FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

MAGDA TERESA SANTOS NEGRÃO

Impact of oncological disease on ovarian stimulation for fertility preservation

ARTIGO CIENTÍFICO ORIGINAL

ÁREA CIENTÍFICA DE MEDICINA DA REPRODUÇÃO

Trabalho realizado sob a orientação de:

PROFESSORA DOUTORA TERESA ALMEIDA SANTOS

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ABRIL/2023

Impact of oncological disease on ovarian stimulation for fertility preservation

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Index

Abstract

With the improvement of diagnostic and treatment techniques in oncology, there has been a significant increase in the survival rates of women of childbearing age with cancer diagnoses, and consequently in women undergoing controlled ovarian stimulation (COS) for fertility preservation (FP). However, the impact of cancer on COS is controversial and studies in this area have produced conflicting results. Thus, the aim of this study was to investigate the impact of cancer prior to gonadotoxic treatments on ovarian reserve and ovarian response to COS.

A retrospective study was conducted using an anonymous database of patients who underwent COS for FP excluding women who had already undergone potentially gonadotoxic treatment. The patients were categorized into cancer and non-cancer group and subcategorized based on cancer type: breast, ovarian, hematologic, colorectal and others.

Some clinical differences were found between groups, such as in age, parity and in patients' smoking habits. Breast and colorectal cancer patients were older than those without a cancer diagnosis (p=0.000 and p=0.001, respectively) and with hematological cancer (p=0.000 and p=0.023, respectively), and also had lower nulliparity rates compared to non-cancer group (p=0.027). There was a higher percentage of women without a cancer diagnosis with smoking habits compared to those with breast and hematological cancers (p=0.003 and p=0.013, respectively). Ovarian reserve and response to stimulation were similar in the two groups. However, a tendency to lower ovarian reserve was observed in women with colorectal cancer, namely anti-Müllerian hormone (AMH) levels were about half compared to the other groups. In this group, there was also a tendency for poorer response to ovarian stimulation, with fewer oocytes available for cryopreservation, and a higher percentage of women in whom no oocyte preservation was possible.

In conclusion, there were no differences in ovarian reserve and response to COS according to cancer type, except for a negative trend in colorectal cancer patients. Additionally, reproductive counseling for cancer patients should consider other factors that can impact fertility, such as age and smoking habits. This knowledge enables us to improve reproductive counseling in this population.

Keywords: Oncofertility, ovarian stimulation, cancer, fertility preservation, oocyte cryopreservation

Introduction

Improvements in cancer diagnosis and treatment protocols have led to an increase in long-term survival rates for women diagnosed with cancer in their childbearing years. According to United Kingdom cancer statistics, between 2016 and 2018, children and young people aged up to 24 years account for less than 1% of all new cases of cancer. In contrast, adults between the ages of 25 and 49 account for about 9% of all new cancer cases.[1] The potential loss of fertility due to cancer treatment can have a significant impact on the quality of life of cancer survivors, especially for young women and it can be more stressful than the cancer diagnosis itself.[2]

Therefore, fertility preservation (FP) has become an important component in the management of these patients, and it is recommended that patients undergo FP counseling prior to gonadotoxic therapy.[3] The strategies to preserve fertility in women may include oocyte cryopreservation, embryo cryopreservation or ovarian tissue cryopreservation.[4,5] Oocyte cryopreservation is the preferred method for fertility preservation in postpubertal patients who can delay chemotherapy. This involves controlled ovarian stimulation (COS), retrieval of oocytes and cryopreservation by vitrification for future use.[4,6] This technique can also be used in patients with hormone-dependent tumors, such as breast cancer, with letrozole protocols.[7,8] Embryo cryopreservation is an alternative option, which has been abandoned because it requires a male partner or the use of donor sperm, which may raise ethical and legal concerns.[4] Ovarian tissue preservation is the only method currently indicated for prepubertal patient or when it is urgent to start treatment.[6,9]

The gonadotoxic effects of chemotherapy and radiotherapy exposure in women have already been shown to severely damage the gonads.[10,11] Even before treatments, cancer itself may negatively affect ovarian function, decreasing ovarian reserve, the number and quality of oocytes retrieved.[12–16] However, it is not consensual, as other studies[17,18] have found no significant impact on ovarian reserve or response to COS in cancer patients. Regarding cancer type, there is also controversy. Alvarez RM *et al.*[19] reported that the type of cancer influences ovarian response to stimulation, once patients with gynecological cancer have fewer of mature oocytes compared with hematological and breast cancer patients. On the other hand, Pavone ME *et al*.[20] found that cancer diagnosis has a significant positive impact on ovarian response, as gynecologic malignancies tended to have a better ovarian response compared to other cancers. Almog B *et al.*[21], in contrast, found that cancer did not affect ovarian reserve or ovarian response, with similar results in women with breast cancer, soft tissue sarcomas, hematologic malignancies, and gastrointestinal tract cancers.

Thus, the effect of cancer itself or even cancer type on ovarian function is not fully understood and there is conflicting evidence in the literature. Further research is needed to understand the relationship between cancer and ovarian function, to offer a better reproductive counseling in cancer patients. Otherwise, it is important to note that the effect of cancer on COS may be influenced by several other factors rather that those related to the cancer type, namely the age of the patient and her general health.

Therefore, the aim of this study was to investigate the effects of cancer on ovarian reserve and response to COS in patients who have not undergone chemotherapy or radiotherapy.

Material and Methods

Population

A retrospective study was conducted, at the Fertility Preservation Center, of *Centro Hospitalar Universitário de Coimbra*. An anonymous database of patients who underwent COS for FP between May 2013 and September 2022 was obtained. The database included clinical information from medical records, including age, indication for FP, type and stage of cancer, body mass index (BMI), smoking history, anti-Müllerian hormone (AMH) levels and the number of mature and immature retrieved oocytes. Women who underwent gonadotoxic therapy (such as chemotherapy or radiotherapy) before FP were excluded.

Study groups

The patients were categorized into two main groups: cancer and non-cancer group. Further subgroups based on cancer type were established: breast cancer, ovarian cancer, hematologic cancer, colorectal cancer, and other tumors (including medulloblastoma, oligodendroglioma, nasopharyngeal carcinoma and cervical cancer).

Outcomes

The main outcomes were ovarian reserve and response to COS. AMH was analyzed to evaluate ovarian reserve, as antral follicle count (AFC) data was insufficient in our sample. Regarding response to COS, the primary outcome was the number of mature oocytes retrieved. Secondary outcomes included total number of oocytes preserved, immature oocytes retrieved, percentage of mature oocytes, and percentage of women with no oocytes retrieved. The sample was also analyzed using the Poseidon criteria[22], which is a set of criteria developed to help clinicians identify and classify patients with low prognosis undergoing assisted reproductive technology and to provide guidance on potential therapeutic strategies to overcome infertility. The criteria are used to predict the response to COS and the chances of achieving a successful pregnancy through in vitro fertilization. They consist of four categories based on age, levels of AMH and AFC (Table 1).

Table 1. Distribution of groups according to the Poseidon criteria adapted from Esteves et al. 2019.

Group 1 (High-prognosis) **AMH ≥ 1.2 ng/mL** **AFC ≥5** **< 35 years**	**Group 2** (Intermediate-prognosis) **AMH ≥ 1.2 ng/mL** **AFC ≥5** **≥ 35 years**
Group 3 (Low-prognosis) **AMH <1.2 ng/mL** **AFC <5** **<35 years**	**Group 4** (Very low-prognosis) **AMH <1.2 ng/mL** **AFC <5** **≥ 35 years**

Statistical analysis

The data were analyzed with SPSS statistics (Statistical Program for the Social Sciences) version 26.0. (IBM Corp., Armonk NY USA). It was evaluated the distribution of the samples for quantitative variables, using the Kolmogorov-Smirnov test when sample number was higher than 10 and Shapiro-Wilk test when less than 10. The quantitative normal results were presented as mean ± standard deviation (SD) and quantitative non-normal variables were expressed as median (interquartile range). the Kruskal-Wallis and Mann-Whitney tests were used to compare the medians of the independent groups to study quantitative variables.

Qualitative variables were expressed as numbers and percentages, with the significance assessed with the chi-square (χ^2) test. Then, to determine which groups had significant

differences, a customized table was used to compare the observed and expected frequencies for each group.

In all tests, a p-value of less than 0.05 was considered statistically significant.

Results

230 of the female patients referred to the Fertility Preservation Center of Reproductive Medicine Unit at *Centro Hospitalar Universitário de Coimbra in the study period* chose to undergo FP and underwent oocyte cryopreservation. Out of these patients, 39 were referred for non-oncologic reasons (non-cancer group), including conditions such as transgenders and X-Fragile syndrome. For oncologic reasons, 191 patients were referred, but 34 were excluded as they had previously undergone cancer treatments. Thus, the cancer group included 157 patients. The most frequent cancer diagnosis was breast cancer in 106 patients (67.5%). Nine patients had ovarian cancer (5.7%), 31 patients had hematologic malignancies (19.7%), 7 had gastrointestinal cancer, namely, colorectal cancer (4.5%), and the remaining 4 had other types of cancer (2.5%).

Patients' characteristics

Analyzing patients' clinical characteristics (Table 2), the comparison between patients with non-cancer and cancer diagnosis showed significant differences in age, parity, and current smoking habits. Non-cancer group included younger patients (p=0.000), with higher nulliparity (p=0.002) and higher percentage of women with smoking habits (p=0.000) compared to those with cancer diagnosis. There were no significant differences in other clinical characteristics.

Table 2. Patient's clinical characteristics according with non-cancer diagnosis and cancer diagnosis

	Non-cancer group (N=39)		Cancer group (N=157)		
	N	%	N	%	*p*
Age at initial diagnostic, mean ± standard deviation (SD) (years old)	24.41 ±0.79		30.57±0.43		**0.000**[a]
Missing data	0	0%	0	0%	
Nulliparous	28	100%	65	73%	**0.002**[b]
Missing data	11	28%	68	43%	
Age of menarche, mean ± SD (years old)	12.51±0.23		12.56±0.12		**0.712**[a]
Missing data	2	5%	9	8%	
Hormonal contraception	18	78%	82	73%	**0.615**[b]
Missing data	16	41%	45	29%	
BMI, mean ± SD (kg/m^2)	22.57±0.70		23.70±0.32		**0.343**[a]
Missing data	7	18%	11	10%	
Current smoking habits	18	50%	17	16%	**0.000**[b]
Missing data	3	8%	49	31%	

[a] Mann-Whitney test
[b] x^2 test

In the subcategories analysis (Table 3), it was observed that non-cancer patients were younger than women with breast (p=0.000) and colorectal cancer (p=0.001). Patients with breast cancer had similar ages to patients with colorectal cancer, and in both groups, patients were older than patients with hematologic cancer (p=0.000 and p=0.023, respectively). It was also observed that patients with breast cancer were older than patients with ovarian cancer (p=0.020). In all other age comparisons, there were no significant differences between groups.

There were no differences between groups in BMI, age at menarche or use of hormonal contraception, but for parity and smoking habits, differences were found (p=0.027 and p=0.015, respectively). The non-cancer, ovarian cancer, hematologic cancer, and other cancer groups all had a higher percentage of nulliparous women (100%, 75%, 88%, 100%, respectively), compared with women with breast cancer (69%) or colorectal cancer (67%). Furthermore, women without cancer diagnosis had higher current smoking habits comparing to those with breast cancer and hematologic cancer diagnosis (p=0.003 e p=0.013, respectively).

Regarding tumor stage at diagnosis in breast cancer and hematologic cancer groups most of the women were at stage II at initial diagnosis, 50% of women with ovarian cancer were diagnosed at stage I and the other 50% at stage III.

Table 3. Patient's clinical characteristics according with non-cancer diagnosis and the subcategories of cancer diagnosis.

	Non-cancer group (N=39)		Cancer group (N=157) Breast (n=106)		Ovarian (n=9)		Hematologic (n=31)		Colorectal (n=7)		Others (n=4)		
	N	%	N	%	N	%	N	%	N	%	N	%	*p*
Age at initial diagnostic. mean ± standard deviation (SD) (years old)	24.41 ±0.79		32.26±0.39		27.11±2.14		25.97±1.13		31.57±1.82		27.50±2.63		**0.000**[a]
Missing data	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	
Nulliparous	28	100%	45	69%	3	75%	14	88%	2	67%	1	100%	**0.027**[b]
Missing data	11	28%	41	39%	5	56%	15	48%	4	57%	3	75%	
Age of menarche. mean ± SD (years old)	12.51±0.23		12.46±0.14		13.44±0.29		12.62±0.29		12.67±0.56		12.50±0.55		**0.335**[a]
Missing data	2	5%	9	8%	0	0%	5	16%	1	14%	0	0	
Hormonal contraception	18	78%	56	71%	6	86%	13	76%	3	60%	4	100%	**0.686**[b]
Missing data	16	41%	27	25%	2	22%	14	45%	2	29%	0	0%	
BMI. mean ± SD (kg/m^2)	22.57±0.70		22.75±0.38		25.60±1.79		23.44±0.66		23.71±1.69		23.59±2.99		**0.486**[a]
Missing data	7	18%	11	10%	3	33%	4	13%	1	14%	0	0	
Current smoking habits	18	50%	13	17%	2	25%	1	6%	0	0%	1	33%	**0.015**[b]
Missing data	3	8%	31	29%	1	11%	13	42%	3	43%	1	25%	
Stage at initial diagnosis													**0.072**[b]
I			12	32%	1	50%	2	20%	0	0%	0	0%	
II			17	45%	0	0%	5	50%	1	100%	1	100%	
III			9	24%	1	50%	0	0%	0	0%	0	0%	
IV			0	0%	0	0%	3	30%	0	0%	0	0%	
Missing data			68	64%	7	78%	21	68%	6	86%	3	75%	

[a]Kruskal–Wallis test.
[b]χ^2 test.

Ovarian reserve assessment

As AFC data were insufficient in our sample, ovarian reserve analysis was performed only with AMH and the Poseidon groups were adapted, using only AMH and age to characterize our sample (Table 4).

Table 4. Adapted Poseidon criteria used in the study.

Group 1 (High-prognosis) **AMH ≥ 1.2 ng/mL < 35 years**	**Group 2** (Intermediate-prognosis) **AMH ≥ 1.2 ng/mL ≥ 35 years**
Group 3 (Low-prognosis) **AMH <1.2 ng/mL <35 years**	**Group 4** (Very low-prognosis) **AMH <1.2 ng/mL ≥ 35 years**

When non-cancer patients were compared to cancer patients regarding adapted/modified Poseidon criteria defined in Table 4, it was observed that the higher percentage of patients in both groups belongs to Poseidon group 1. In the non-cancer patient group (Table 5), 68.4% of

women were classified as Poseidon group 1, 2.6% were classified as Poseidon group 2 and 29.0% were classified as Poseidon group 3.

Table 5. Distribution of non-cancer patients according to Poseidon criteria (group 1 – AMH≥1.2ng/mL and <35 years; group 2 – AMH≥1.2ng/mL and ≥35 years; group 3 – AMH<1.2ng/mL and <35 years; group 4 – AMH<1.2ng/mL and ≥35 years).

Non-cancer group N=38 (1 missing value)				
	< 35 years		≥35 years	
	N	**%**	**N**	**%**
AMH≥1.2ng/ml	26	**68.4%**	1	**2.6%**
AMH<1.2ng/ml	11	**29.0%**	0	**0.0%**

In the cancer group (Table 6), 65.1% of women were classified as Poseidon group 1, 15.1% were classified as Poseidon group 2, 11.8% were classified as Poseidon group 3 and 7.9% of women were classified as Poseidon group 4.

Table 6. Distribution of patients with cancer diagnosis according to Poseidon criteria (group 1 – AMH≥1.2ng/mL and <35 years; group 2 – AMH≥1.2ng/mL and ≥35 years; group 3 – AMH<1.2ng/mL and <35 years; group 4 – AMH<1.2ng/mL and ≥35 years).

Cancer group N=152 (5 missing values)				
	< 35 years		≥35 years	
	N	**%**	**N**	**%**
AMH≥1.2ng/ml	99	**65.1%**	23	**15.1%**
AMH<1.2ng/ml	18	**11.8%**	12	**7.9%**

Moreover, the percentage of women in Poseidon group 3 was significantly higher (p=0.010), and in Poseidon group 2 significantly lower (p=0.030) in the non-cancer group compared to the cancer group (Fig.1).

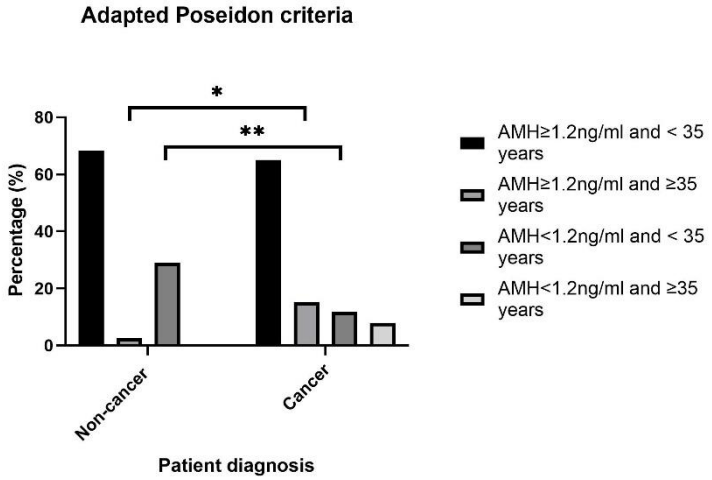

Figure 1. Graphical representation of the distribution of women without cancer diagnosis and with cancer diagnosis, categorized according to adapted Poseidon criteria (group 1 – AMH≥1.2ng/mL and <35 years; group 2 – AMH≥1.2ng/mL and ≥35 years; group 3 – AMH<1.2ng/mL and <35 years; group 4 – AMH<1.2ng/mL and ≥35 years).

In the subcategories analysis (Fig.2), it was observed that the highest percentage of women were classified as Poseidon group 1 in all cancer groups: 61.9% in patients with breast cancer, 62.5% in ovarian cancer, 78.6% in hematologic cancer, 57.1% in colorectal cancer, and 75.0% in other types of cancer. Except for the group of women diagnosed with breast cancer (11.4%) in the other four subcategories of cancer there were no women in the Poseidon group 4. The comparison between subcategories did not show any significant difference. However, comparing the non-cancer group with each cancer subcategories, it was observed that non cancer group had a significant higher percentage of women with low prognosis (Poseidon group 3 = 29.0%) compared to women diagnosed with breast cancer (8.6%, p=0.034).

Regarding the isolated analysis of AMH serum levels, it was found that there were no differences between groups. The non-cancer group had a mean level of AMH of 4.05±0.68 ng/mL, while cancer group had 3.48±0.24 ng/mL (Table 7). It was found that there were no differences between cancer categories in AMH serum levels: 3.39±0.28 ng/mL for breast, 3.63±1.42 ng/mL for ovarian cancer, 3.87±0.47 ng/mL for hematologic cancer, 1.58±0.49 ng/mL for colorectal cancer and 6.22±3.97 ng/mL for the others. Interestingly, all subcategories had AMH levels above 3 ng/mL, except colorectal cancer group who tend to have lower AMH values (Table 8).

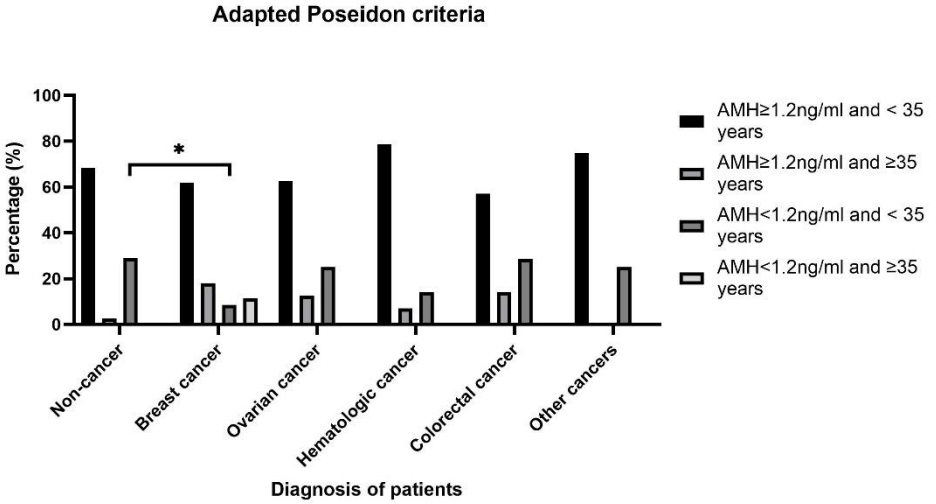

Figure 2. Graphical representation of the distribution of women, categorized according to adapted Poseidon criteria (group 1 – AMH≥1.2ng/mL and <35 years; group 2 – AMH≥1.2ng/mL and ≥35 years; group 3 – AMH<1.2ng/mL and <35 years; group 4 – AMH<1.2ng/mL and ≥35 years), showing non-cancer groups and subcategories of cancer diagnosis.

Ovarian response to COS

In Table 7 it is observed that there were no significant differences in the number of mature oocytes retrieved and total oocytes preserved between non-cancer group (7.11±0.92 and 9.00±1.14, respectively) and cancer group (7.83±0.48 and 8.79±0.53, respectively). However, non-cancer group had higher number of immature oocytes retrieved and lower percentage of mature oocytes (p=0.024 and p=0.027 respectively).

Table 7. AMH levels and ovarian response by non-cancer and cancer diagnosis.

	Non-cancer group (N=39)		**Cancer group (N=157)**		***p***
	N	%	N	%	
AMH, mean ± SD (ng/mL)	4.05±0.68		3.48±0.24		**0.563**[a]
Missing data	0	0%	5	3%	
Fertility preservation					
Total oocytes preserved, mean ± SD	9.00±1.14		8.79±0.53		**0.864**[a]
Mature oocytes (MII), mean ± SD	7.11±0.92		7.83±0.48		**0.607**[a]
Immature oocytes (MI), mean ± SD	1.89±0.52		0.88±0.13		**0.024**[a]
Percentage of mature oocytes (%)	77.51%		84.80%		**0.027**[a]
No oocytes retrieved	2	6%	13	8%	**0.693**[b]
Missing data	4	10%	0	0%	

[a] Mann-Whitney test.
[b] x^2 test.

The subcategories analysis (Table 8) showed that the non-cancer group had higher number of total oocytes preserved (9.00±1.14) compared with patients with breast cancer (8.64±0.60), ovarian cancer (6.00±0.91), colorectal cancer (5.00±2.08), and others (7.75±2.14), except when compared with women with hematologic cancer (9.96±1.54). There were no significant differences in the number of mature oocytes retrieved and immature oocytes retrieved or percentage of mature oocytes between groups, however non-cancer group tend to have a lower percentage of mature oocytes retrieved (82.21%) compared with breast cancer (91.13%), ovarian cancer (89.15%), hematologic cancer (91.15%), colorectal cancer (90.63%), and others (84.19%).

Within cancer subcategories, patients with ovarian and colorectal cancer tend to have a lower number of mature oocytes (5.22±0.80 and 4.57±2.02, respectively) than patients with breast (7.77±0.53) or hematologic cancer (9.96±1.54) and others (6.25±2.02).

Overall, all parameters analyzed in the evaluation of ovarian response did not have significant differences between groups, as it can be observed in Table 8.

Table 8. Subcategories analysis of AMH levels and ovarian response by type of cancer.

	Non-cancer group (N=39)	**Cancer group (N=157)**										***p***
		Breast (n=106)		Ovarian (n=9)		Hematologic (n=31)		Colorectal (n=7)		Others (n=4)		
	N %	N	%	N	%	N	%	N	%	N	%	
AMH, mean ± SD (ng/mL)	4.05±0.68	3.39±0.28		3.63±1.42		3.87±0.47		1.58±0.49		6.22±3.97		**0.220**[a]
Missing data	0 0%	1	1%	1	11%	3	10%	0	0%	0	0%	
Fertility preservation												
Total oocytes preserved, mean ± SD	9.00±1.14	8.64±0.60		6.00±0.91		11.28±1.65		5.00±2.08		7.75±2.14		**0.382**[a]
Mature oocytes (MII), mean ± SD	7.11±0.92	7.77±0.53		5.22±0.80		9.96±1.54		4.57±2.02		6.25±2.02		**0.318**[a]
Immature oocytes (MI), mean ± SD	1.89±0.52	0.87±0.16		0.78±0.36		1.00±0.32		0.43±0.43		1.50±1.19		**0.244**[a]
Percentage of mature oocytes (%)	78%	85%		90%		88%		91%		84%		**0.193**[a]
No oocytes retrieved	2 6%	8	7%	0	0%	2	7%	3	43%	0	0%	**0.120**[b]
Missing data	4 10%	2	2%	0	0%	3	10%	1	15%	0	0%	

[a]Kruskal–Wallis test.
[b]x^2 test.

Interestingly, as it can be observed, there was a higher percentage of patients with colorectal cancer (43%) in whom no oocytes were retrieved despite ovarian stimulation comparing with non-cancer group (6%), breast cancer (7%), ovarian cancer (0%), hematologic cancer (7%) or others (0%), but without significant differences (Fig.3).

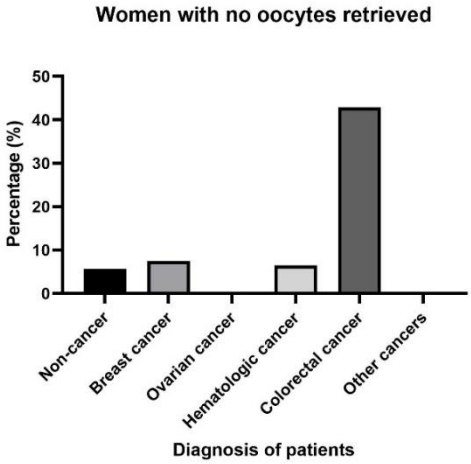

Figure 3. Graphical representation showing the percentage of women in each cancer subcategory for whom no oocytes were retrieval.

Discussion and conclusion

FP counselling is challenging, namely for oncological patients. The knowledge regarding the influence of cancer, such as cancer type, in ovarian function is scarce and contradictory. Thus, this study presents additional data regarding this topic to offer a personalized counseling for cancer patients.

According to similar studies, this research found that breast cancer patients are among the most frequent groups that undergo FP[23]. Additionally, patients with hematologic malignancies were generally younger compared to other cancer types, which could be attributed to the prevalence of Hodgkin's lymphoma in this group, typically diagnosed at younger ages.[24] Non-cancer patients, on the other hand, were younger than all the other groups.

In this study, differences in parity and smoking habits, among different cancer types were found. Patients with breast and colorectal cancers had lower percentages of nulliparous women, likely due to these cancers being more commonly diagnosed at an older age. Interestingly, patients diagnosed with cancer may have fewer current smokers, potentially due to lifestyle changes made to improve their overall health and well-being. Variations among cancer groups in clinical parameters, such as smoking habits and age, were found in previous studies and it may have a negative influence in the fertility capacity of women.[25–27] As a result, the non-cancer group's higher percentage of younger women and with smoking habits may contribute to bias in the results.

Regarding ovarian function, previous research has shown that there are no consistent results. While some studies suggested that cancer may have a negative impact, others suggested the opposite or that there are no differences at all between cancer and non-cancer diagnosis.

In what concerns ovarian reserve assessment, this is the first study to apply adapted Poseidon criteria to categorize patients based on FP prognosis. By comparing non-cancer patients with cancer group, the results suggested that cancer patients may have a better prognosis than non-cancer patients, as they had a significantly higher percentage of women with intermediate prognosis (Poseidon group 2) and patients in the non-cancer group had a higher percentage of women with low prognosis (Poseidon group 3). However, these differences are possibly due to the significant differences that were found in patients' clinical characteristics, namely age.

Regarding the AMH value alone, similar mean levels were seen in the groups. This agrees with other studies that have found no significant impact on ovarian reserve in cancer patients.[18,21] In contrast, a negative impact in ovarian reserve was previous demonstrated by Friedler *et al*.[15], who found that women with ovarian cancer had lower levels of AMH, compared to healthy controls. Quintero *et al.*[16] also found that patients with ovarian cancer had lower levels of follicle-stimulating hormone, another marker of ovarian reserve, compared to healthy controls.

Oocyte cryopreservation is an stablished technique for FP, which usually involves ovarian stimulation to produce multiple oocytes, that are then retrieved and cryopreserved by vitrification.[4] In the future, the oocytes can be thawed and fertilized with sperm, and the resulting embryos can be transferred to the uterine cavity for pregnancy.[6] Considering ovarian response to COS, there are also conflicting data, several studies reported that malignant disease has a negative impact on oocyte quality[13], that fewer oocytes are retrieved from patients with breast cancer than from control subjects[14]. The first study reported in 1998 showed that malignant disease has a negative impact on oocyte quality.[13] Later, some studies suggested that fewer oocytes are retrieved from patients with breast cancer than from control subjects.[14] Almog *et al.* [21] observed significantly lower estradiol levels in cancer patients compared to the control group, suggesting a possible negative impact of cancer on granulosa cell performance. However, the same study also found that ovarian response parameters were similar in women with breast cancer, soft tissue sarcomas, hematologic malignancies, and gastrointestinal tract cancers.[21] In contrast, Alvarez & Ramanathan[19] reported that there were significant differences in the number of mature oocytes retrieved in different cancer types, with patients with hematologic malignancies having a higher number of mature oocytes retrieved comparing with patients with gynecologic malignancies. Pavone *et al.* [20] also found that cancer diagnosis had a significant positive impact on ovarian response. Contrary to previous study he

described that gynecologic malignancies tended to have a better ovarian response compared to other cancers. However, they included women exposed to chemotherapy prior to FP which is known to have a gonadotoxic effect. Recently, it was demonstrated that gynecological cancer patients have a lower number of retrieved mature oocytes compared to patients with hematological and breast cancer.[19] In this study, there were no differences in all analyzed parameters regarding ovarian response to COS, nor in the comparation between gynecologic cancer, namely ovarian cancer, with the other groups.

An interesting result obtained in our study that was not reported in previous studies, is that patients with colorectal cancer tended to have lower ovarian reserve (lower AMH levels) and also a tendency for a worse response to COS, with lower total numbers preserved oocytes, fewer mature oocytes and higher percentage of women in whom no oocytes could be preserved.

There are several limitations that must be considered when interpreting the results of this study. Including the difficult to assess the true ovarian reserve or number of primordial follicles with *in vivo* imaging due to its poor resolution. To overcome this limitation, AMH was used as an indicator of ovarian reserve, because it correlates with the number of primordial follicles, and it is detectable in girls of all age.[28,29] In addition, the number of female cancer patients undergoing FP, although increasing, is still low, making studies and statistics difficult. In fact, the small sample size and the big differences between the various cancer categories may also affect our results and may not have been sufficient to reach statistical significance in ovarian reserve and ovarian response to stimulation. The retrospective design of the study is another significant limitation, as it does not allow for the collection of missing data.

In conclusion, these findings suggest that the impact of cancer type on fertility may vary depending on other factors, such as patient age and smoking habits. It was also found that colorectal cancer may have a negative impact on ovarian reserve and response to COS, suggesting that patients with colorectal cancer might be at risk of experiencing negative outcomes during ovarian stimulation, indicating the need for personalized care and management. However, more research is needed to fully understand the impact of different cancer types on FP.

References

1. Cancer Research UK [Internet]. [cited 2023 Mar 1]. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/incidence/age#heading-Zero

2. Letourneau JM, Ebbel EE, Katz PP, Katz A, Ai WZ, Chien AJ, *et al*. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. Cancer. 2012;118(6):1710–7.

3. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, *et al*. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. Journal of Clinical Oncology. 2006;24(18):2917–31.

4. Donnez J, Dolmans MM. Fertility preservation in women. Nature Reviews Endocrinology. 2013;9(12):735–49.

5. Donnez J, Dolmans MM. Fertility Preservation in Women. New England Journal of Medicine. 2017;377(17):1657–65.

6. Del-Pozo-Lérida S, Salvador C, Martínez-Soler F, Tortosa A, Perucho M, Giménez-Bonafé P. Preservation of fertility in patients with cancer (Review). Oncology Reports. 2019;41(5):2607–14.

7. Requena A, Herrero J, Landeras J, Navarro E, Neyro JL, Salvador C, *et al*. Use of letrozole in assisted reproduction: A systematic review and meta-analysis. Human Reproduction Update. 2008;14(6):571–82.

8. Qin Y. Effects of using letrozole in combination with the GnRH antagonist protocol for patients with poor ovarian response: A meta-analysis. Journal of Gynecology Obstetrics and Human Reproduction. 2021; 50(8): 102139.

9. Mahajan N. Fertility preservation in female cancer patients: An overview. Journal of Human Reproductive Sciences. Medknow Publications; 2015;8(1):3–12.

10. Noyes N, Knopman JM, Melzer K, Fino ME, Friedman B, Westphal LM. Oocyte cryopreservation as a fertility preservation measure for cancer patients. In: Reproductive BioMedicine Online. 2011;23(3):323–33.

11. Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? Human Reproduction Update. 2012;18(5):525–35.

12. Agarwal A, Said TM. Implications of systemic malignancies on human fertility. Reprod Biomed Online. 2004;9(6):673–9.

13. Pal L, Leykin L, Schifren JL, Isaacson KB, Chang YC, Nikruil N, *et al*. Malignancy may adversely influence the quality and behaviour of oocytes. Human Reproduction. 1998;13(7):1837-40

14. Moria A, Das M, Shehata F, Holzer H, Son WY, Tulandi T. Ovarian reserve and oocyte maturity in women with malignancy undergoing in vitro maturation treatment. Fertility and Sterility. 2011;95(5):1621–3.

15. Friedler S, Koc O, Gidoni Y, Raziel A, Ron-El R. Ovarian response to stimulation for fertility preservation in women with malignant disease: A systematic review and meta-analysis. Fertility and Sterility. 2012;97(1):125–33.

16. Quintero RB, Helmer A, Huang JQ, Westphal LM. Ovarian stimulation for fertility preservation in patients with cancer. Fertility and Sterility. 2010;93(3):865–8.

17. Tulandi T, Holzer H. Effects of malignancies on the gonadal function. Vol. 98, Fertility and Sterility. Elsevier Inc.; 2012;98(4):813–5.

18. Knopman JM, Noyes N, Talebian S, Krey LC, Grifo JA, Licciardi F. Women with cancer undergoing ART for fertility preservation: a cohort study of their response to exogenous gonadotropins. Fertility and Sterility. 2009;91(4, Suppl.):1476–8.

19. Alvarez RM, Ramanathan P. Fertility preservation in female oncology patients: The influence of the type of cancer on ovarian stimulation response. Human Reproduction. 2018;33(11):2051–9.

20. Pavone ME, Hirshfeld-Cytron J, Lawson AK, Smith K, Kazer R, Klock S. Fertility preservation outcomes may differ by cancer diagnosis. Journal of Human Reproductive Sciences. 2014;7(2):111–8.

21. Almog B, Azem F, Gordon D, Pauzner D, Amit A, Barkan G, *et al*. Effects of cancer on ovarian response in controlled ovarian stimulation for fertility preservation. Fertility and Sterility. 2012;98(4):957–60.

22. Esteves SC, Alviggi C, Humaidan P, Fischer R, Andersen CY, Conforti A, *et al*. The POSEIDON Criteria and Its Measure of Success Through the Eyes of Clinicians and Embryologists. Frontiers in Endocrinology. 2019;10:814

23. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. CA: A Cancer Journal for Clinicians. 2009;59(4):225–49.

24. Smith A, Howell D, Patmore R, Jack A, Roman E. Incidence of haematological malignancy by sub-type: A report from the Haematological Malignancy Research Network. British Journal of Cancer. 2011;105(11):1684–92.

25. Plante BJ, Cooper GS, Baird DD, Steiner AZ. The impact of smoking on antimüllerian hormone levels in women aged 38 to 50 years. Menopause. 2010;17(3):571–6.

26. Lawrenz B, Jauckus J, Kupka M, Strowitzki T, Von Wolff M. Efficacy and safety of ovarian stimulation before chemotherapy in 205 cases. Fertility and Sterility. 2010;94(7):2871–3.

27. Tinkanen H, Blä M, Laippala P, Tuohimaa P, Kujansuu E. Prognostic factors in controlled ovarian hyperstimulation. Fertility and sterility.1999;72(5):932-36

28. Anderson RA, Wallace WHB. Antimüllerian hormone, the assessment of the ovarian reserve, and the reproductive outcome of the young patient with cancer. Fertility and Sterility. Elsevier Inc.; 2013;99(6):1469–75.

29. Brougham M, Crofton P, Johnson E, Evans N, Anderson R, Wallace H. Anti-Müllerian Hormone Is a Marker of Gonadotoxicity in Pre-and Postpubertal Girls Treated for Cancer Citation for published version. Journal of Clinical Endocrinology & Metabolism. 2012;97(6):2059–67.

Annexes

I. Related publications

a) This work has been submitted in abstract form for the *XXXVIII Jornadas de Estudos da Reprodução* and is awaiting review by the event's organizing committee.

II. Acknowledgements

“Somos do tamanho de nossos sonhos”

Fernando Pessoa

I would like to start my acknowledgement with the sentence above, which was an engine to get me through these 5 years of medical school. During the last 10 years I pursued my dream of becoming a physician. Sometimes it can be a long and challenging journey, as it has been for me, but it can also be extremely rewarding.

The last year has been particularly gratifying for me and my dream of becoming an obstetrician was growing. Consequently, I have been trying to find a topic for my master’s thesis that aligns with my passion for generating life rather than trying to overcome death.

For this reason, I would like to first thank to Dr. Ana Sofia Pais for listening to me and suggesting the theme of my master’s thesis which matched with my interests and passion. Then, I would like to thank Professor Teresa Almeida Santos who trusted on me and who had confidence in my abilities by accepting me in her team.

I also want to refer my husband, my mum, my dad, my brother and all my family for always being by my side and for never letting me give up, even on bad days.

Last, I would like to thank my girls (you know who you are), who kept me on board, who believed in me, encourage me, and remembered me every day that I am as bigger as my dreams.