OXFORD

human reproduction

Human Reproduction, 2023, **38(9)**, 1856–1860
https://doi.org/10.1093/humrep/dead128
Advance Access Publication Date: June 16, 2023
Peer Perspectives

Going back to the start: do cancer and haematological disorders affect germ cells in prepubertal boys?

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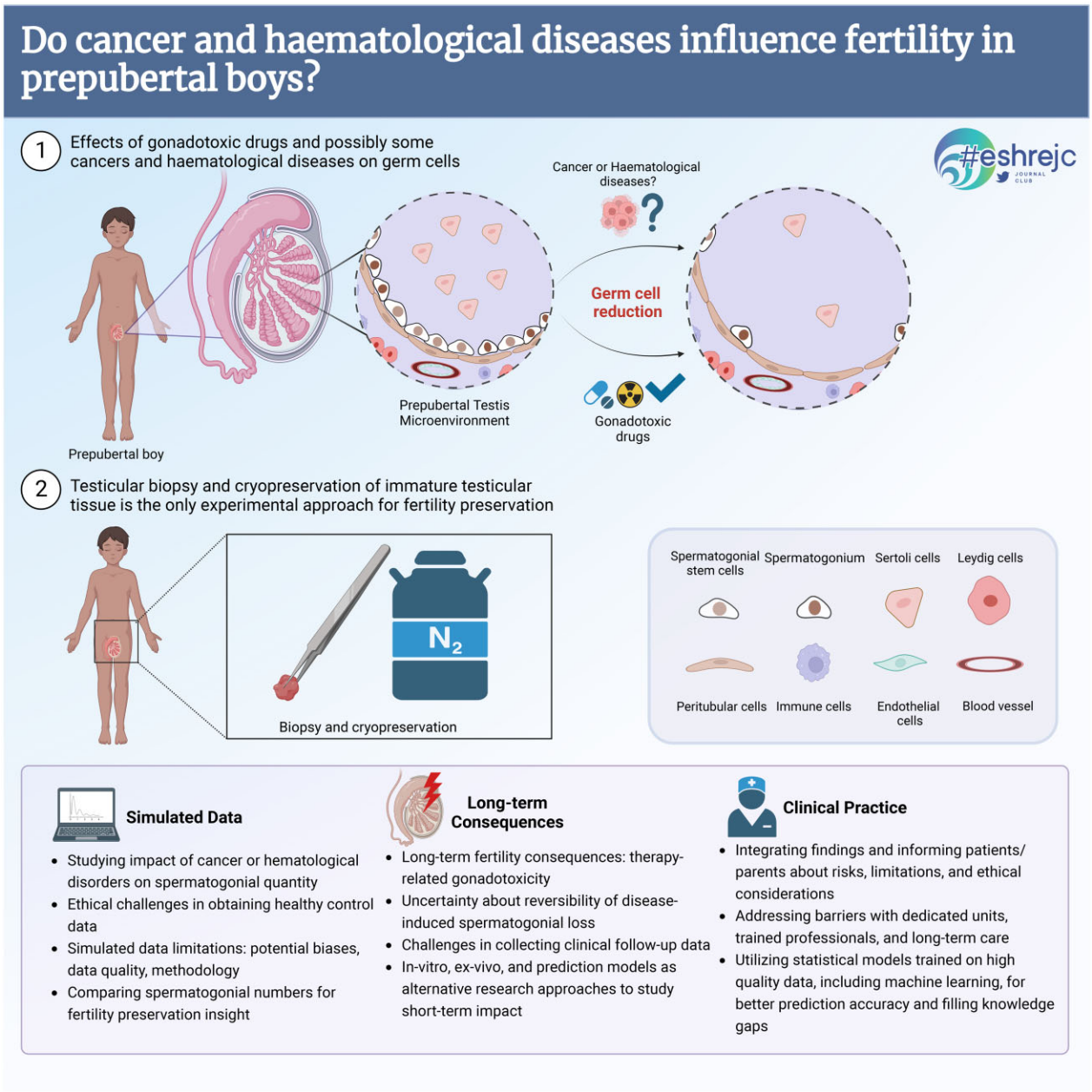
Keywords: fertility preservation / childhood cancer / testicular tissue cryopreservation / spermatogonia / male fertility / prepubertal boys / haematological disorders

Graphical Abstract



The March 2023 ESHRE Journal Club was dedicated to a study by Masliukaite *et al.* (2023) discussing the effect of cancer or severe haematological disorders on prepubertal boys' fertility potential and fertility preservation options.

Recent evidence shows the need for optimal counselling about the adverse effects of cancer treatments on reproductive tissues and options for preserving fertility in prepubertal paediatric males (Mulder *et al.*, 2021). Some of the current challenges in the field are the urgency in decision-making for fertility preservation, the necessary involvement of parents (or legal guardians), and the uncertainty about the degree of treatment gonadotoxicity. Male fertility preservation programmes include cryopreservation of immature testicular tissue, although the restoration procedures are still at an experimental stage without any live births in humans (Kanbar *et al.*, 2022). In this context, it is imperative to determine whether the disease itself adversely affects the male gonad thereby reducing fertility potential before treatment is initiated. Subsequently, it is crucial to pinpoint whether such damage is age and/or disease dependent, and if it is reversible. Masliukaite and colleagues recently published a retrospective study aiming to evaluate spermatogonial quantity in testes of 101 prepubertal boys with cancer or severe haematological disorders admitted for fertility preservation, and to compare the measurements with simulated control data generated from original studies in healthy populations (Masliukaite *et al.*, 2023). They reported the highest prevalence of reduced spermatogonial quantity in boys with central nervous system tumours and haematological diseases, especially in patients <7 years of age. These results, obtained before disease treatment, carry relevant implications for fertility preservation counselling and could inform the selection criteria for fertility preservation, which currently varies across different institutions and countries (Anderson *et al.*, 2015;

Braye et al., 2019; Valli-Pulaski et al., 2019; Kanbar et al., 2021; Mincheva and Schlatt, 2021; Newton et al., 2022). These topics were the central focus during the March 2023 edition of the ESHRE Journal Club.

Do cancer and haematological diseases influence fertility in prepubertal boys?

The question on whether a primary pathology (cancer or other diseases like sickle cell disease) has an impact, even before gonadotoxic treatments, on fertility is not new. In adults, a reduced quality of ejaculate samples collected for sperm cryopreservation in patients with cancer was hypothesized to be immunomediated or a result of DNA damage (Barr et al., 1993; Stigliani et al., 2021). In prepubertal boys, it is known that spermatogonial quantity is significantly reduced after treatment with alkylating agent therapies or with hydroxyurea for sickle cell disease (Stukenborg et al., 2018), but the hypothesis that the disease itself causes damage to spermatogonia even before therapies is not yet confirmed; nor is it ruled out. For example, a reduced number of spermatogonia was observed in boys diagnosed with single gene mutations such as thalassaemia major (Stukenborg et al., 2018) and sickle cell disease (Gille et al., 2021). The genetic and epigenetic landscape that can influence cancer predisposition and onset is multifactorial and could impact the delicate balance needed for puberty (Manotas et al., 2022). Furthermore, the negative effect of the primary disease on different organs and systems could impact on fertility: for example, it has been shown that fertility is affected in patients with kidney diseases, mainly due to impaired function of the hypothalamic–pituitary–gonadal axis (Dumanski and Ahmed, 2019).

The data presented by Masliukaite and colleagues report an age-dependent effect of cancer or severe haematological cancer on spermatogonial quantity in prepubertal boys that is more pronounced in patients ≤7 years of age (Masliukaite et al., 2023). These data suggest that a detrimental effect on spermatogonial quantity is present at an early age, before treatment, although it does not provide definitive evidence about the onset: is it at the onset of the primary pathology or is it present from birth or even *in utero*? Without definitive proof, the hypotheses cannot be anything other than speculative.

The quest for the right model: advantages and challenges of using simulated data choosing the correct controls is of paramount importance to better understand the possible impact of cancer or haematological disorders on spermatogonial quantity in testes of prepubertal boys. However, it is extremely difficult to obtain good quality data on healthy individuals due to ethical concerns. Using simulated controls, such as in Masliukaite et al. (2023), is a good option to circumvent this conundrum, despite inevitably introducing biases. The resulting controls are limited by the quality of the original data they are derived from, and then by the methodology used to collect and analyse them. The simulated control data used by Masliukaite and colleagues are based on six cohort studies describing original quantitative data about the number of spermatogonia per transverse tubular cross section (S/T) and the spermatogonial density per cm^3 of testicular volume (S/V) in healthy boys (described in Masliukaite et al., 2016). Because not all the original studies had reported both measurements, and it was not possible to retrieve every original data point, Masliukaite et al. (2023) applied polynomial meta-regression analyses to describe their patterns throughout prepubertal life. To account for data uncertainty, they performed multiple simulations. The expected pattern in healthy boys consisted of a S/V and S/T decline during the first 3 years of life, a gradual increase until the ages of 6–7 years, a plateau until the age of 11 years, and a sharp incline reaching puberty. Notably, the time points at which a major reduction in S/T and S/V were reported in cancer patients corresponded with those at which an increase would have been expected.

Another possible limitation of using simulated data is that the model implied that 30% of the supposed healthy controls had low spermatogonial numbers, which seems a higher prevalence than expected. Journal club participants suggested that the difference in spermatogonial number between simulated controls and patients may be more informative than the prevalence of low spermatogonial numbers itself. Indeed, this difference is not large, but if it is shown to be predictive of severe damage after therapies it could be useful to inform patient selection for fertility preservation.

Long-term consequences on fertility

Regardless of whether the spermatogonial quality is reduced from the underlying condition, the therapies used, such as chemotherapy and radiotherapy, have a known gonadotoxicity effect (Wallace et al., 2005). We still do not know if the observed partial loss in spermatogonial population is reversible or not; more data would be of extreme relevance for oncofertility counselling. A complete loss of spermatogonia is not reversible (Lopes et al., 2021), but there are animal studies showing re-population after a partial reduction in spermatogonia following chemotherapy exposure (Zohni et al., 2012). What is the quality of remaining spermatogonia, for example in terms of DNA integrity, represents another relevant question.

Data from clinical follow-up of patients are essential but present multiple challenges such as varying healthcare provision, loss of patients from follow-up once they transition from paediatrics to adult medicine, and difficulties in determining the contribution of each drug to subsequent testicular function (Howard et al., 2018). Are *in vitro* and *ex vivo* approaches an option to explore the unanswered questions? Prediction models and *in silico* platforms are a possible option to explore both the correlations between initial S/T and S/V (combined with predictors such as age, disease, treatment) and the reproductive outcomes in adulthood and could provide a better understanding of the possible role of the crosstalk between spermatogonia and somatic cells in the maturing testis. Such models would need the application of appropriate statistical and machine learning methods, informed by data on the development of germ and somatic cells in the testis of healthy boys from birth, through puberty and into adulthood (Masliukaite et al., 2016), combined with data about the initial testicular content in childhood cancer patients, such as those collected from Masliukaite et al. (2023). *In vitro* and *xeno* transplantation models are another possibilities to study human testicular development and the impact of cancer or its treatment; however, these are primarily aimed at assessing short-term impact on spermatogonial stem cells as these models do not currently support spermatogenesis over the longer term, as opposed to other species including non-human primates (Ntemou et al., 2019).

How to incorporate the study results into the fertility preservation counselling of prepubertal boys?

Incorporating the experimental results, suggesting that spermatogonial quantity is already reduced before gonadotoxic therapies

in boys with cancer or haematological diseases, into the fertility preservation counselling is never easy, especially because immature testicular tissue cryopreservation is the only possible fertility preservation option. Though it is generally safe (Kanbar *et al.*, 2021), the clinical efficacy of fertility restoration strategies has not been shown yet in humans (Kanbar *et al.*, 2022). It is mandatory to inform patients and their parents about the risk of treatment gonadotoxicity and to discuss with them available fertility preservation options and their limitations (Mulder *et al.*, 2021). Additional possible barriers to effective fertility counselling include insufficient knowledge of the treating physicians and the absence of standard shared pathways of care and protocols (Newton *et al.*, 2022). It has been argued that prepubertal testicular tissue cryopreservation should be considered ethical if the possible benefits outweigh the burdens, if it is generally safe, and if patient/parents are adequately informed, supported, and guided in their decision-making process (McDougall *et al.*, 2018). Childhood cancer survivors would use their cryopreserved tissue in years/decades, if ever, with the expectation that the clinical procedures to restore fertility will be established by then (Goossens *et al.*, 2020). The fact that there is no guarantee for future parenthood and that potentially fertility may never be restored should be discussed as well. As with the situation in adult fertility preservation programmes, the establishment of dedicated units with trained professionals and the availability of a fertility specialist for urgent comprehensive counselling have been deemed to be important for successful prepubertal fertility preservation programmes (Anazodo *et al.*, 2019). The possibility of long-term care, including fertility follow-up visits, is also essential, both to give patients awareness about their fertility potential and to collect high-quality data (Massarotti *et al.*, 2019). Statistical models, including machine learning, are powerful tools that may help fill the gaps in knowledge of the biological mechanisms of the observed results and to improve the accuracy of the prediction of treatment gonadotoxicity.

Data availability

No datasets were generated or analysed in the current manuscript.

Acknowledgements

The authors would like to thank all the participants of ESHRE journal club on Twitter for their contribution in the discussion.

Authors' roles

M.M., J.J.F.-Z, G.L., and O.F.A. conceptualized and wrote the discussion questions; M.M. organized the discussion; M.M., J.J.F.-Z., G.L., O.F.A., and C.M. moderated the discussion; M.M. prepared and led the discussion material and moderation; K.D., M.K., R.T.M., M.M.-R. contributed intellectually to the discussion as an expert; O.F.A. prepared the graphical abstract; J.J.F.Z., G.L., and O.F.A. provided outlines for the manuscript content; and C.M. compiled and wrote the manuscript and led the publication process. All authors provided critical revision to the article and approved the final version.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

All authors declare no conflict of interest.

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