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Review

Fertility on ice: an overview of fertility preservation for children and adolescents with cancer

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Key content
- Continued advances in oncology treatments have led to better survival rates for children and young adults with cancer.
- An important 'late effect' of cancer treatment is the loss of fertility.
- Although many survivors of childhood cancers go on to conceive without difficulty, the potential loss of fertility is a concern for children and young adults with cancer, their parents and caregivers.
- We review current options for fertility preservation (FP) for prepubertal and postpubertal girls and boys, including oocyte cryopreservation, ovarian tissue cryopreservation, sperm cryopreservation and testicular tissue cryopreservation.

Learning objectives
- To understand the different FP methods available for children and adolescents, the barriers to FP and ethical and psychological considerations
- To raise awareness of the importance of early discussions about FP with patients who are at risk of infertility from their cancer treatment.

Ethical Issues
- Autotransplantation of ovarian tissue in survivors of haematological malignancies, particularly leukaemia, carries a strong risk of re-introducing the malignancy and should be avoided.
- Clinicians must consider suitability of the patient for FP, taking into account emotional maturity, patient and parent desire for FP and the physical fitness of the patient, including their immunosuppressive state.

Keywords: child and young adult cancer / fertility preservation / oocyte cryopreservation / ovarian tissue cryopreservation

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Introduction

Continued advances in oncology treatments have led to better survival rates for children (0–14 years), adolescent (15–19 years) and young adult (20–24 years) cancer patients.[1] Overall survival is now greater than 80%. Because of this, there is increasing emphasis on improving the long-term quality of life of cancer survivors. Efforts to reduce the downstream sequelae of treatment are increasingly a priority. When a patient who has not yet reached their reproductive goals is given a cancer diagnosis, it is best practice to discuss their future fertility because chemotherapy, radiotherapy and some surgical treatments may lead to a reduction or loss of gonadal function. Thus, one of the most important 'late effects' of cancer treatment is the loss of fertility.[2]

Fertility preservation (FP) is the preservation of an individual's oocytes, sperm or gonadal tissue so that the individual may use them to have their own biological children in future. Consideration of FP is indicated if there is a risk of future gonadal failure for any aetiology;[3] however, this review article focuses on FP options for children and young adults with cancer.

Effects of oncology treatment on subsequent fertility and reproductive function

Childhood cancers are treated using various regimens, such as chemotherapy, surgery, haematopoietic stem cell transplant and radiotherapy including proton therapy. More recently,

immune-based therapies have also been adopted. Conventional treatment modalities can negatively affect reproductive potential through inadvertent injury to the hypothalamic–pituitary axis and to the reproductive organs themselves; for example, the ovary, testes, uterus and vagina.[4]

The effects of radiotherapy depend on the dose received, the fractionation schedule and the targeted field of radiation. The effects of chemotherapy are related to the type of agent used, as well as the cumulative dose received.[5]

Total body irradiation, radiotherapy to a field that includes the ovaries or testes, and the use of alkylating agents including cyclophosphamide, busulfan and chlorambucil, pose the greatest risk of gonadotoxicity.[6] Higher accumulated doses of alkylating agents lower anti-müllerian hormone (AMH) levels and decrease the chance of pregnancy.[7] Radiation therapy damages granulosa cells, with subsequent damage to ovarian follicles. Irradiation of the ovaries of 10 Gy and above is associated with premature ovarian insufficiency.[7]

Methods of fertility preservation

Sperm cryopreservation

Thirty percent of male survivors of childhood cancer suffer from azoospermia (no sperm) and 18% suffer from oligospermia (reduced sperm).[8] Where appropriate, postpubertal boys who are given a cancer diagnosis should be given the option of sperm cryopreservation before commencing treatment. This method of FP is well established, relatively noninvasive and, usually, does not delay oncology treatment to any significant degree.

However, sperm cryopreservation has its limitations. It is only suitable for postpubertal boys, some boys may suffer anxiety and be unable to produce a sperm sample by masturbation, or there may be religious or cultural concerns.[9] Limitations may also be imposed by the nature of the disease itself (for example, patients with cord compression display impairment of the normal neurological pathways required for ejaculation) and by medication side effects (such as analgesic narcotics required for pain control). Nevertheless, several studies have demonstrated that most adolescent cancer patients can produce a semen sample, including boys as young as 12 years of age.[10,11]

Sperm quantity and quality may also be adversely affected by the primary tumour, most notably in cases of malignant testicular neoplasms.[8] A UK study published in 2002[11] looked at 238 adolescent patients referred for sperm cryopreservation before cancer treatment. Diagnoses included testicular cancer, leukaemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, osteosarcoma, Ewing's sarcoma, acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML). Of these 238 patients, 33 (13.9%) were unable to produce a sample, while 205 (86.1%) successfully provided a sample suitable for cryopreservation. No cases of azoospermia were reported pre-treatment. Sperm parameters were broadly uniform across all cancer types, with sperm counts increasing in an age-dependent manner.[11]

Oocyte cryopreservation

For postpubertal females, the established options for FP are embryo or oocyte cryopreservation.[12] However, given the young age of child and young adult patients, oocyte cryopreservation is usually most appropriate.

Cryopreservation refers to the cooling of cells and tissues to sub-zero temperatures, thus halting all biological activity so that they can be preserved for future use.[13] The human metaphase II oocyte is a particularly fragile cell, owing to its large size, large cytosolic water content and chromosomal rearrangement.[14] Initial oocyte freezing efforts using a 'slow freezing' technique were hampered by cellular damage, ice crystal formation or excessive dehydration, so this technique has now been replaced by vitrification. Vitrification is a process of cryopreservation using high concentrations of cryoprotectants and rapid cooling to avoid the formation of ice crystals. This has markedly improved the viability of cryopreserved cells.[13] The first human birth from a frozen oocyte was reported in 1986.[15]

Embryo and oocyte vitrification require at least one cycle of ovarian stimulation with subsequent oocyte retrieval, thus are not appropriate for prepubertal girls.

Ovarian stimulation usually takes 2 weeks and involves self-administration of follicle-stimulating hormone injections. Development of ovarian follicles, each containing an oocyte, is tracked using ultrasound scans and serum hormone levels. Ideally, the ovarian stimulation regime begins at the start of the menstrual cycle. However, random-start protocols are also used, with obvious benefits for oncology patients who need to commence cancer treatment as soon as possible. Recently, the idea of 'back-to-back' stimulation protocols has been introduced. This involves a double ovarian stimulation during both the follicular and luteal phases, with the intention of achieving a greater oocyte yield in less time.[16] Oocyte retrieval is typically performed by ultrasound-guided transvaginal needle aspiration under sedation. Increasing evidence for the safety of the procedure led oocyte vitrification to be reclassified from experimental to nonexperimental in 2013 by both the American Society of Reproductive Medicine (ASRM) and the European Society for Human Reproduction and Embryology (ESHRE).[17] The procedure is now in routine use in clinical assisted reproduction.

Risks associated with ovarian stimulation for oocyte cryopreservation in postpubertal females are 1) delayed initiation of cancer treatment, 2) ovarian hyperstimulation syndrome (OHSS) in girls with high ovarian reserve and 3) the effect of stimulation protocols that increase estrogen levels on hormone-dependent malignancies. For estrogen-dependent breast and gynaecologic cancers (rare in under 25-

year-olds), current recommendations indicate use of aromatase inhibitor-based protocols for ovarian stimulation, because these may mitigate the risk of cancer recurrence.[18] Furthermore, it is important to take into account that the process of ovarian stimulation and oocyte retrieval requires ultrasound scans; these are usually performed transvaginally, so require a certain level of physical and psychological maturity.

Notably, a recent large multicentre study of oocyte vitrification and in vitro fertilisation (IVF) outcomes revealed markedly lower success rates in young women (≤35 years) who used this fertility preservation method after cancer diagnosis compared with age-matched women seeking elective fertility preservation for non-oncological reasons.[19] This included significantly lower oocyte survival (81.2% versus 91.4%), and reduced cumulative live birth rates (40% versus 70%). This important study suggests that primary malignancy may affect reproductive potential and should be taken into consideration when counselling patients.

Ovarian tissue cryopreservation

Ovarian tissue cryopreservation (OTC) involves laparoscopic surgery to remove all or part of the ovary, followed by cryopreservation of the excised tissue with a view to autotransplantation in the future. The tissue may be transplanted back to the patient's pelvic cavity (orthotopically), or to a site outside of the pelvic cavity; for example, to the forearm or rectus muscle (heterotopically).[20] Typically, strips of ovarian cortex are laparoscopically grafted back to the exposed ovarian medulla or an adjacent site, thus making spontaneous pregnancy or use of assisted reproduction techniques possible. Oocyte retrieval and embryo development have been demonstrated following heterotopic transplantation of ovarian tissue; however, live birth rates are very low and natural conception is not possible with this technique.[20]

A key benefit of OTC is that ovarian stimulation is not necessary, so treatment for cancer patients is not delayed. Additionally, retrieval of tissue for OTC does not require sexual maturity, so it is suitable for prepubertal girls. A further advantage is that autotransplantation of ovarian tissue after puberty can restore general ovarian endocrine function in addition to preserving fertility.[20]

The first successful pregnancy after replacement of cryopreserved human ovarian tissue in an adult female was reported in 2004.[21] Since then, there have been more than 130 live births recorded using this procedure.

Ovarian activity has been reported to resume in over 90% of women after replacement of their ovarian tissue; this occurs a median of 4 months after transplantation. Ovarian activity is sustained for a variable duration of time, but has been reported to last several years in some cases.[22] A recent publication reported on 95 orthotopic cryopreserved ovarian tissue transplantations in 74 adult women treated for cancer in in the European FertiProtekt Network.[23] Mean age at cryopreservation and transplantation was 30 years and 34 years, respectively, with the two most common diagnoses being breast cancer and Hodgkin's lymphoma. Of women with premature ovarian insufficiency (POI) at the time of first transplantation, 62.5% showed evidence of ovarian activity 1 year post-transplantation, 27.5% achieved a pregnancy, and 22.5% had a live birth. Importantly, most of these pregnancies resulted from natural conception. The potential for natural conception is a primary advantage of OTC over oocyte or embryo cryopreservation. True success rates were, however, confounded by several factors, including residual ovarian activity and transplantations for endocrine function rather than fertility restoration.[22]

To date, there have been just two case reports of a successful live birth following autotransplantation of ovarian tissue that was cryopreserved pre-menarche.[24,25] The first case involved a woman, originally from the Republic of Congo, who was diagnosed with sickle-cell anaemia at the age of five. Her right ovary was laparoscopically removed and cryopreserved at the age of 13 years and 11 months, before having curative therapy with haematopoetic stem cell transplant (HSCT). As expected, following the treatment, the patient developed primary ovarian failure, with elevated gonadotrophins. Menarche was induced at the age of 15.5 years.

Ten years later, the patient wished to become pregnant. The patient underwent ovarian tissue transplantation. Menstruation occurred 5 months later and was followed by regular menstrual cycles thereafter.[24] Two years post-transplantation, the patient became pregnant and delivered a healthy boy in November 2014.[24]

The youngest worldwide reported case of ovarian tissue cryopreservation before puberty with subsequent transplantation of the tissue was a 9-year-old girl suffering from β-thalassaemia. Her ovarian tissue was cryopreserved for 14 years before being transplanted back at the age of 23. Subsequently, she conceived following IVF treatment with an oocyte derived from the transplanted tissue and delivered a healthy baby.[25]

One significant potential disadvantage of OTC is that ovarian tissue stored prior to cancer treatment may harbour malignant cells.[17] Numerous methods can be used to determine the extent of malignant cell contamination, including immunohistochemistry and molecular analysis. However, these tests are all destructive to tissue, so cannot be applied to the ovarian tissue intended for transplantation. It is generally accepted that, where there is no evidence of metastatic disease of solid cancers, there is low risk of ovarian malignant contamination.[22] However, in the case of leukaemia, several studies have indicated that the risk that of malignant cells in the ovary is high.[20,21] The use of OTC in cases of leukaemia is therefore controversial.

OTC is now considered non-experimental in some countries (for example, Denmark and Israel). A 2018 FP guideline published by the American Society of Clinical Oncology (ASCO) indicated that the experimental status of OTC is under evaluation in the USA, based on accumulating evidence of successful pregnancy outcomes.[18]

In vitro maturation

In vitro maturation (IVM) of oocytes is another technique that avoids ovarian stimulation, but is classified as experimental. This concept was first introduced to reduce risk by avoiding ovarian stimulation in patients who had severe OHSS in their previous IVF treatments.[26] IVM oocyte cryopreservation involves the retrieval of immature oocytes from ovaries after minimal or no gonadotrophin stimulation and their subsequent maturation in the laboratory. IVM may be done at the time of oocyte collection, or immature oocytes may be cryopreserved for use in IVM at a later stage.[27]

Very few live births have been reported after IVM oocyte cryopreservation. The first live birth was reported following cryopreservation using the slow-cooling method of oocytes retrieved at the immature germinal vesicle (GV) stage in conventional IVF cycles.[28] Subsequently, five live births were reported following vitrification at metaphase-II (MII) stage after human chorionic gonadotrophin (hCG)-primed IVM cycles.[29] Live births have also been achieved using the IVM of immature oocytes obtained from resected ovarian cortex.[30] A retrospective study of 267 patients, which compared fresh and vitrified IVM-oocytes, showed that vitrification resulted in lower clinical pregnancy (36.1% versus 10.7%) and live birth rates (25.9% versus 8.9%).[26]

IVM is particularly advantageous for oncology patients. As IVM does not require ovarian stimulation, it does not delay cancer treatment, and it does not carry the risk of malignant contamination, as is the case in OTC (described above). The first live birth following IVM for a cancer patient was recently reported by Professor Grynberg's group in France. In brief, seven immature follicles were retrieved from a 29-year-old patient and matured in vitro for 48 hours, resulting in six MII oocytes suitable for vitrification. After 5 years, all six oocytes were thawed and fertilised (by intracytoplasmic sperm injection, ICSI), and a single cleavage stage embryo transfer resulted in pregnancy and healthy live birth.[31]

Testicular tissue cryopreservation

For prepubertal boys, the only potential option for fertility preservation is testicular tissue cryopreservation (TTC), which is still considered to be an experimental technique. In males, puberty heralds the maturation of germinal epithelium towards spermatids and mature sperm. Therefore, retrieval options before puberty are unlikely to produce cells that can currently be used for assisted reproductive techniques. Thus, the efficacy of TTC has yet to be proven; no live births from frozen tissue have been reported in humans to date. In a recent milestone study, Fayomi et al.[32] reported a successful pregnancy in rhesus macaques using transplanted prepubertal cryopreserved testicular tissue in conjunction with IVF. Importantly, complete spermatogenesis was confirmed in all transplanted testicular tissue grafts.[32]

Despite the experimental nature of the technique, research indicates that parents and survivors are undeterred and remain interested in pursuing this option.[9] The procedure of testicular tissue retrieval is straightforward and can be coordinated with other procedures requiring general anaesthetic. For most cases, a 3–5-mm incision of the tunica albuginea permits collection of three to four small (1–2 mm^3) biopsies, which are placed in media and immediately transferred to the tissue bank for processing and storage.[9] To generate sperm cells, suggested strategies are IVM or tissue transplantation, by either grafting onto an existing testicle or injecting germ cell preparations into the rete testes.[33]

Ovarian transposition and the use of gonadotrophin-releasing hormone agonists

Efforts to reduce the risk of gonadotoxicity include ovarian transposition or oophoropexy, which is an effective method of FP for both prepubertal and postpubertal girls requiring pelvic radiation for non-ovarian tumours. In this technique, one or both ovaries and fallopian tubes are separated from the uterus and attached to the wall of the abdomen, away from the radiation target area.[34] Barriers to success with this method include scattered radiation and alterations in ovarian blood supply. Overall efficacy is thought to be around 50%.[35] In terms of preserving ovarian function in paediatric patients, success rates are difficult to establish given the limited study number and follow-up but are estimated to be between 60% and 83%.[36] Large-scale follow-up studies of clinical pregnancy and livebirth outcomes following ovarian transposition in child and young adult cancer have yet to be completed.

Administration of gonadotrophin-releasing hormone (GnRH) agonists during chemotherapy to suppress ovarian activity is another example of a strategy to reduce the negative impact of chemotherapy on ovarian reserve. Meta-analyses have demonstrated that GnRH agonist use during chemotherapy in an adult population with breast cancer improves return of ovarian function and pregnancy rates.[37] However, in malignancies other than breast cancer, there is limited evidence to suggest their role in the prevention of gonadotoxicity.[38,39] GnRH agonists are not useful in a prepubertal cohort owing to inherent hypogonadotropic function.[7] They could be considered for those who are postpubertal, although the efficacy of this option remains

uncertain. In fact, the recently published draft of ESHRE guidance on female FP[40] advises that, in malignancies other than breast cancer, GnRH agonists should not be offered as an option for ovarian function protection and fertility preservation.

Patient selection for fertility preservation

Appropriate patient selection for FP is essential. It is not always appropriate for patients to take steps to preserve their fertility. Many patients will be too unwell to consider FP methods, or will decide that retaining fertility is not a priority for them.

For all FP methods, the patient must have a realistic chance of survival from their disease. If a patient's condition is considered palliative, there is potential harm and no benefit to preserving their fertility. Clinicians must consider if the patient is medically fit enough to undergo the FP procedure involved. Consent must be obtained – and this can be a challenging ethical consideration in the case of minors.

Another key factor to consider before invasive procedures such as OTC, oocyte retrieval or testicular tissue freezing, is the nature of the required oncology treatment; that is, whether or not the proposed treatment is of sufficiently high risk to a patient's fertility to justify the FP procedure. Whole body, pelvic (or abdominopelvic in children) radiotherapy and high-dose alkylating agents carry the greatest risk of gondadotoxicity.

Wallace et al.[41] developed the Edinburgh selection criteria for suitability for OTC as outlined in Box 1. These criteria have been shown to accurately predict which girls and young women will or will not develop premature ovarian insufficiency. They therefore aid appropriate patient selection for OTC before the start of cancer treatment.[41]

Reproductive outcomes for survivors

Research has found that most adult survivors of childhood cancer express the wish to have children.[42,43] Many cancer survivors will go on to conceive spontaneously. However, if they have trouble conceiving and wish to proceed with use of their stored sperm, oocytes, embryos or cryopreserved ovarian tissue, further treatment will be necessary to achieve a pregnancy.

Anderson and colleagues[44] used linkable databases of cancer registrations and pregnancy-related outcome records in Scotland (1981–2014) to investigate whether women who have had a cancer diagnosis are as likely to achieve pregnancy as their age-comparable counterparts who have not had cancer.[44] Over 23 000 women aged 39 or younger at diagnosis (including those treated as children) were included in their assessment. The authors found that overall, cancer survivors had a lower than expected number of pregnancies compared with the general population: 6627 observed compared to 10 736 expected pregnancies. Thus, survivors of cancer were approximately 38% less likely to become pregnant, highlighting that at-risk patients should ideally be provided timely access to fertility counselling and fertility preservation treatments.[44]

Box 1. The Edinburgh selection criteria[41]

- Age younger than 35 years
- No previous chemotherapy or radiotherapy if aged 15 years or older at diagnosis, but mild, nongonadotoxic chemotherapy acceptable if younger than 15 years
- A realistic chance of surviving for 5 years
- A high risk of premature ovarian insufficiency (>50%)
- Informed consent (from parents and, where possible, the patient)
- Negative serology results for HIV, syphilis and hepatitis B
- Not pregnant and no existing children

Fertility preservation counselling and discussions

It is essential to provide patients with information about FP in a timely fashion, as soon as possible after a cancer diagnosis. A systematic review by Taylor et al.[45] summarised that children and young adults with a new cancer diagnosis, and their parents, value the opportunity to discuss fertility concerns and preservation options.[45] Unfortunately, research indicates that discussions about fertility and fertility preservation are not taking place.[46–48] children and young adults with cancer and their parents often report that they have no recollection of conversations about fertility options with their clinician.[49]

A mixed methods systematic review by Vindrola-Padros and colleagues[50] studied healthcare professionals' (HCPs) views on discussing FP with children, adolescents and young cancer patients (aged 0–24). Sixteen papers reporting 14 studies were reviewed, most of which took place in North America and Western Europe. These authors found that HCPs had a general awareness of the risks to fertility associated with oncology treatments, but that they lacked knowledge of the various FP options. This, naturally, affected the discussion about FP with children and young adults. Further reported barriers to adequate discussions about FP included sense of comfort, patient factors (for example, those patients who had a poor prognosis or were considered too young), parent factors, and the lack of availability of written information.

In Europe, efforts to improve knowledge of oncofertility preservation options have included the establishment of a pan-European Consortium (PanCareLIFE) that aims to develop FP guidelines for children and adolescents diagnosed with cancer.[51] Funding of FP represents an

additional barrier in many healthcare systems. A recent study of 27 European countries revealed that just over 50% (14/27) cover the cost of oocyte cryopreservation for medical reasons, either through funding by the state or a compulsory insurance system.[52]

Conclusion

The impact of loss of fertility and unintended childlessness on young men and women who have been previously treated for cancer cannot be underestimated. Recent technological advances, such as oocyte vitrification and OTC offer increasing hope to this group.

In this era of improving survival rates of childhood cancer, as well as major scientific developments to circumvent reproductive aging, medical disciplines must carefully ensure that those who would benefit most have the necessary information and access to fertility preservation methods.

Disclosure of interests

There are no conflicts of interest.

Contribution to authorship

LH researched and wrote the article. LEG and MW reviewed and edited the article. All authors approved the final version.

References

1 Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. *CA Cancer J Clin* 2016;**66**:271–89.
2 Mancini J, Rey D, Preau M, Malavolti L, Moatti JP. Infertility induced by cancer treatment: inappropriate or no information provided to majority of French survivors of cancer. *Fertil Steril* 2008;**90**:1616–25.
3 Donnez J, Dolmans MM. Fertility preservation in women. *N Engl J Med* 2017;**377**:1657–65.
4 Gelson E, Prakash A, Macdougall J, Williams D. Reproductive health in female survivors of childhood cancer. *Obstet Gynaecol* 2016;**18**: 315–22.
5 Brougham MF, Crofton PM, Johnson EJ, Evans N, Anderson RA, Wallace WH. Anti-Mullerian hormone is a marker of gonadotoxicity in pre- and postpubertal girls treated for cancer: a prospective study. *J Clin Endocrinol Metab* 2012;**97**:2059–67.
6 Anderson RA, Mitchell RT, Kelsey TW, Spears N, Telfer EE, Wallace WH. Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults. *Lancet Diabetes Endocrinol* 2015;**3**:556–67.
7 Roeca C, Dovey S, Polotsky AJ. Recommendations for assessing ovarian health and fertility potential in survivors of childhood cancer. *Maturitas* 2019;**122**:57–9.
8 Thomson AB, Campbell AJ, Irvine DC, Anderson RA, Kelnar CJ, Wallace WH. Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case-control study. *Lancet* 2002;**360**:361–7.
9 Romao RL, Lorenzo AJ. Fertility preservation options for children and adolescents with cancer. *Can Urol Assoc J* 2017;**11** 1-2 Suppl 1: S97–102.
10 Halpern JA, Thirumavalavan N, Kohn TP, Patel AS, Leong JY, Cervellione RM, et al. Distribution of semen parameters among adolescent males undergoing fertility preservation in a multicenter international cohort. *Urology* 2019;**127**:119–23.
11 Bahadur G, Ling KL, Hart R, Ralph D, Wafa R, Ashraf A, et al. Semen quality and cryopreservation in adolescent cancer patients. *Hum Reprod* 2002;**17**:3157–61.
12 Leader A, Lishner M, Michaeli J, Revel A. Fertility considerations and preservation in haemato-oncology patients undergoing treatment. *Br J Haematol* 2011;**153**:291–308.
13 Iussig B, Maggiulli R, Fabozzi G, Bertelle S, Vaiarelli A, Cimadomo D, et al. A brief history of oocyte cryopreservation: arguments and facts. *Acta Obstet Gynecol Scand* 2019;**98**:550–8.
14 Bromfield JJ, Coticchio G, Hutt K, Sciajno R, Borini A, Albertini DF. Meiotic spindle dynamics in human oocytes following slow-cooling cryopreservation. *Hum Reprod* 2009;**24**:2114–23.
15 Chen C. Pregnancy after human oocyte cryopreservation. *Lancet* 1986;**1**:884–6.
16 Kuang Y, Chen Q, Hong Q, Lyu Q, Ai A, Fu Y, et al. Double stimulations during the follicular and luteal phases of poor responders in IVF/ICSI programmes (Shanghai protocol). *Reprod Biomed Online* 2014;**29**: 684–91.
17 ESHRE Task Force on Ethics and Law, Dondorp W, de Wert G, Pennings G, Shenfield F, Devroey P, et al. Oocyte cryopreservation for age-related fertility loss. *Hum Reprod* 2012;**27**:1231–7.
18 Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, et al. Fertility preservation in patients with cancer: ASCO clinical practice guideline update. *J Clin Oncol* 2018;**36**:1994–2001.
19 Cobo A, Garcia-Velasco J, Domingo J, Pellicer A, Remohi J. Elective and onco-fertility preservation: factors related to IVF outcomes. *Hum Reprod* 2018;**33**:2222–31.
20 Dolmans MM, Manavella DD. Recent advances in fertility preservation. *J Obstet Gynaecol Res* 2019;**45**:266–79.
21 Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 2004;**364**:1405–10.
22 Anderson RA, Wallace WHB, Telfer EE. Ovarian tissue cryopreservation for fertility preservation: clinical and research perspectives. *Hum Reprod Open* 2017;**2017**:hox001.
23 van der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krussel J, et al. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. *Hum Reprod* 2016;**31**:2031–41.
24 Demeestere I, Simon P, Dedeken L, Moffa F, Tsepelidis S, Brachet C, et al. Live birth after autograft of ovarian tissue cryopreserved during childhood. *Hum Reprod* 2015;**30**:2107–9.
25 Matthews SJ, Picton H, Ernst E, Andersen CY. Successful pregnancy in a woman previously suffering from beta-thalassemia following transplantation of ovarian tissue cryopreserved before puberty. *Minerva Ginecol* 2018;**70**:432–5.
26 Hatirnaz S, Ata B, Hatirnaz ES, Dahan MH, Tannus S, Tan J, et al. Oocyte in vitro maturation: a sytematic review. *Turk J Obstet Gynecol* 2018;**15**:112–25.
27 Son WY, Henderson S, Cohen Y, Dahan M, Buckett W. Immature oocyte for fertility preservation. *Front Endocrinol (Lausanne)* 2019;**10**:464.
28 Tucker MJ, Wright G, Morton PC, Massey JB. Birth after cryopreservation of immature oocytes with subsequent in vitro maturation. *Fertil Steril* 1998;**70**:578–9.
29 Cohen Y, St-Onge-St-Hilaire A, Tannus S, Younes G, Dahan MH, Buckett W, et al. Decreased pregnancy and live birth rates after vitrification of in vitro matured oocytes. *J Assist Reprod Genet* 2018;**35**:1683–9.
30 Shirasawa H, Terada Y. In vitro maturation of human immature oocytes for fertility preservation and research material. *Reprod Med Biol* 2017;**16**:258–67.
31 Grynberg M, Mayeur Le Bras A, Hesters L, Gallot V, Frydman N. First birth achieved after fertility preservation using vitrification of in vitro matured oocytes in a woman with breast cancer. *Ann Oncol* 2020;**31**:541–2.
32 Fayomi AP, Peters K, Sukhwani M, Valli-Pulaski H, Shetty G, Meistrich ML, et al. Autologous grafting of cryopreserved prepubertal rhesus testis produces sperm and offspring. *Science* 2019;**363**:1314–9.
33 Bahadur G, Chatterjee R, Ralph D. Testicular tissue cryopreservation in boys. Ethical and legal issues: case report. *Hum Reprod* 2000;**15**: 1416–20.

34 Noyes N, Knopman JM, Long K, Coletta JM, Abu-Rustum NR. Fertility considerations in the management of gynecologic malignancies. *Gynecol Oncol* 2011;**120**:326–33.
35 Fisch B, Abir R. Female fertility preservation: past, present and future. *Reproduction* 2018;**156**:F11–27.
36 Sauvat F, Binart N, Poirot C, Sarnacki S. Preserving fertility in prepubertal children. *Hormone Res* 2009;**71** Suppl 1:82–6.
37 Munhoz RR, Pereira AA, Sasse AD, Hoff PM, Traina TA, Hudis CA, et al. 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.
38 Elgindy E, Sibai H, Abdelghani A, Mostafa M. Protecting ovaries during chemotherapy through gonad suppression: a systematic review and meta-analysis. *Obstet Gynecol* 2015;**126**:187–95.
39 Lambertini M, Horicks F, Del Mastro L, Partridge AH, Demeestere I. Ovarian protection with gonadotropin-releasing hormone agonists during chemotherapy in cancer patients: from biological evidence to clinical application. *Cancer Treat Rev* 2019;**72**:65–77.
40 ESHRE Female Fertility Preservation Guideline Development Group. *Female fertility preservation*. Guideline of the European Society of Human Reproduction and Embryology (ESHRE). Review Report. Grimberg: ESHRE; 2020.
41 Wallace WH, Smith AG, Kelsey TW, Edgar AE, Anderson RA. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. *Lancet Oncol* 2014;**15**:1129–36.
42 Nilsson J, Jervaeus A, Lampic C, Eriksson LE, Widmark C, Armuand GM, et al. 'Will I be able to have a baby?' Results from online focus group discussions with childhood cancer survivors in Sweden. *Hum Reprod* 2014;**29**:2704–11.
43 Reinmuth S, Liebeskind AK, Wickmann L, Bockelbrink A, Keil T, Henze G, et al. Having children after surviving cancer in childhood or adolescence - results of a Berlin survey. *Klin Padiatr* 2008;**220**:159–65.
44 Anderson RA, Brewster DH, Wood R, Nowell S, Fischbacher C, Kelsey TW, et al. The impact of cancer on subsequent chance of pregnancy: a population-based analysis. *Hum Reprod* 2018;**33**:1281–90.
45 Taylor JF, Ott MA. Fertility preservation after a cancer diagnosis: a systematic review of adolescents', parents', and providers' perspectives, experiences, and preferences. *J Pediatr Adolesc Gynecol* 2016;**29**:585–98.
46 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;**108**:1602–15.
47 Goldfarb SB, Kamer SA, Oppong BA, Eaton A, Patil S, Junqueira MJ, et al. Fertility preservation for the young breast cancer patient. *Ann Surg Oncol* 2016;**23**:1530–6.
48 Hohmann C, Borgmann-Staudt A, Rendtorff R, Reinmuth S, Holzhausen S, Willich SN, et al. Patient counselling on the risk of infertility and its impact on childhood cancer survivors: results from a national survey. *J Psychosoc Oncol* 2011;**29**:274–85.
49 Anazodo A, Laws P, Logan S, Saunders C, Travaglia J, Gerstl B, et al. How can we improve oncofertility care for patients? A systematic scoping review of current international practice and models of care. *Hum Reprod Update* 2019;**25**:159–79.
50 Vindrola-Padros C, Dyer KE, Cyrus J, Lubker IM. Healthcare professionals' views on discussing fertility preservation with young cancer patients: a mixed method systematic review of the literature. *Psychooncology* 2017;**26**:4–14.
51 Byrne J, Grabow D, Campbell H, O'Brien K, Bielack S, Am Zehnhoff-Dinnesen A, et al. PanCareLIFE: The scientific basis for a European project to improve long-term care regarding fertility, ototoxicity and health-related quality of life after cancer occurring among children and adolescents. *Eur J Cancer* 2018;**103**:227–37.
52 ESHRE Working Group on Oocyte Cryopreservation in Europe, Shenfield F, de Mouzon J, Scaravelli G, Kupka M, Ferraretti AP, et al. Oocyte and ovarian tissue cryopreservation in European countries: statutory background, practice, storage and use. *Hum Reprod Open* 2017;**2017**:hox003.

