE PROJECTO DE INVESTIGAÇÃO

CHUC	020	14

NOME DO PROJECTO:

EFEITOS DA TERAPÊUTICA ANTINEOPLÁSICA NA FERTILIDADE DE MULHERES COM CANCRO DA MAMA: CONTRIBUIÇÃO DE NOVOS MARCADORES NA IDENTIFICACÃO DE RISCO

INVESTIGADOR PRINCIPAL:

Mestre Cristina Miranda da Silva

Tendo por base o parecer da Comissão de Ética, é autorizada a realização, no Centro Hospitalar e Universitário de Coimbra, do Projecto de Investigação supracitado.

DATA: 10 / 7 / 14

Presidente do Conselho de Administração

Dr. José Martins Nunes
Presidente do Conselho de Administração
C.H.U.C.

(Dr. José Martins Nunes)

Annex 4.4 Declaration of authorization from the Gynaecology Unit of CHUC, EPE

CHUC
CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA

SERVIÇO DE GINECOLOGIA
CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA, EPE (CHUC-EPE)

(Diretora: Professora Doutora Isabel Torgal)

Declaração

Declara-se, para os devidos efeitos, que o **Serviço de Ginecologia do Centro Hospitalar e Universitário de Coimbra, CHUC, EPE**, acolherá e proporcionará as condições institucionais necessárias à realização do projeto de investigação Efeitos da terapêutica antineoplásica na fertilidade de mulheres com cancro da mama: contribuição de novos marcadores na identificação do risco.

O projeto de investigação a ser desenvolvido afigura-se-nos com qualidade científica e interesse para esta Instituição, motivo pelo qual passo e assino esta declaração.

Coimbra, 12 de Fevereiro de 2014

Isabel Torgal

Professora Doutora Isabel Torgal
Diretora do Serviço de Ginecologia

Annex 4.5 Declaration of authorization from the Reproductive Medicine Unit of CHUC, EPE

PARECER CIENTIFICO

Projecto Investigação: Efeitos da terapêutica antineoplásica na fertilidade de mulheres com cancro da mama: contribuição de novos marcadores na identificação do risco.

Investigador: Cristina Miranda da Silva.

Serviço: Serviço de Reprodução Humana do CHUC, EPE.

Parecer Científico
Objectivos. Metodologia. Resultados esperados.
Outros Centros ou Serviços envolvidos.

O estudo "Efeitos da terapêutica antineoplásica na fertilidade de mulheres com cancro da mama: contribuição de novos marcadores na identificação do risco" afigura-se muito relevante no contexto da identificação do risco de infertilidade associado à terapêutica antineoplásica para tratamento do cancro da mama. Os resultados vão contribuir para **informar melhor as doentes com cancro da mama** sobre as possibilidades de virem a ter filhos depois dos tratamentos.
Este é um **estudo observacional prospetivo** que pretende fundamentalmente avaliar o efeito de diferentes esquemas de terapêutica antineoplásica na reserva ovárica de mulheres com cancro da mama. São ainda objetivos do estudo identificar fatores associados ao doente e/ou aos tratamentos que contribuam para um maior risco de reserva ovárica diminuída após terapêutica antineoplásica e verificar a capacidade dos níveis iniciais de marcadores de reserva ovárica se relacionarem com a recuperação da função ovárica após terapêutica antineoplásica, em mulheres com cancro da mama.

O Director do Serviço

(Professora Doutora Ana Teresa Almeida Santos)

HUC – Gab. Ap. Inv.

Annex 4.6 Patient information document and informed consent

Efeitos da terapêutica antineoplásica na fertilidade de mulheres com cancro da mama: contribuição de novos marcadores na identificação do risco

Informação ao doente

Equipa de investigação

Investigadora responsável: Mestre Cristina Miranda da Silva, Farmacêutica, doutoranda da Faculdade de Farmácia da Universidade de Coimbra.
Carteira profissional n.º 12255 pela Ordem dos Farmacêuticos.
E-mail: tina.silva@sapo.pt
Orientadores:
Professora Doutora Ana Cristina Ribeiro Rama, Serviços Farmacêuticos do CHUC, EPE; Centro de Estudos Farmacêuticos da Faculdade de Farmácia da Universidade de Coimbra.
Professora Doutora Ana Teresa Almeida Santos, Diretora do Serviço de Reprodução Humana do CHUC, EPE, Faculdade de Medicina da Universidade de Coimbra.

Objetivos do estudo

1. Observar os efeitos dos tratamentos do cancro da mama na fertilidade da mulher.
2. Conhecer os fatores que influenciam esses efeitos e que poderão incluir características das doentes (por exemplo a idade, características genéticas ou os níveis de hormonas reprodutivas) e aspetos relacionados com o cancro e seus tratamentos (por exemplo o tipo de tratamentos e a dose administrada).
3. Utilizar novos testes, chamados testes de reserva ovárica, para avaliar o potencial reprodutivo de mulheres com cancro da mama antes, durante e após tratamentos.

Papel das participantes

A sua **colaboração é voluntária** e consiste em:

1. Autorizar a **consulta do seu processo clínico hospitalar** pela investigadora responsável e, quando necessário, responder a **entrevistas** para recolha de diversas informações sociodemográficas, sobre estilos de vida, reprodutivas e clínicas.
2. Deslocar-se ao Centro de Preservação da Fertilidade do CHUC, EPE para **consultas em diversos momentos (antes, durante e após terminarem os tratamentos)**; nestas consultas serão recolhidas amostras sanguíneas e realizadas ecografias, para **avaliar a sua fertilidade; quando se revele adequado,** poderá ainda **ser informada sobre formas de preservar a sua fertilidade**, através das técnicas disponíveis no centro.

Em qualquer momento, e por qualquer motivo, **pode desistir de colaborar nesta investigação**. A sua decisão sobre colaborar, ou não, com esta investigação, não irá prejudicar o atendimento que lhe é disponibilizado nem a relação com o seu médico. Este estudo mereceu o **parecer favorável da Comissão de Ética do CHUC, EPE**.

Todas as participantes serão consultadas antes, durante e após terminarem os tratamentos do cancro, no Centro de Preservação da Fertilidade, Serviço de Reprodução Humana do CHUC, EPE. Nestas consultas, quando adequado à sua situação, vai receber informação sobre **formas de preservar a sua fertilidade**, recorrendo às técnicas disponíveis.

Potenciais riscos

A participação no estudo envolve alguns riscos ligeiros associados à colheita de amostras sanguíneas, como dor local e hematomas ("nódoas negras"). Complicações mais graves como hemorragias, inflamação ou infeções são raras. Para minimizar estes riscos, as colheitas serão realizadas por profissionais qualificados e experientes. Não existe qualquer risco associado à realização de ecografias endovaginais.

Confidencialidade dos dados

Todas as informações recolhidas no âmbito deste estudo são **confidenciais** e os dados recolhidos serão utilizados **exclusivamente para o presente estudo**. Todos os contactos serão feitos em ambiente de privacidade. Foi solicitada e obtida **autorização da Comissão Nacional de Proteção de Dados** garantindo, em qualquer caso, que a identificação das participantes nunca será tornada pública.

Muito obrigada pela sua participação!

A investigadora

_____

Consentimento Informado

Eu, _____, declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pela investigadora. Foi-me garantida a possibilidade de, em qualquer altura, recusar participar neste estudo sem qualquer tipo de consequências. Desta forma, aceito participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade que me são dadas pela investigadora.

Coimbra, _____ de _____ de 201__.

Assinatura da participante

_____

Este documento é composto de 2 páginas e feito em duplicado: uma via para a investigadora, outra para a pessoa que consente.

2

Annex 4.7 Data collection form

"Efeitos da terapêutica antineoplásica na fertilidade de mulheres com cancro da mama: contribuição de novos marcadores na identificação do risco."

Ficha de Recolha de Dados

Critérios inclusão/exclusão

☐ 18 Anos ≤ Idade ≤ 40 Anos
☐ Cancro da mama inicial, não metastizado
☐ Indicação para QT (neo)adjuvante
☐ Não está grávida
☐ Nunca fez QT e/ou RT potencialmente gonadotóxica
☐ Sem história de ooforectomia e/ou histerectomia
☐ Níveis iniciais de HAM detetáveis

Identificação da Participante

Código: 2015

Nome: _____

Instituição de saúde: _____

Serviço e Médico assistente: _____

N.º processo clínico: _____

Contactos da participante

Telefone: _____

E-mail: _____

Data de inclusão no estudo (1.ª consulta):

___/___/_____

Data 1ª avaliação intermédia

___/___/_____

Data 2ª avaliação intermédia

___/___/_____

Data avaliação final (até 1M após)

___/___/_____

Data avaliação final (6M após)

___/___/_____

2015

A - Dados a recolher na 1.ª consulta (antes de iniciar terapêutica sistémica)

Data: ___/___/_____

1. Dados sociodemográficos

Idade: _____ anos.

Estado civil:

Solteira ☐ Casada ☐ Em união de facto ☐ Divorciada ☐ Viúva ☐

Companheiro:

Sim ☐ Não ☐

Habilitações:

1º ciclo ☐ 2º ou 3º ciclo ☐ Ensino secundário ☐ Ensino superior ☐

2. Informação sobre hábitos de vida

Tabagismo:

Nunca fumou ☐ Ex-fumadora ☐ Fumadora ☐

Consumo de bebidas alcoólicas:

Consome ☐ Não consome ☐

3. Dados clínicos

História familiar de infertilidade: Sim ☐ Não ☐

História pessoal de infertilidade: Sim ☐ Não ☐

História cirúrgica relevante: _____

Problemas de saúde: _____

História farmacoterapêutica:

Medicamento	Posologia	Data início	Data fim

Peso e altura/índice de massa corporal

Peso: _____ kg; Altura: _____ cm; IMC: _____

Dados ginecológicos e reprodutivos

História obstétrica

Nunca engravidou ☐

Já engravidou mas nunca foi mãe ☐

Tem filhos biológicos ☐

Se já engravidou, quantas vezes? _____

Períodos menstruais no último ano:

Sim ☐ Não ☐

Regularidade: Sim ☐ Não ☐

Data da última menstruação: ___/___/_____ (para identificar fase do ciclo)

Uso de contraceção hormonal:

Nunca utilizou ☐ Ex-utilizadora ☐ Utiliza atualmente ☐

2015

Se utiliza, qual o medicamento? _____.
Se utiliza ou utilizou, durante quanto tempo? _____ meses/anos.
Se ex-utilizadora, data de suspensão: ___/___/_____

Marcadores de reserva ovárica

Marcador	Data	Valor
HAM	___/___/_____	
CFA	___/___/_____	
FSH	___/___/_____	

B - Dados a recolher no processo clínico / junto do médico assistente

Dados relativos à doença oncológica
Data de diagnóstico: ___/___/_____
Caracterização do tumor:
Envolvimento ganglionar: Sim ☐ Não ☐
Tipo histológico: _____
Grau: _____
Estadio (TNM): _____
Expressão de recetores: _____

Dados relativos aos tratamentos da doença oncológica:

Cirurgia: Prevista ☐ Realizada ☐

Tipo: _____; Data: ___/___/_____.

Radioterapia: Prevista ☐ Realizada ☐

Data: ___/___/_____

Quimioterapia: Neoadjuvante ☐ Adjuvante ☐

Data início: ___/___/_____; Data fim: ___/___/_____

Protocolo de quimioterapia (nº ciclos, fármacos, dose, via de administração): _____

_____

Taxano concomitante ☐ Taxano sequencial ☐

Terapêutica anti-HER2: Sim ☐ Não ☐

Data prevista para início: ___/___/_____

Início concomitante ao taxano ☐ Início sequencial ao taxano ☐

Terapêutica hormonal: Sim ☐ Não ☐

Data prevista para início: ___/___/_____

Fármaco, posologia: _____

Agonista GnRH: Sim ☐ Não ☐

2015

Data prevista para início: ___/___/_____

Fármaco, posologia: _____

C - Dados a recolher no follow-up, durante a terapêutica sistémica

Marcadores de reserva ovárica e período menstrual

Marcador	Data	Valor	Observações
HAM	___/___/_____		
	___/___/_____		
	___/___/_____		
	___/___/_____		
	___/___/_____		
CFA	___/___/_____		
	___/___/_____		
	___/___/_____		
	___/___/_____		
	___/___/_____		
Período menstrual	___/___/_____	Sim ☐ Não ☐	
	___/___/_____	Sim ☐ Não ☐	
	___/___/_____	Sim ☐ Não ☐	

D - Dados a recolher no follow-up, após a terapêutica sistémica

Data em que terminou terapêutica sistémica: ___/___/_____

DUM: ___/___/_____

Marcadores de reserva ovárica e período menstrual

Marcador	Data	Valor	Observações
HAM	___/___/_____		
	___/___/_____		
	___/___/_____		
	___/___/_____		
	___/___/_____		
CFA	___/___/_____		
	___/___/_____		
	___/___/_____		
	___/___/_____		
	___/___/_____		
Período menstrual	___/___/_____	Sim ☐ Não ☐	
	___/___/_____	Sim ☐ Não ☐	
	___/___/_____	Sim ☐ Não ☐	

2015