Update on fertility preservation from the Barcelona International Society for Fertility Preservation–ESHRE–ASRM 2015 expert meeting: indications, results and future perspectives

Francisca Martinez, on behalf of the International Society for Fertility Preservation–ESHRE–ASRM Expert Working Group

Hospital Universitario Dexeus, Gran Via Carlos III, 71-75, 08208, Barcelona, Spain

Study Question: What progress has been made in fertility preservation (FP) over the last decade?
Summary Answer: FP techniques have been widely adopted over the last decade and therefore the establishment of international registries on their short- and long-term outcomes is strongly recommended.
What Is Known Already: FP is a fundamental issue for both males and females whose future fertility may be compromised. Reproductive capacity may be seriously affected by age, different medical conditions and also by treatments, especially those with gonadal toxicity. There is general consensus on the need to provide counselling about currently available FP options to all individuals wishing to preserve their fertility.
Study Design, Size, Duration: An international meeting with representatives from expert scientific societies involved in FP was held in Barcelona, Spain, in June 2015.
Participants/Materials, Setting, Methods: Twenty international FP experts belonging to the American Society of Reproductive Medicine, ESHRE and the International Society of Fertility Preservation reviewed the literature up to June 2015 to be discussed at the meeting, and approved the final manuscript. At the time this manuscript was being written, new evidence considered relevant for the debated topics was published, and was consequently included.
Main Results and the Role of Chance: Several oncological and non-oncological diseases may affect current or future fertility, either caused by the disease itself or the gonadotoxic treatment, and need an adequate FP approach. Women wishing to postpone maternity and transgender individuals before starting hormone therapy or undergoing surgery to remove/alter their reproductive organs should also be counselled accordingly. Embryo and oocyte cryopreservation are first-line FP methods in postpubertal women. Metaphase II oocyte cryopreservation (vitrification) is the preferred option. Cumulative evidence of restoration of ovarian function and spontaneous pregnancies after ART following orthotopic transplantation of cryopreserved ovarian tissue supports its future consideration as an open clinical application. Semen cryopreservation is the only established method for FP in men. Testicular tissue cryopreservation should be recommended in prepubertal boys even though fertility restoration strategies by autotransplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans. The establishment of international registries on the short- and long-term outcomes of FP techniques is strongly recommended.
Limitations, Reasons for Caution: Given the lack of studies in large cohorts or with a randomized design, the level of evidence for most of the evidence reviewed was 3 or below.
Wider Implications of the Findings: Further high quality studies are needed to study the long-term outcomes of FP techniques.
Study Funding/Competing Interest(s): None.
Trial Registration Number: N/A.
Key Words: Fertility preservation, semen cryopreservation, testicular tissue cryopreservation, embryo cryopreservation, oocyte cryopreservation, ovarian tissue cryopreservation, oncological fertility preservation, non-oncological fertility preservation, fertoprotection

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/16938-24298

Received March 27, 2017; accepted May 19, 2017; published online July 21, 2017.
This article has not been externally peer reviewed.
This article is simultaneously published in *Human Reproduction*.
Participants of the Expert Working Group are listed in the Appendix.
Correspondence: Francisca Martinez, Hospital Universitario Dexeus, Barcelona, Spain (E-mail: pacmar@dexeus.com).

Fertility and Sterility® Vol. 108, No. 3, September 2017 0015-0282

http://dx.doi.org/10.1016/j.fertnstert.2017.05.024

INTRODUCTION

Reproductive capacity may be seriously affected by age, different conditions, including genetic syndromes, and also by treatments, especially those with gonadal toxicity. Fertility preservation (FP) is a fundamental issue for individuals of reproductive age (both male and female) or prepubescent boys and girls whose future fertility may be compromised. There is general consensus on the need to provide counselling about currently available FP options to all individuals wishing to preserve their fertility. Timely referral to a fertility specialist for informed FP decisions becomes essential.

Several techniques for FP are nowadays well established while others are still considered experimental. These techniques have been subject of continuous review by experts.

Reviews have been mostly focused on FP and cancer. However, the need for FP in other pathologic situations, either due to the disease itself or to gonadotoxic treatment, and even in non-medical indications, is on the rise. Moreover, new perspectives to tackle FP are being developed, and evidence about the results of spontaneous pregnancy and ART after current FP procedures is growing. With the aim of reviewing all these aspects and drawing recommendations, an international meeting with representatives from expert scientific societies involved in FP was held in Barcelona (Spain) in June 2015. This paper summarizes the topics debated, with a special focus on indications for FP, current outcomes and future perspectives. A more detailed summary can be found at *Fertility and Sterility* online.

MATERIALS AND METHODS

Twenty international FP experts belonging to the American Society of Reproductive Medicine (ASRM), European Society of Human Reproduction and Embryology (ESHRE) and the International Society of Fertility Preservation (ISFP) attended the meeting. Experts conducted a review of the literature up to June 2015 to be discussed at the meeting. At the time this manuscript was being written, new evidence considered relevant for the debated topics was published, and has consequently been included. Given the lack of studies in large cohorts or with a randomized design, the quality of evidence, according to the European Society for Medical Oncology Clinical Practice Guidelines for fertility preservation in cancer patients (1) for most of the evidence reviewed was 3 or below.

INDICATIONS FOR FP

Cancer

Many forms of cancer are associated with impaired semen quality or ovarian function at the time of cancer diagnosis. However, the main effect on fertility arises from commonly used treatments such as chemotherapy with alkylating agents and pelvic radiation. Gonadal failure resulting from these treatments may affect different aspects of reproductive health, including pubertal development, hormone production, and sexual function in adults (2). More than 80% of children and adolescents with cancer become long-term survivors (3), raising the interest in the long-term effects of cancer treatment on fertility.

Male. Spermatogonia are especially sensitive to chemotherapy and radiotherapy. The effect, which is dose-dependent, may not be permanent if the spermatogonial stem cell (SSC) population is not fully depleted. Data on the impact of recent biological or targeted cancer therapies on male fertility is limited (4). For most of these therapies, the effects seem to be mild, mostly involving reproductive endocrinology. Finally, surgical pelvic interventions for malignant or benign disease may affect the anatomy or normal functioning of reproductive organs.

Female. Chemotherapy and radiotherapy may induce premature ovarian insufficiency (POI) in women. Ovarian damage is drug- and dose- dependent and increases as the patient ages (5). Radiotherapy may also affect the uterus, leading to reduced vascularity, myometrium damage (fibrosis) and hormone- dependent insufficiency. Data on the impact of recent biological or targeted cancer female fertility therapies is also limited except for bevacizumab, with a rate of 34% POI reported (4). Fertility may also be impaired by surgical removal or damage to reproductive organs.

Non-oncological Medical Indications

FP options should also be discussed with adult and younger women and men affected by several non-oncological medical conditions. Table 1 summarizes the most common non-oncological conditions requiring FP.

Autoimmune diseases. Table 2 summarizes autoimmune diseases reported to benefit from immunosuppressive therapy with alkylating agents (cyclophosphamide). Continuous POI in women with chronic autoimmune diseases also increases the risk of hypoestrogenism-related comorbidities (23). Males with systemic lupus erythematosus show a high frequency of testicular Sertoli cell dysfunction (24). New treatment approaches are changing the prognosis of patients with autoimmune diseases, although information about toxicity for reproduction is still limited.

Hematopoietic stem cell transplantation. Hematopoietic stem cell transplantation (HSCT, autologous or allogeneic) has been an important therapeutic tool for some oncological and non- oncological systemic diseases. Patients undergoing HSCT are at particularly high risk of developing ovarian (64–85%) or testicular (50–90%) failure since aggressive chemotherapy and radiotherapy is needed to destroy pre- existing bone marrow (8) (Table 1).

Medical conditions causing POI. POI may result from several causes, either due to their extensive or progressive nature, or bilateral adnexectomy (10, 11). Patients at risk of POI by a known genetic cause or family history (Turner's syndrome, fragile X mental retardation (FMR)1 pre-mutation, classic galactosaemia) may also benefit from FP (9). Women of reproductive age with endometriosis may benefit from FP before surgical treatment as they are at increased risk of POI and infertility (15).

Male genetic disorders and testicular tissue damage. Klinefelter's syndrome is the most common sex chromosomal disorder in humans, causing hypogonadism and azoospermia in >90% cases (6), and the benefit of FP is currently unclear.

TABLE 1

Non-oncological conditions requiring fertility preservation.

Indication	Disease
Autoimmune diseases (6, 7)	Systemic lupus erythematosus (SLE) Behcet's disease Churg-Strauss syndrome (eosinophilic granulomatosis) Steroid resistant glomerulonephritis Granulomatosis with polyangiitis (formerly Wegener's granulomatosis) Inflammatory bowel diseases Rheumatoid arthritis Pemphigus vulgaris
Hematopoietic stem cell transplantation (7, 8)	Autoimmune diseases unresponsive to immunosuppressive therapy Haematological diseases (sickle cell anaemia, thalassaemia major, plastic anaemia)
Medical conditions causing POI (9)	Altered hypothalamic–pituitary–gonadal axis (10, 11) Ovarian oophoritis Benign ovarian tumours Mosaic Turner's syndrome Fragile X Mental Retardation 1 (12) Galactosaemia (13) Beta-thalassaemia (14) Endometriosis (15)
Male genetic disorders	Klinefelter's syndrome (6)
Testicular damage (16)	
Gender reassignment procedures (17)	
Severe body trauma requiring surgical intervention	

Note: POI = premature ovarian insufficiency.

Martinez. Update on fertility preservation. Fertil Steril 2017.

Testicular injury may also result in irreparable damage to the testicular tissue leading to infertility (16). Salvage, even in cases of subjectively dead testicle, and FP options can be offered (25).

Gender reassignment procedures. Removal of testicles or ovaries destroy the ability to have genetically- related children, while feminizing/masculinizing medications used in gender reassignment procedures may lead to diminished fertility. It is necessary to discuss and provide counselling about FP and fertility treatment 'before starting hormone therapy or undergoing surgery to remove/alter their reproductive organs' even at a younger age (26), although there are ethical concerns (27). Evidence regarding reproductive health issues in individuals receiving treatments for gender dysphoria is scarce. Currently, there are no established techniques for preserving gonadal function in pre-pubertal or pubertal adolescents, who will never develop reproductive function in their natal sex owing to blockers or cross-gender hormones.

Delayed Childbearing

Female fertility decreases gradually but significantly after age 32 years, and faster after 37 years, which compromises fertility when delaying childbearing. The term 'AGE banking' (oocyte banking for anticipated gamete exhaustion) has been proposed for oocyte cryopreservation in these cases (28).

TABLE 2

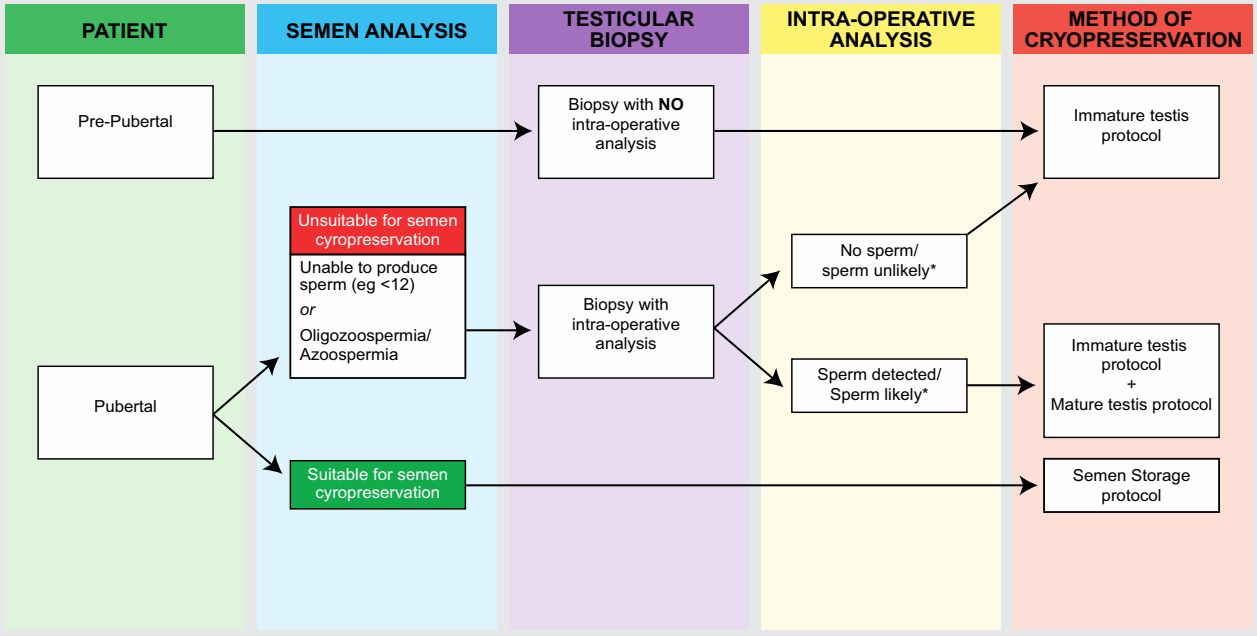
Clinical outcomes from fertility preservation techniques in women.

Author	FP technique	Women/Indication	Outcome
Dolmans et al., (18)	Embryo cryopreservation	54/Cancer 33 returned/20 ET	22% LBR per ET nine pregnancies Four deliveries
Oktay et al., (19)	Embryo cryopreservation	33/Breast cancer 18 returned/55 ET	45% LBR per ET 26 pregnancies 18 deliveries
Cobo et al., (20)	Oocyte vitrification	Ovum donation programme	6.5% oocyte-to-baby rate. CLBR increased with the number of oocytes used
Cobo et al., (21)	Oocyte vitrification	Delaying childbearing or non-oncological medical conditions	50% LBR per patient in women ≤35 years old 22.9% LBR per patient in women >36 years old
Donnez et al., (22)	Ovarian tissue cryopreservation		N = 111 cases, 32 conceived 29.0% LBR per patient

Note: FP = fertility preservation; ET = embryo transfer; LBR = live birth rate; CLBR = cumulative live birth rate.

Martinez. Update on fertility preservation. Fertil Steril 2017.

FIGURE 1



Algorithm for the cryopreservation of testicular tissue/sperm in pre-pubertal and adolescent patients at high risk of infertility. Clinical assessment for puberty should be carried out by a clinician with experience in pubertal assessment. It must be stressed that no clinical parameter can accurately predict the presence of sperm. The proven treatment option for pubertal and adolescent boys who are considered capable of producing a semen sample is semen collection and cryopreservation. If sufficient sperm are recovered, the gametes can be banked using commercial glycerol-based sperm cryomedia. For those young patients who are clinically pre-pubertal and for whom semen cryopreservation is not possible, the fertility preservation strategy should include collection of a testicular biopsy by an experienced surgeon. The tissue should be cryopreserved with a protocol optimised for preserving immature germ cells, (immature testis protocol). Patients who are pubertal but are unable to produce a suitable semen sample may proceed to testicular biopsy, with intra-operative analysis. Techniques for intra-operative analysis may vary between institutions but should be aimed at identifying tissue containing (or likely to contain) sperm. This should be carried out by an individual with experience in analysis of testicular tissue (e.g. surgeon, embryologist or andrologist). When sperm are not identified or deemed unlikely the tissue should be frozen with the immature testis protocol used for pre-pubertal patients. For patients in whom sperm are identified or considered likely to be present, tissue should be split into two portions for storage. One portion should be cryopreserved using the immature testis preservation protocol, while the second portion should be stored using a protocol aimed at preserving mature sperm cells with glycerol as the main cryoprotectant. As stated in the text, at present there are several protocols for cryopreservation of immature testicular tissue and there is no clear evidence at the time of writing to demonstrate which is optimal. Reprinted with permissions from (2).

Martinez. Update on fertility preservation. Fertil Steril 2017.

AVAILABLE PROCEDURES FOR FP

Women

Both embryo and oocyte cryopreservation (slow freezing or vitrification) are first-line FP methods (5) (Fig. 1). However, oocyte cryopreservation is increasingly preferred (4). Mature oocyte vitrification is preferred in post- pubertal women when gonadotoxic treatment can be delayed to allow time for controlled ovarian stimulation (COS) (29). Harvesting of immature oocytes would be an option for patients unable to undergo COS such as prepubertal girls or women with aggressive or hormone-sensitive cancers (30). IVM improves outcomes in breast cancer patients undergoing COS for FP (31).

Ovarian tissue cryopreservation (OTC) is a COS-independent experimental technique which also allows immediate cancer treatment, and is currently the only FP option in paediatric patients (7) and in hormone-dependent diseases (32). Reimplantation of this tissue either in the pelvic cavity (orthotopic) or elsewhere (heterotopic) has the potential of restoring fertility and ovarian hormone secretion. Reimplantation of frozen–thawed ovarian tissue in the pelvic cavity is usually carried out by laparoscopy. The surgical technique is contingent on the presence (or not) of at least one ovary (33).

Ovarian tissue could also be preserved as an entire ovary with its vascular pedicle, preventing ischaemic damage occurring between transplantation and revascularization (34). Fertility restoration after whole ovary preservation requires retransplantation of the whole organ accompanied by vascular anastomoses of the blood vessels. Natural fertility has been fully restored following autotransplantation of whole ovaries and their supporting vascular pedicle after slow freezing and thawing in sheep (35). Cryopreservation of the whole ovary is likely to be more problematic in adult women due to the increased size of their ovaries, the difficulty of achieving adequate perfusion and penetration of the cryoprotectants agents through the whole organ, and the inherently different freezing and thawing optima for the different cell types in both the ovary and blood vessels (36). More research is needed before this technique can be translated into clinical practice.

Fertoprotective agents. GnRH analogues/agonists (GnRHa) may protect follicles from destruction during chemotherapy, probably by suppression of gonadotrophin levels and reduction of utero-ovarian perfusion (37). These agents have long been used for the prevention of ovarian damage, despite their debatable efficacy. Two meta-analyses of randomised trials using GnRHa have found an overall significantly reduced risk of POI in young breast cancer patients (38, 39). This protective effect of GnRHa is less clear in other cancer patients (ovarian) or not present at all, as in young patients with lymphoma (40). Still, the quality of evidence is relatively low given the number of women included, relatively short term follow-up hitherto and significant heterogeneity. Further high quality studies are needed to study the long-term effects of GnRHa on the prevention of POI.

Men

Sperm cryopreservation is the only established FP method in adult and adolescent males. Alternatives to the procurement of semen samples by masturbation include assisted ejaculation methods such as penile vibratory stimulation or electroejaculation. An algorithm for sperm and testicular tissue cryopreservation in prepubertal boys and adolescent males at high risk of fertility loss has been recently recommended (2) (Fig. 1).

RESULTS OF ART AFTER FP

Women

As a well-established technology, embryo cryopreservation has high pregnancy success rates (41). However, outcomes in cancer patients are scarce. Table 2 summarizes recently reported clinical outcomes from FP techniques in women. A similar live birth rate (LBR) per patient among women with cancer undergoing IVF and embryo cryopreservation, and cumulative live birth rate (CLBR) to that achieved with fresh embryos in non-cancer patients has been reported (18) (Table 2). Success rates associated with oocyte vitrification are superior to slow freezing (42). Among women undergoing oocyte vitrification because of age or because of non-oncological medical conditions, a LBR per patient of 50% among women aged $\leq$35 years was found, and of 22.9% among those aged >36 years after the transfer of embryos obtained from vitrified oocytes. CLBR was higher and increased faster among younger women (21) (Table 2). These success rates are comparable to those achieved with fresh oocytes (43, 44). Outcomes after oocyte vitrification among female cancer patients are scarce. Martinez et al., (36) reported fertilization rates up to 76.6% and a mean ($\pm$SD) number of embryos transferred of 1.8–0.7 among 11 women with cancer, four of whom gave birth at term with no negative perinatal outcomes. Alvarez et al., (45) first reported a successful birth in a woman with invasive ovarian cancer.

Despite being considered an experimental technique, both restoration of ovarian function and spontaneous pregnancies after ART have been reported after orthotopic transplantation of cryopreserved ovarian tissue (46). Only one case of a live birth after heterotopic transplantation has been reported up to 2013 (47). Recently, Demestree et al. have reported the first live birth following re- grafting of ovarian tissue that had been cryopreserved during childhood in a 13-year old girl undergoing HSCT (48). A large series of 60 live births after transplantation of cryopreserved ovarian tissue (OTT) has been reported, showing that by repeating the procedure, ovarian activity can be restored for more than 11 years (49). In a series of 111 cases, the conception rate after OTT was 29%. Two women delivered three babies each, proving the efficacy of the technique, as well as the possibility of conceiving naturally several times after only one transplantation procedure (22, 50). Although it is impossible to provide a fixed success rate for OTT as long as it remains active (51), given these encouraging results, ovarian cortex transplantation is proposed as an open clinical application (22).

Men

Success rates of semen cryopreservation have greatly increased with advances in ICSI, with pregnancy rates of up to 57% (2). With ICSI (52), reported a LBR of 62.1% in a cohort of 272 men with cancer, which was significantly higher than that of the comparative normospermic non-cancer population. To date, no clinical outcomes have been reported with other FP techniques.

FUTURE PERSPECTIVES

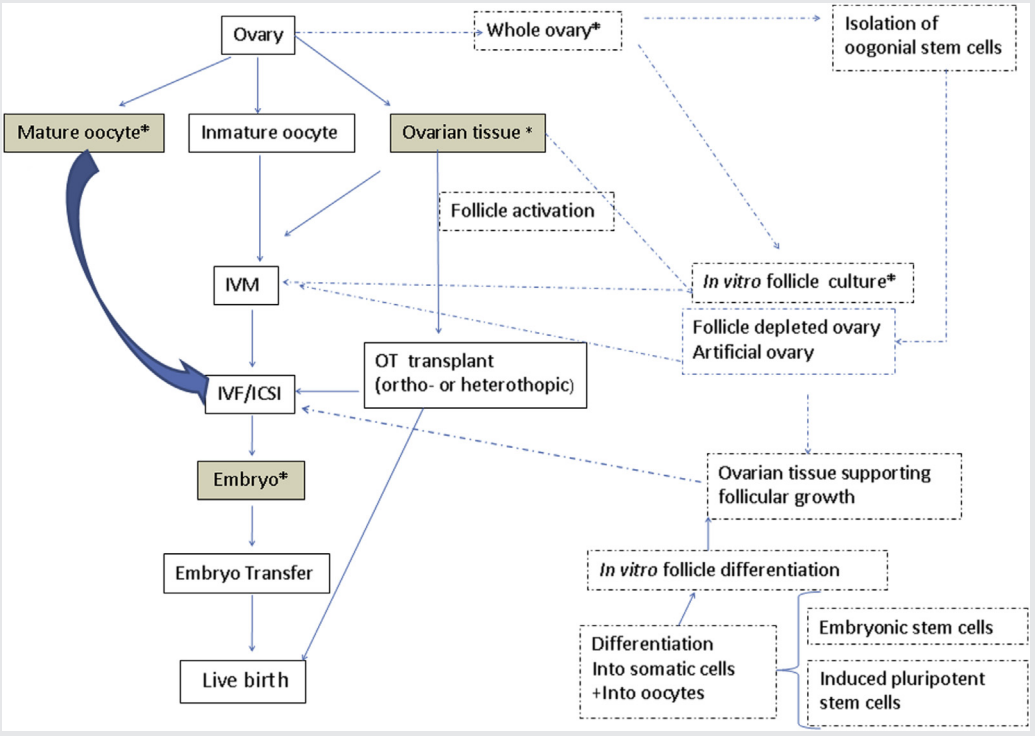
Figure 2 summarizes all FP techniques that are currently under study.

Women

Activation of ovarian follicles. Cryopreserved ovarian tissue from prepuberal patients and patients at risk of primary POI contains immature primordial follicles that must be activated in order to start developing. Activation of primordial follicles can be induced in vivo by mechanically interrupting the Hippo signalling pathway (by ovarian fragmentation, drilling, laser) (53). Follicle activation may also be achieved in vitro before autotransplantation by acting on the PI3K-PTEN-AKT-FOXO3 pathway [phosphatidylinositol 3-kinase (PI3K) activators and phosphatase and tensin homologue enzyme (PTEN) inhibitors, protein kinase B (AKT) stimulators, transcriptional factor forkhead box O3 (FOXO3)] which regulates primordial follicle dormancy at oocyte level (53). Using this double approach in women with POI (54), found rapid follicle growth after grafting ovarian tissue back to patients, obtaining mature eggs. A live birth was achieved after IVF and embryo transfer. In vitro activation protocols are under development with the aim of increasing the pool of viable activated follicles available for in vitro growth (IVG) procedures (55).

In vitro follicle culture. Transplantation of cryopreserved tissue carries the risk of re-seeding original cancer cells into the patient (56, 57). This risk can be minimised by using complete IVG and maturation of oocytes as the means of fertility restoration (58).

FIGURE 2



Fertility preservation techniques in women. Experimental procedures are indicated in discontinuous boxes, while established ones (i.e. those proven to restore fertility, with live births reported) are indicated in shaded boxes. Vitrified-thawed oocytes can be fertilized by IVF/ICSI for embryo transfer. Immature oocytes can be matured in vitro (IVM) for IVF/ICSI. Research is undergoing on the potential use of oogonial stem cells to repopulate follicle-depleted ovaries or differentiating follicle somatic cells and oocytes from embryonic stem cells or induced pluripotent stem cells to assemble de novo follicles for transplantation or IVM and IVF/ICSI. *Cryopreserved. OT = ovarian tissue.

Martinez. Update on fertility preservation. Fertil Steril 2017.

To date, three-dimensional (3D) culture methods have proven most successful in supporting the demands of human follicle activation and IVG as these approaches are best able to mimick the in vivo ovarian growth environment. Several multi-step culture systems have succeeded in culturing human follicles (59, 60, 61). Critically, all IVG systems used for fertility restoration for FP patients must start with the in situ culture of primordial follicles from cryopreserved tissue. The recent production of meiotically competent metaphase II non-human primate and human oocytes following IVG of freshly isolated secondary follicles (62, 63) is encouraging. However, considerable further research effort is needed to confirm the safety and efficacy of oocytes derived following extended IVG and maturation of human oocytes from cryopreserved tissue before this technology can used to restore fertility in FP patients (64).

Artificial ovaries. An alternative to the in vitro culture of primordial follicles is their development into an engineered 'artificial ovary', consisting of isolated preantral follicles along with other ovarian cells assembled in a structure- 3D matrix, or scaffold, which allows follicles to grow and develop in an ovarian-like environment (65, 66). Once transplanted to the patient, this 'artificial ovary' would potentially restore fertility and endocrine function (32).

New fertoprotective agents. The most recent theory of chemotherapy-induced follicle loss suggests that, simultaneously with large follicle apoptosis, chemotherapy also triggers activation of dormant follicle growth. Current research focuses thus on both agents with anti-apoptotic properties (imatinib, sphingosine-1-phosphate, thyroid hormone T3, granulocyte colony- stimulating factor and tamoxifen) that have been shown to reduce follicle loss in animal models and on agents that also prevent follicle activation such as AS101, an immune modulator that acts on the PI3K/PTEN/AKT follicle activation pathway (67), and the anti-Mullerian hormone (68). Clinical applicability of these agents depends not only on their fertoprotective capacity, but also on their potential interaction with cancer treatments.

Men

The risk of reintroducing malignant cells via the graft might be overcome by in vitro spermatogenesis. SSCs (whole testicular biopsy or isolated SSCs) are cultured in 3D systems that resemble the in vivo situation (2). The genetic stability of a long-term culture of human SSCs from two prostate cancer patients has been reported, although changes in the methylation status were observed (69). The consequences of these

epigenetic changes on the functionality of the sperm of the health of the offspring are unknown. The fertilizing ability of in vitro-cultured sperm is to be established before assessing the clinical value of this technique. Moreover, fertility restoration strategies by autotransplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans (2).

Both Genders

Artificial gametes. The use of primordial germ cells (PGC) present in the gonads, such as SSC, is a promising approach for treating infertility in patients lacking functional oocytes or sperm. However, the population of PGC is scarce and decreases with age. Pluripotent stem cells (PSC), such as embryonic stem cells or induced PSC, constitute other potential sources of gametes (70).

Hayashi and Saitou (71) derived functional PGCs from PSC potentially able to generate functional oocytes and sperm. Recently (72), have reported the generation of haploid mouse spermatid-like cells able to produce viable and fertile offspring. Notwithstanding these advances, 'to date there are no proven stem cell-based means to improve reproductive function, either by producing functional gametes in vitro, or stimulating the resident stem cell population in the ovary to elicit de novo oocyte production' (70).

SUMMARY

The Expert Working Group made the following recommendations:

- Several oncological and non-oncological diseases may affect current or future fertility, either due to the disease itself or to gonadotoxic treatment, and need an adequate FP approach. These patients should be counselled regarding potential fertility loss and should be referred to fertility specialists to discuss options for FP and current results.
- Women wishing to postpone maternity and transgender individuals before starting hormone therapy or undergoing surgery to remove/alter their reproductive organs should also be counselled accordingly.
- Embryo and oocyte cryopreservation are first-line FP methods in postpubertal women. Metaphase II oocyte cryopreservation (vitrification) is the preferred option.
- Cumulative evidence for restoration of ovarian function and spontaneous pregnancies after ART following orthotopic transplantation of cryopreserved ovarian tissue supports its future consideration as an open clinical application.
- Semen cryopreservation is the only established FP technique in men.
- Testicular tissue cryopreservation should be recommended in pre- pubertal boys even though fertility restoration strategies by autotransplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans.
- The establishment of international registries on the short- and long- term outcomes of FP techniques is strongly recommended.

ACKNOWLEDGMENTS

We would like to gratefully acknowledge all the participants in the meeting, and especially to the ASRM, ESHRE and the ISFP for supporting the project. We also thank Beatriz Viejo Ph.D. for editorial assistance in the preparation of this report.

AUTHORS' ROLES

F.M. drafted the article. C.Y.A., C.G., M.M.D., F.M., D.M., P.P., H.P., M.R., P.deS., A.V. and H.W. drafted and revised the article. C.Y.A., P.N.B.; R.B., A.C., J.D., M.M.D., H.E., A.F., C.G., M.G., S.K., F.M., D.M., P.P., A.P., H.P., M.R., P.deS., A.V. and H.W. reviewed and discussed the evidence. All authors approved the final version of the manuscript.

FUNDING

None.

CONFLICT OF INTEREST

None.

APPENDIX

ISFP–ESHRE–ASRM Expert Working Group

List of Participants

Claus Yding Andersen; Copenhagen University Hospital Rigshospitalet, Denmark. yding@rh.dk

PN Barri; Servicio de Medicina de la Reproducción, Hospital Universitario Dexeus, Barcelona, Spain. perbar@dexeus.com

Robert Brannigan; Feinberg School of Medicine, Northwester University, Chicago, USA. rebrannigan@gmail.com

A Cobo; Instituto Valenciano de Infertilidad, Valencia, Spain. ana.cobo@ivi.es

Jacques Donnez; Society for Research into Infertility (SRI) Brussels, Belgium. jacques.donnez@gmail.com

Marie Madeleine Dolmans; Gynaecology Department, Cliniques Universitaires Saint-Luc; Pôle de Gynécologie, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium. mmdolmans@gmail.com/Marie-madeleine.dolmans@uclouvain.be

J.L.H. (Hans) Evers; *Human Reproduction* Editor-in-Chief (ESHRE). Jh.evers@gmail.com

Anis Feki; Department of Obstetrics and Gynecology, HôpitalCantonal, Fribourg Switzerland. Anis.Feki@h-fr.ch

Mariëtte Goddijn; Center for Reproductive Medicine, Department of Obstetrics and Gynecology, University of Amsterdam, Amsterdam, Netherlands. m.goddijn@amc.uva.nl

Clarisa Gracia; School of Medicine, University of Pennsylvania, Pennsylvania, USA. Cgracia@uphs.upenn.edu

Sam Kim; Department of Obstetrics and Gynecology; University Kansas School of Medicine, Kansas, USA. skim2@kumc.edu

Francisca Martinez, Servicio de Medicina de la Reproducción, Hospital Universitario Dexeus, Barcelona, Spain. pacmar@dexeus.com

Dror Meirow; Sheba medical Center, Tel-Hashomer, Israel. Meirow@post.tau.ac.il

Pasquale Patrizio; Yale University Fertility Center, New Haven, CT 06511, USA. pasquale.patrizio@yale.edu

Antonio Pellicer; *Fertility and Sterility* Editor-In-Chief. American Society for Reproductive Medicine (ASRM). apellicer@ivi.es

Helen Picton; Division of Reproduction and Early Development, Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, UK LS2 9JT. H.M.Picton@leeds.ac.uk

Mitchel Rosen; University of California, San Francisco, USA. Mitchell.Rosen@ucsf.edu

Petra de Sutter; Department of Reproductive Medicine, University of Gent, Gent, Belgium. Petra.DeSutter@uzgent.be

Anna Veiga; Servicio de Medicina de la Reproducción, Hospital Universitario Dexeus, Barcelona, Spain. anavei@dexeus.com

HamishWallace; Royal Hospital for Sick Children, Edinburgh, Scotland. Hamish.wallace@nhs.net

REFERENCES

1. Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim HA Jr, Peccatori FA, et al. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med 2016;14:1.
2. Picton HM, Wyns C, Anderson RA, Goossens E, Jahnukainen K, Kliesch S, et al. A European perspective on testicular tissue cryopreservation for fertility preservation in prepubertal and adolescent boys†. Hum Reprod 2015;30:2463–75.
3. Phillips SM, Padgett LS, Leisenring WM, Stratton KK, Bishop K, Krull KR, et al. Survivors of childhood cancer in the United States: prevalence and burden of morbidity. Cancer Epidemiol Biomarkers Prev 2015;24:653–63.
4. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, et al, Oncology AsoC. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500–10.
5. Ethics Committee of ASRM. Fertility preservation and reproduction in patients facing gonadotoxic therapies: a committee opinion. Fertil Steril 2013;100:1224–31.
6. Bedaiwy MA, Botros R. Fertility Preservation. Advances and Controversies. Daryaganj, New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd; 2014.
7. Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol 2013;9:735–49.
8. Joshi S, Savani BN, Chow EJ, Gilleece MH, Halter J, Jacobsohn DA, et al. Clinical guide to fertility preservation in hematopoietic cell transplant recipients. Bone Marrow Transplant 2014;49:477–84.
9. ESHRE POI Guideline Development Group. Guideline on the management of premature ovarian insufficiency [Online]. European Society of Human Reproduction and Embryology. Available at: https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Management-of-premature-ovarian-insufficiency.aspx; 2015. Accessed July 2, 2016.
10. Donnez J, Kim SS. Principles and Practice of Fertility Preservation. New York, US: Cambridge University Press; 2011.
11. Harward LE, Mitchell K, Pieper C, Copland S, Criscione-Schreiber LG, Clowse ME. The impact of cyclophosphamide on menstruation and pregnancy in women with rheumatologic disease. Lupus 2013;22:81–6.
12. Gleicher N, Yu Y, Himaya E, Barad DH, Weghofer A, Wu Y-G, et al. Early decline in functional ovarian reserve in young women with low (CGGn < 26) FMR1 gene alleles. Transl Res 2015;166, 502–507.e1–2.
13. Fridovich-Keil JL, Gubbels CS, Spencer JB, Sanders RD, Land JA, Rubio-Gozalbo E. Ovarian function in girls and women with GALT-deficiency galactosemia. J Inherit Metab Dis 2011;34:357–66.
14. Roussou P, Tsagarakis NJ, Kountouras D, Livadas S, Diamanti-Kandarakis E. Beta-thalassemia major and female fertility: the role of iron and iron-induced oxidative stress. Anemia 2013, Article ID 617204.
15. Somigliana E, Viganò P, Filippi F, Papaleo E, Benaglia L, Candiani M, et al. Fertility preservation in women with endometriosis: for all, for some, for none? Hum Reprod 2015;30:1280–6.
16. Stahl PJ, Stember DS, Hsiao W, Schlegel PN. Indications and strategies for fertility preservation in men. Clin Obstet Gynecol 2010;53:815–27.
17. Darney PD. Hormonal contraception. In: Kronenberg HM, Melmer S, Polonsky KS, Larsen PR, editors. Williams Textbook of Endocrinology. Philadelphia, PA: Saunders/Elsevier; 2008:615–44.
18. Dolmans MM, Hollanders de Ouderaen S, Demylle D, Pirard C. Utilization rates and results of long-term embryo cryopreservation before gonadotoxic treatment. J Assist Reprod Genet 2015;32:1233–7.
19. Oktay K, Turan V, Bedoschi G, Pacheco FS, Moy F. Fertility preservation success subsequent to concurrent aromatase inhibitor treatment and ovarian stimulation in women with breast cancer. J Clin Oncol 2015;33:2424–9.
20. Cobo A, Garrido N, Pellicer A, Remohí J. Six years' experience in ovum donation using vitrified oocytes: report of cumulative outcomes, impact of storage time, and development of a predictive model for oocyte survival rate. Fertil Steril 2015;104:1426–34.e8.
21. Cobo A, García-Velasco JA, Coello A, Domingo J, Pellicer A, Remohí J. Oocytes vitrification as an efficient option for elective fertility preservation. Fertil Steril 2016;105:755–64.
22. Donnez J, Dolmans M-M, Diaz C, Pellicer A. Ovarian cortex transplantation: time to move on from experimental studies to open clinical application. Fertil Steril 2015;104:1097–8.
23. Marder W, Fisseha S, Ganser MA, Somers EC. Ovarian damage during chemotherapy in autoimmune diseases: broad health implications beyond fertility. Clin Med Insights Reprod Health 2012;2012:9–18.
24. Suehiro RM, Borba EF, Bonfa E, Okay TS, Cocuzza M, Soares PM, et al. Testicular Sertoli cell function in male systemic lupus erythematosus. Rheumatology (Oxford) 2008;47:1692–7.
25. Woodruff DY, Horwitz G, Weigel J, Nangia AK. Fertility preservation following torsion and severe ischemic injury of a solitary testis. Fertil Steril 2010;94:352.e4–5.
26. Coleman E, Bockting W, Botzer M, Cohen-Kettenis P, DeCuypere G, Feldman J, et al. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. Int J Transgenderism 2012;13:165–232.
27. De Wert G, Dondorp W, Shenfield F, Barri P, Devroey P, Diedrich K, et al. ESHRE Task Force on Ethics and Law 23: medically assisted reproduction in singles, lesbian and gay couples, and transsexual peopledagger. Hum Reprod 2014;29:1859–65.
28. Stoop D, van der Veen F, Deneyer M, Nekkebroeck J, Tournaye H. Oocyte banking for anticipated gamete exhaustion (AGE) is a preventive intervention, neither social nor nonmedical. Reprod Biomed Online 2014;28:548–51.
29. Cobo A, Garcia-Velasco JA, Domingo J, Remohi J, Pellicer A. Is vitrification of oocytes useful for fertility preservation for age-related fertility decline and in cancer patients? Fertil Steril 2013;99:1485–95.
30. Dahhan T, Dancet EA, Miedema DV, van der Veen F, Goddijn M. Reproductive choices and outcomes after freezing oocytes for medical reasons: a follow-up study. Hum Reprod 2014;29:1925–30.
31. Oktay K, Buyuk E, Rodriguez-Wallberg KA, Sahin G. In vitro maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation. Reprod Biomed Online 2010;20:634–8.

32. Kim SY, Kim SK, Lee JR, Woodruff TK. Toward precision medicine for preserving fertility in cancer patients: existing and emerging fertility preservation options for women. J Gynecol Oncol 2016;27:e22.
33. Donnez J, Jadoul P, Pirard C, Hutchings G, Demylle D, Squifflet J, et al. Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. Fertil Steril 2012;98:720–5.
34. Donnez J, Dolmans MM, Martinez-Madrid B, Demylle D, Van Langendonckt A. The role of cryopreservation for women prior to treatment of malignancy. Curr Opin Obstet Gynecol 2005;17:333–8.
35. Campbell BK, Hernandez-Medrano J, Onions V, Pincott-Allen C, Aljaser F, Fisher J, et al. Restoration of ovarian function and natural fertility following the cryopreservation and autotransplantation of whole adult sheep ovaries. Hum Reprod 2014;29:1749–63.
36. Martinez M, Rabadan S, Domingo J, Cobo A, Pellicer A, Garcia-Velasco JA. Obstetric outcome after oocyte vitrification and warming for fertility preservation in women with cancer. Reprod Biomed Online 2014;29:722–8.
37. Meirow D, Dor J, Kaufman B, Shrim A, Rabinovici J, Schiff E, et al. Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms of ovarian injury. Hum Reprod 2007;22:1626–33.
38. Del Mastro L, Ceppi M, Poggio F, Bighin C, Peccatori F, Demeestere I, et al. 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 2014;40:675–83.
39. Lambertini M, Ceppi M, Poggio F, Peccatori FA, Azim HA, Ugolini D, et al. 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 2015;26:2408–19.
40. Demeestere I, Brice P, Peccatori FA, Kentos A, Dupuis J, Zachee P, et al. 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 2016;34:2568–74.
41. Bedoschi G, Oktay K. Current approach to fertility preservation by embryo cryopreservation. Fertil Steril 2013;99:1496–502.
42. Cil AP, Bang H, Oktay K. Age-specific probability of live birth with oocyte cryopreservation: an individual patient data meta-analysis. Fertil Steril 2013;100:492–9.e3.
43. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling- oocyte study. Hum Reprod 2010;25:66–73.
44. Solé M, Santaló J, Boada M, Clua E, Rodríguez I, Martínez F, et al. How does vitrification affect oocyte viability in oocyte donation cycles? A prospective study to compare outcomes achieved with fresh versus vitrified sibling oocytes. Hum Reprod 2013;28:2087–92.
45. Alvarez M, Solé M, Devesa M, Fábregas R, Boada M, Tur R, et al. Live birth using vitrified–warmed oocytes in invasive ovarian cancer: case report and literature review. Reprod Biomed Online 2014;28:663–8.
46. Oktay K, Bedoschi G, Pacheco F, Turan V, Emirdar V. First pregnancies, live birth, and in vitro fertilization outcomes after transplantation of frozen-banked ovarian tissue with a human extracellular matrix scaffold using robot-assisted minimally invasive surgery. Am J Obstet Gynecol 2016;214:94.e1–9.
47. Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, et al. First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. Hum Reprod 2013;28:2996–9.
48. Demeestere I, Simon P, Dedeken L, Moffa F, Tsépélidis S, Brachet C, et al. Live birth after autograft of ovarian tissue cryopreserved during childhood. Hum Reprod 2015;30:2107–9.
49. Donnez J, Dolmans M-M. Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. J Assist Reprod Genet 2015;32:1167–70.
50. Jensen AK, Kristensen SG, Macklon KT, Jeppesen JV, Fedder J, Ernst E, et al. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. Hum Reprod 2015;30:2838–45.
51. Andersen CY. Success and challenges in fertility preservation after ovarian tissue grafting. Lancet 2015;385:1947–8.
52. Garcia A, Herrero MB, Holzer H, Tulandi T, Chan P. Assisted reproductive outcomes of male cancer survivors. J Cancer Surviv 2015;9:208–14.
53. Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. Endocr Rev 2015;36:1–24.
54. Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. Proc Natl Acad Sci U S A 2013;110:17474–9.
55. Novella-Maestre E, Herraiz S, Rodriguez-Iglesias B, Diaz-Garcia C, Pellicer A. Short-term PTEN inhibition improves in vitro activation of primordial follicles, preserves follicular viability, and restores AMH levels in cryopreserved ovarian tissue from cancer patients. PLoS One 2015;10:e0127786.
56. Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen–thawed ovarian tissue. Fertil Steril 2013;99:1514–22.
57. Yding Andersen C, Ernst E, Baerentzen S, Birkebaek NH, Clausen N. No malignancy detected in surplus ovarian tissue from a former Ewing sarcoma patient who experienced relapse four years after being grafted with frozen/thawed ovarian tissue. J Assist Reprod Genet 2014;31:1567–8.
58. Smitz J, Dolmans MM, Donnez J, Fortune JE, Hovatta O, Jewgenow K, et al. Current achievements and future research directions in ovarian tissue culture, in vitro follicle development and transplantation: implications for fertility preservation. Hum Reprod Update 2010;16:395–414.
59. McLaughlin M, Telfer EE. Oocyte development in bovine primordial follicles is promoted by activin and FSH within a two-step serum-free culture system. Reproduction 2010;139:971–8.
60. Picton HM, Harris SE, Muruvi W, Chambers EL. The in vitro growth and maturation of follicles. Reproduction 2008;136:703–15.
61. Skory RM, Xu Y, Shea LD, Woodruff TK. Engineering the ovarian cycle using in vitro follicle culture. Hum Reprod 2015;30:1386–95.
62. Xiao S, Zhang J, Romero MM, Smith KN, Shea LD, Woodruff TK. In vitro follicle growth supports human oocyte meiotic maturation. Sci Rep 2015;5:17323.
63. Xu M, Fazleabas AT, Shikanov A, Jackson E, Barrett SL, Hirshfeld-Cytron J, et al. In vitro oocyte maturation and preantral follicle culture from the luteal-phase baboon ovary produce mature oocytes. Biol Reprod 2011;84:689–97.
64. Anckaert E, De Rycke M, Smitz J. Culture of oocytes and risk of imprinting defects. Hum Reprod Update 2013;19:52–66.
65. Laronda MM, Jakus AE, Whelan KA, Wertheim JA, Shah RN, Woodruff TK. Initiation of puberty in mice following decellularized ovary transplant. Biomaterials 2015;50:20–9.
66. Vanacker J, Dolmans MM, Luyckx V, Donnez J, Amorim CA. First transplantation of isolated murine follicles in alginate. Regen Med 2014;9:609–19.
67. Kalich-Philosoph L, Roness H, Carmely A, Fishel-Bartal M, Ligumsky H, Paglin S, et al. Cyclophosphamide triggers follicle activation and 'burnout'; AS101 prevents follicle loss and preserves fertility. Sci Transl Med 2013;5:185ra62.
68. Roness H, Kashi O, Meirow D. Prevention of chemotherapy-induced ovarian damage. Fertil Steril 2016;105:20–9.
69. Nickkholgh B, Mizrak SC, van Daalen SK, Korver CM, Sadri-Ardekani H, Repping S, et al. Genetic and epigenetic stability of human spermatogonial stem cells during long-term culture. Fertil Steril 2014;102:1700–7.e1.
70. Vassena R, Eguizabal C, Heindryckx B, Sermon K, Simon C, van Pelt AM, et al. Stem cells in reproductive medicine: ready for the patient? Hum Reprod 2015;30:2014–21.
71. Hayashi K, Saitou M. Stepwise differentiation from naïve state pluripotent stem cells to functional primordial germ cells through an epiblast-like state. Methods Mol Biol 2013;1074:175–83.
72. Zhou Q, Wang M, Yuan Y, Wang X, Fu R, Wan H, et al. Complete meiosis from embryonic stem cell-derived germ cells in vitro. Cell Stem Cell 2016;18:330–40.

Update on fertility preservation from the Barcelona International Society for Fertility Preservation–ESHRE–ASRM 2015 expert meeting: indications, results and future perspectives

Francisca Martinez, on behalf of the International Society for Fertility Preservation–ESHRE–ASRM Expert Working Group

Hospital Universitario Dexeus, Gran Via Carlos III, 71-75, 08208, Barcelona, Spain

Study Question: What progress has been made in fertility preservation (FP) over the last decade?

Summary Answer: FP techniques have been widely adopted over the last decade and therefore the establishment of international registries on their short- and long-term outcomes is strongly recommended.

What Is Known Already: FP is a fundamental issue for both males and females whose future fertility may be compromised. Reproductive capacity may be seriously affected by age, different medical conditions and also by treatments, especially those with gonadal toxicity. There is general consensus on the need to provide counselling about currently available FP options to all individuals wishing to preserve their fertility.

Study Design, Size, Duration: An international meeting with representatives from expert scientific societies involved in FP was held in Barcelona, Spain, in June 2015.

Participants/Materials, Setting, Methods: Twenty international FP experts belonging to the American Society of Reproductive Medicine, ESHRE and the International Society of Fertility Preservation reviewed the literature up to June 2015 to be discussed at the meeting, and approved the final manuscript. At the time this manuscript was being written, new evidence considered relevant for the debated topics was published, and was consequently included.

Main Results and the Role of Chance: Several oncological and non-oncological diseases may affect current or future fertility, either caused by the disease itself or the gonadotoxic treatment, and need an adequate FP approach. Women wishing to postpone maternity and transgender individuals before starting hormone therapy or undergoing surgery to remove/alter their reproductive organs should also be counselled accordingly. Embryo and oocyte cryopreservation are first-line FP methods in post-pubertal women. Metaphase II oocyte cryopreservation (vitrification) is the preferred option. Cumulative evidence of restoration of ovarian function and spontaneous pregnancies after ART following orthotopic transplantation of cryopreserved ovarian tissue supports its future consideration as an open clinical application. Semen cryopreservation is the only established method for FP in men. Testicular tissue cryopreservation should be recommended in pre- pubertal boys even though fertility restoration strategies by autotransplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans. The establishment of international registries on the short- and long-term outcomes of FP techniques is strongly recommended.

Limitations, Reasons for Caution: Given the lack of studies in large cohorts or with a randomized design, the level of evidence for most of the evidence reviewed was three or below.

Wider Implications of the Findings: Further high quality studies are needed to study the long-term outcomes of FP techniques.

Study Funding/Competing Interest(s): None.

Trial Registration Number: N/A.

Key Words: Fertility preservation, semen cryopreservation, testicular tissue cryopreservation, embryo cryopreservation, oocyte cryopreservation, ovarian tissue cryopreservation, oncological fertility preservation, non-oncological fertility preservation, fertoprotection

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/16938-24298

Received March 27, 2017; accepted May 19, 2017; published online July 21, 2017.
This article has not been externally peer reviewed.
This article is simultaneously published in *Human Reproduction*.
Participants of the Expert Working Group are listed in the Appendix.
Correspondence: Francisca Martinez, Hospital Universitario Dexeus, Barcelona, Spain (E-mail: pacmar@dexeus.com).

Fertility and Sterility® Vol. 108, No. 3, September 2017 0015-0282

http://dx.doi.org/10.1016/j.fertnstert.2017.05.024

INTRODUCTION

Reproductive capacity may be seriously affected by age, different conditions, including genetic syndromes, and also by treatments, especially those with gonadal toxicity. Fertility preservation (FP) is a fundamental issue for individuals of reproductive age, both male and female, or prepubescent boys and girls whose future fertility may be compromised. There is general consensus on the need to provide counselling about currently available FP options to all individuals wishing to preserve their fertility. Timely referral to a fertility specialist for informed FP decisions becomes essential.

Several techniques for FP are nowadays well established while others are still considered experimental. These techniques have been the subject of continuous review by experts with the aim of providing physicians involved in FP with up-to-date knowledge and counselling. Given the particular nature of FP, recommendations are largely based on cohort studies, case series, small non-randomized clinical trials, or case reports, which further makes FP a challenging but rather controversial field.

Reviews have been mostly focused on cancer, which is probably the main indication for FP given its high incidence and impact on reproductive health (1–6). However, the need for FP in other pathologic situations, either due to the disease itself or to gonadotoxic treatment, and even in non-medical indications, is on the rise. Moreover, new perspectives to tackle FP are being developed, and evidence about the results of spontaneous pregnancy and ART after current FP procedures is growing, which may further help clinicians provide adequate counselling. With the aim of reviewing all these aspects and drawing recommendations, an international meeting with representatives from expert scientific societies involved in FP was held in Barcelona (Spain) in June 2015. This paper summarizes the topics debated, with a special focus on indications for FP, current outcomes and future perspectives. A condensed version of this summary has been included in the print issue of *Fertility and Sterility*.

MATERIALS AND METHODS

Twenty international FP experts belonging to the American Society of Reproductive Medicine (ASRM), ESHRE and the International Society of Fertility Preservation (ISFP) attended the meeting. Experts conducted a review of the literature and evidence presented in scientific meetings up to June 2015 to be discussed at the meeting. At the time this manuscript was being written, new evidence considered relevant for the debated topics was published, and has consequently been included. Given the lack of studies in large cohorts or with a randomized design, the quality of evidence according to the European Society for Medical Oncology Clinical Practice Guidelines for fertility preservation in cancer patients (3) for most of the evidence reviewed was three or below.

INDICATIONS FOR FP

Cancer

Many forms of cancer are associated with impaired semen quality or ovarian function at the time of cancer diagnosis. However, the main effect on fertility arises from commonly used treatments such as chemotherapy with alkylating agents and pelvic radiation that present well-known gonadotoxic side effects. Gonadal failure resulting from these treatments may affect different aspects of reproductive health, including pubertal development, hormone production, and sexual function in adults (6, 7). The fact that more than 80% of children and adolescents with cancer become long-term survivors (8) has raised an increased interest in the long-term effects of cancer treatment on fertility.

Male. Spermatogonia are especially sensitive to chemotherapy and radiotherapy. The effect, which is dose-dependent, may not be permanent if the spermatogonial stem cell (SSC) population is not fully depleted (6). Data about the impact of recent biological or targeted cancer therapies on male fertility are limited (4). For most of these therapies, the effects seem to be mild, mostly involving reproductive endocrinology (9). Finally, surgical pelvic interventions for malignant or benign disease may affect the anatomy or normal functioning of reproductive organs (10).

Female. Chemotherapy and radiotherapy may induce premature ovarian insufficiency (POI) in women (11). Ovarian damage is drug- and dose-dependent and increases as the patient ages (1). Radiotherapy may also affect the uterus, leading to reduced vascularity, myometrium damage (fibrosis) and hormone-dependent insufficiency (5). Recent evidence in female survivors of childhood cancer shows that chemotherapy without radiotherapy to the brain or the pelvis has few effects on future pregnancy or live births (12). In any case, FP should be considered prior to chemotherapy to maximize future reproductive potential. Data about the impact of recent biological or targeted cancer female fertility therapies is also limited except for bevacizumab, with a 34% rate of POI reported (4). Fertility may also be impaired by surgical removal or damage to reproductive organs.

Non-oncological Medical Indications

FP options should also be discussed with adult and younger women and men affected by several non-oncological medical conditions. Non- oncological systemic diseases, such as haematological and autoimmune conditions, usually require chemotherapy or radiotherapy, especially for those in need of a bone marrow or haematopoietic stem cell transplantation (HSCT) (11). In other conditions reproductive function may be compromised by genetic causes or by surgical interventions. Finally, FP may be offered to patients at high risk of fertility loss as a result of severe body trauma requiring surgical intervention. Table 1 summarizes the most common non-oncological conditions requiring FP.

Autoimmune diseases. Table 1 summarizes autoimmune diseases reported to benefit from immunosuppressive therapy with alkylating agents (cyclophosphamide) (11, 13). POI in these women is also affected by disease duration and presence of anti-Ro and anti-U1RNP (ribonucleoprotein) antibodies (17). Continuous POI in women with chronic autoimmune diseases also increases the risk of hypoestrogenism-related

TABLE 1

Non-oncological conditions requiring fertility preservation.

Indication	Disease
Autoimmune diseases (11, 13)	Systemic lupus erythematosus (SLE) Behcet's disease Churg-Strauss syndrome (eosinophilic granulomatosis) Steroid resistant glomerulonephritis Granulomatosis with polyangiitis (formerly Wegener's granulomatosis) Inflammatory bowel diseases Rheumatoid arthritis Pemphigus vulgaris
Hematopoietic stem cell transplantation (11, 14)	Autoimmune diseases unresponsive to immunosuppressive therapy Haematological diseases (sickle cell anaemia, thalassaemia major, plastic anaemia)
Medical conditions causing POI (15)	Altered hypothalamic–pituitary–gonadal axis (16, 17) Ovarian oophoritis Benign ovarian tumours Mosaic Turner's syndrome Fragile X Mental Retardation 1 (18) Galactosemia (19) Beta-thalassaemia (20) Endometriosis (21)
Male genetic disorders	Klinefelter's syndrome (13)
Testicular damage (22)	
Gender reassignment procedures (23)	
Severe body trauma requiring surgical intervention	

Note: POI = premature ovarian insufficiency.

Martinez. Update on fertility preservation. Fertil Steril 2017.

comorbidities (including cardiovascular disease and osteoporosis) (24). Males with systemic lupus erythematosus show a high frequency of testicular Sertoli cell dysfunction associated with semen abnormalities (25). New treatment approaches are changing the prognosis of patients with autoimmune diseases, although information about toxicity for reproduction is still limited.

Hematopoietic stem cell transplantation. HSCT (autologous or allogeneic) has been an important therapeutic tool for some oncological and non-oncological systemic diseases. Patients undergoing HSCT are at particularly high risk of developing ovarian (64–85%) or testicular (50–90%) failure since aggressive chemotherapy and radiotherapy is needed to destroy pre-existing bone marrow (14). The most common non-oncological diseases benefiting from HSTC are autoimmune diseases that are unresponsive to immunosuppressive therapy or benign haematological diseases (Table 1) (11).

Medical conditions causing POI. POI may also result from several other causes, including an altered hypothalamic–pituitary–gonadal axis, ovarian oophoritis, benign ovarian tumours, either due to their extensive or progressive nature, or bilateral adnexectomy (16, 17). POI is also common in Turner's syndrome. However, FP may not be feasible for most patients with this disease since by the time they reach puberty their primordial follicle reserve may already be depleted. FP may only be offered to young patients with mosaic Turner's syndrome after careful consideration of increased pregnancy-associated risks (15, 26).

Other conditions associated with POI include FMR1 gene mutations (Fragile X Mental Retardation 1) as a result of premature ovarian aging (18), classic galactosemia (19) or beta-thalassaemia in female patients who suffer from hypogonadotrophic hypogonadism associated with amenorrhoea, anovulation and infertility (20).

It is well documented that women with endometriosis are at increased risk of POI and that about half of them will experience infertility (21). The causal relationship between endometriosis and infertility is unclear (27). FP may be of interest for reproductive-age women at risk of impaired fertility due to progression or surgical treatment of this condition (28). Nevertheless, some authors restrict FP to women with bilateral unoperated endometriomas and those with previous unilateral endometrioma removal requiring surgery for a contralateral recurrence (21).

Male genetic disorders and testicular tissue damage. Klinefelter's syndrome is the most common sex chromosomal disorder in humans. This syndrome causes hypogonadism and azoospermia in >90% cases (13, 22). Testicular injury may also result in irreparable damage to the testicular tissue leading to infertility (22). A recent study has highlighted the relevance of attempting salvage, even in cases of subjectively dead testicle, and to offer the patient FP options (29).

Gender reassignment procedures. Removal of testicles or ovaries destroys the ability to have genetically- related children, while feminizing/masculinizing medications used in gender reassignment procedures may lead to diminished fertility (23). The World Professional Association for Transgender Health has emphasized the need to discuss and provide counselling about FP and fertility treatment 'before starting hormone therapy or undergoing surgery to remove/alter their reproductive organs' even at a younger age (30). Further, the Endocrine Society recommends providing counsel about FP 'prior to initiation of puberty suppression in adolescents and before treatment with sex hormones of the desired sex in both adolescents and adults' (31). Evidence regarding

reproductive health issues in individuals receiving treatments for gender dysphoria is scarce. Currently, there are no established techniques for preserving gonadal function in pre- pubertal or pubertal adolescents, who will never develop reproductive function in their natal sex owing to blockers or cross-gender hormones (30). There is an ongoing ethical debate on whether FP should or should not be offered to transgender individuals (32).

Delayed Childbearing

Female fertility decreases gradually but significantly after age 32 years, and faster after 37 years, which compromises fertility when delaying childbearing (33). This is important, since an increasing proportion of couples in developed countries choose to have children later in life ($\geq$35 years). Given that delaying childbearing is considered a non-medical indication for FP, the term 'AGE banking' (oocyte banking for anticipated gamete exhaustion) has been proposed for oocyte cryopreservation in these cases (34).

AVAILABLE PROCEDURES FOR FP

The most recent practice guidelines issued for cancer patients by the ASRM (1) and the American Society of Clinical Oncology (4) provide an exhaustive review of all evidence supporting currently available FP procedures, which may be adaptable to all scenarios where fertility may be compromised.

Women

Both, embryo and oocyte cryopreservation (slow freezing or vitrification) are first-line FP methods (Fig. 1). However, oocyte cryopreservation is increasingly preferred in adolescent girls or young women without a life partner given that it overcomes religious, ethical or practical issues related to embryo storage (4, 35). Mature oocyte vitrification is preferred in post-pubertal women when gonadotoxic treatment can be delayed to allow time for controlled ovarian stimulation (COS) (36). Harvesting of immature oocytes by aspiration would be an option for patients unable to undergo COS such as pre-pubertal girls, women with aggressive or hormone- sensitive cancers, or those with polycystic ovary syndrome (37). The benefits of adding tamoxifen or letrolozole to COS regimens administered to women with breast cancer are still unclear (38). IVM has been shown to improve outcomes in breast cancer patients undergoing COS for FP (39).

Ovarian tissue cryopreservation (OTC) is a COS-independent experimental technique which also allows immediate cancer treatment, and is currently the only FP option in paediatric patients (11) and in hormone-dependent diseases (37). Reimplantation of this tissue either in the pelvic cavity (orthotopic) or elsewhere (heterotopic) has the potential of restoring fertility and ovarian hormone secretion. Reimplantation of frozen–thawed ovarian tissue in the pelvic cavity is usually carried out by laparoscopy, but the surgical technique is contingent on the presence (or not) of at least one ovary. If an ovary is present, the remaining atrophic cortex is removed using scissors, thereby creating a grafting bed onto which the thawed ovarian fragments are placed. If no ovaries remain, the ovarian pieces are placed in a peritoneal window created in the peritoneum of the broad ligament, in an area where retroperitoneal capillaries are visible (40).

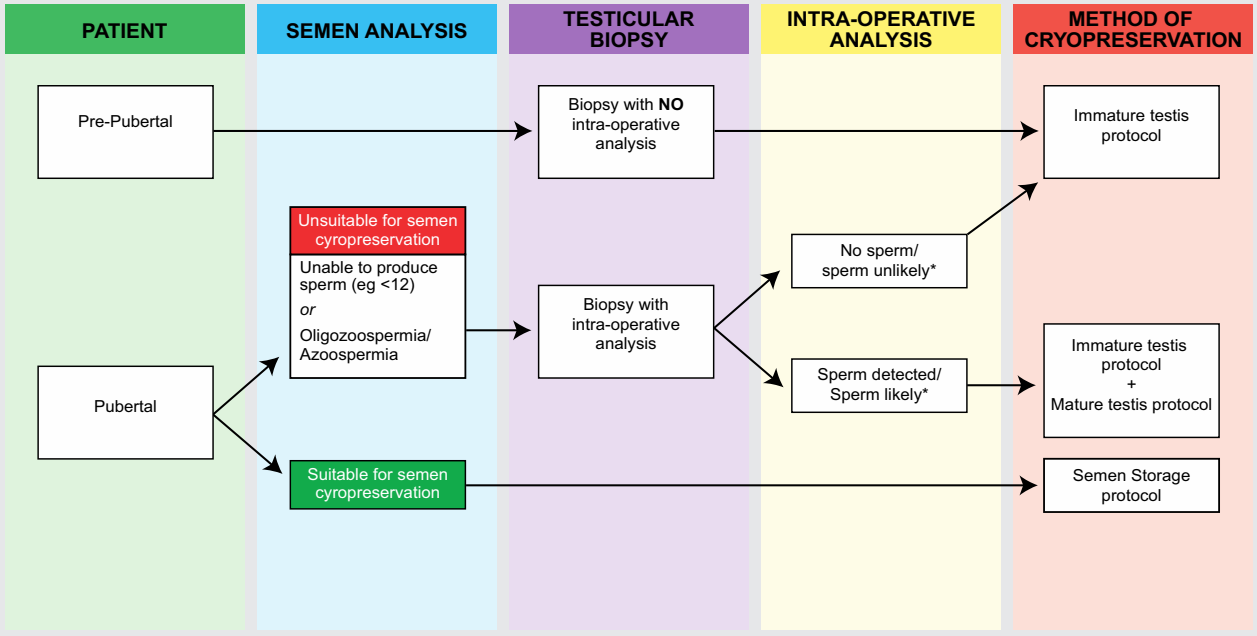
Ovarian tissue could also be preserved as an entire ovary with its vascular pedicle, preventing ischaemic damage occurring between transplantation and revascularization (41). Fertility restoration after whole ovary preservation requires retransplantation of the whole organ accompanied by vascular anastomoses of the blood vessels. However, although recent evidence from large animals, such as sheep, suggests that natural fertility can be fully restored following autotransplantation of whole ovaries and their supporting vascular pedicle after slow freezing and thawing (42, 43), cryopreservation of the whole ovary is likely to be more problematic in adult women owing to the increased size of their ovaries, the difficulty of achieving adequate perfusion and penetration of the cryoprotectants agents through the whole organ, and the inherently different freezing and thawing optima for the different cell types in both the ovary and blood vessels. Although several methods have been developed to help overcome these barriers (42–45), more research is needed before this technique can be translated into clinical practice.

Fertoprotective agents. GnRH analogues/agonists (GnRHa) may protect follicles from destruction during chemotherapy, probably by suppression of gonadotrophin levels and reduction of utero-ovarian perfusion (46). These agents have long been used for the prevention of ovarian damage, despite their efficacy being a subject of debate owing to inconsistent results from randomized trials using GnRHa (47–54). Two meta-analyses of randomized trials have found an overall significant reduced risk of POI in young breast cancer patients (55, 56). GnRHa increased the pregnancy rate and had no negative impact on prognosis (56). This protective effect was not as clear in other cancer patients (ovarian and lymphoma) (55). Recently, no protective effect at all was found in young patients with lymphoma (57). Still, the quality of evidence is relatively low given the number of women included, relatively short-term follow-up hitherto and significant heterogeneity. Further high quality studies are needed to study the (long-term) effects of GnRHa use on POI.

Men

Sperm cryopreservation is the only established FP method in adult and adolescent males. Alternatives to the procurement of semen samples by masturbation include assisted ejaculation methods such as penile vibratory stimulation or electroejaculation. A recent paper on the European perspective on testicular tissue cryopreservation for FP in pre-pubertal and adolescent boys by the ESHRE Task Force on Fertility Preservation for severe diseases recommends an algorithm for sperm and testicular tissue cryopreservation in pre-pubertal boys and adolescent males at high risk of fertility loss (7). This algorithm includes alternative experimental techniques, such as testicular sperm extraction (TESE), for patients presenting oligo- or azoospermia at the time of cryopreservation or those with necrozoospermia or ejaculation disorders, or

FIGURE 1



Algorithm for the cryopreservation of testicular tissue/sperm in pre-pubertal and adolescent patients at high risk of infertility. Clinical assessment for puberty should be carried out by a clinician with experience in pubertal assessment. It must be stressed that no clinical parameter can accurately predict the presence of sperm. The proven treatment option for pubertal and adolescent boys who are considered capable of producing a semen sample is semen collection and cryopreservation. If sufficient sperm are recovered the gametes can be banked using commercial glycerol-based sperm cryomedia. For those young patients who are clinically pre-pubertal and for whom semen cryopreservation is not possible the fertility preservation strategy should include collection of a testicular biopsy by an experienced surgeon. The tissue should be cryopreserved with a protocol optimized for preserving immature germ cells, (immature testis protocol). Patients who are pubertal but are unable to produce a suitable semen sample may proceed to testicular biopsy, with intra-operative analysis. Techniques for intra-operative analysis may vary between institutions but should be aimed at identifying tissue containing (or likely to contain) sperm. This should be carried out by an individual with experience in analysis of testicular tissue (e.g. surgeon, embryologist or andrologist). When sperm are not identified or deemed unlikely the tissue should be frozen with the immature testis protocol used for pre-pubertal patients. For patients in whom sperm are identified or considered likely to be present, tissue should be split into two portions for storage. One portion should be cryopreserved using the immature testis preservation protocol, whilst the second portion should be stored using a protocol aimed at preserving mature sperm cells with glycerol as the main cryoprotectant. As stated in the text at present there are several protocols for cryopreservation of immature testicular tissue and there is no clear evidence at the time of writing to demonstrate which is optimal. Reprinted with permission from (7).

Martinez. Update on fertility preservation. Fertil Steril 2017.

immature testicular tissue cryopreservation or SSC cryopreservation when no sperm can be collected (Fig. 1).

RESULTS OF ART AFTER FP

Women

As a well-established technology, embryo cryopreservation has high pregnancy success rates (35). However, outcomes in cancer patients are scarce. Recently (58), reported a 44% live birth rate (LBR) per patient among 54 women with cancer undergoing IVF and embryo cryopreservation, with a cumulative live birth rate (CLBR) similar to that achieved with fresh embryos in non-cancer patients (Table 2). Similarly, Oktay et al. reported 18 pregnancies and 25 live births among 33 women with breast cancer, with a LBR of 45% per embryo transfer (59). Success rates associated with oocyte cryopreservation have significantly improved in recent years, with vitrification success rates being superior to slow freezing (63). A recent report of the outcomes achieved in oocyte donation showed a 6.5% oocyte-to-baby rate, with CLBR increasing with the number of oocytes used (60). A similar report among women undergoing oocyte vitrification because of age or because of non-oncological medical conditions revealed a LBR per patient of 50% among women aged $\leq$35 years, and of 22.9% among those aged >36 years after the transfer of embryos obtained from vitrified oocytes. The CLBR was higher and increased faster among younger women (61) (Table 2). It should be noted that these figures arise from patients with a good prognosis who are managed by a highly experienced team, and therefore may not be representative of other FP programmes (64). These success rates are comparable to those achieved with fresh oocytes (65, 66). Outcomes after oocyte vitrification among female cancer patients are scarce. Martinez et al., (45) reported fertilization rates up to 76.6% and a mean number of embryos transferred of 1.8 $\pm$ 0.7 SD among 11 women with cancer, four of whom gave birth at term without negative perinatal outcomes. Alvarez et al., (67) first reported a successful birth in a woman with invasive ovarian cancer.

Despite being considered an experimental technique both restoration of ovarian function and spontaneous pregnancies

TABLE 2

Clinical outcomes from fertility preservation techniques in women.

Author	FP technique	Women/Indication	Outcome
Dolmans et al., (58)	Embryo cryopreservation	54/Cancer 33 returned/20 ET	22% LBR per ET Nine pregnancies Four deliveries
Oktay et al., (59)	Embryo cryopreservation	33/Breast cancer 18 returned/55 ET	45% LBR per ET 26 pregnancies 18 deliveries
Cobo et al., (60)	Oocyte vitrification	Ovum donation programme	6.5% oocyte-to-baby rate. CLBR increased with the number of oocytes used
Cobo et al., (61)	Oocyte vitrification	Delaying childbearing or non-oncological medical conditions	50% LBR per patient in women ≤35 year old 22.9% LBR per patient in women >36 years old
Donnez et al., (62)	Ovarian tissue cryopreservation		N = 111 cases, 32 conceived 29.0% LBR per patient

FP = fertility preservation; ET = embryo transfer; LBR = live birth rate; CLBR = cumulative live birth rate.

Martinez. Update on fertility preservation. Fertil Steril 2017.

after ART have been reported after orthotopic transplantation of cryopreserved ovarian tissue (68–71). Only one case of a live birth after heterotopic transplantation has been reported up to 2013 (72). Recently, Demestree et al. have reported the first live birth following re-grafting of ovarian tissue that had been cryopreserved during childhood in a 13-year old girl undergoing HSCT (68). To date, a large series of 60 live births after transplantation of cryopreserved ovarian tissue has been reported, also showing that by repeating the procedure ovarian activity can be restored for more than 11 years (69). In a series of 111 cases, the conception rate was 29%. Two women delivered three babies each, proving the efficacy of the technique, as well as the possibility of conceiving naturally several times after only one transplantation procedure (62, 70). Although it is impossible to provide a fixed success rate for transplantation of ovarian tissue as long as it remains active (73), given these encouraging results, ovarian cortex transplantation is proposed as an open clinical application (62).

Men

Success rates of semen cryopreservation have greatly increased with advances in ART, especially in ICSI, with pregnancy rates up to 57% (7). With this technique (74), reported a LBR of 62.1% in a cohort of 272 men with cancer, which was significantly higher than that of the comparative normospermic non-cancer population. To date, no clinical outcomes have been reported with other FP techniques.

FUTURE PERSPECTIVES

Figure 2 summarizes all FP techniques that are currently under study.

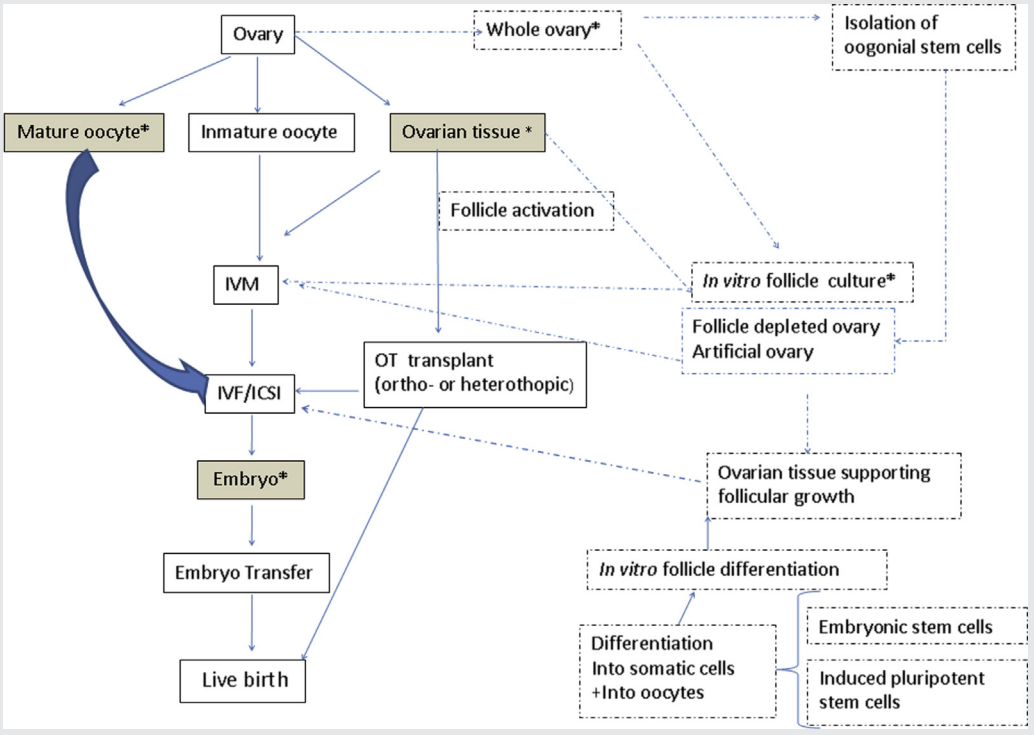
Women

Activation of ovarian follicles. Cryopreserved ovarian tissue from prepuberal patients and patients with primary POI contains immature primordial follicles that must be activated in order to start developing. It has been recently described that activation of primordial follicles can be induced in vivo by mechanically interrupting the Hippo signalling pathway (by ovarian fragmentation, drilling, laser) (75). Follicle activation may also be achieved in vitro before autotransplantation by acting on the PI3K- PTEN-AKT-FOXO3 pathway [phosphatidylinositol 3-kinase(PI3K) activators and phosphatase and tensin homologue enzyme (PTEN) inhibitors, Protein Kinase B (AKT) stimulators, transcriptional factor forkhead box O3(FOXO3)] which has been shown to regulate primordial follicle dormancy at oocyte level (75). This pathway has also a fundamental role in FSH stimulation of granulosa cell differentiation of antral follicles and in oocyte maturation of preovulatory follicles (75). Using this double approach in women with POI (76), found rapid follicle growth after grafting ovarian tissue back to patients, obtaining mature eggs. A live birth was achieved after IVF and embryo transfer. In vitro activation protocols are under development with the aim of increasing the pool of viable activated follicles available for in vitro growth (IVG) procedures (77).

In vitro follicle culture. Transplantation of cryopreserved tissue carries the risk of re-seeding original cancer cells into the patient, as recently highlighted in a series of recent reviews and reports (72, 78–83). This risk can be minimized by using complete IVG and maturation of oocytes as the means of fertility restoration (84, 85). The goal of complete IVG and maturation of oocytes is particularly challenging in human follicles because of the greatly extended developmental time-frame for follicles and oocytes, and increased size of ovulatory follicles and mature gametes (86). Furthermore, recent evidence suggests that the dynamics of the in vivo and hence in vitro growth environments may differ between the pre- and post-pubertal human ovary (87).

To date, three-dimensional (3D) culture methods have proved most successful in supporting the demands of human follicle activation and IVG, as these approaches are best able to maintain the morphology of the follicles and preserve critical cell–cell interactions both between the different cellular compartments in the follicle and between the follicle and its surrounding stromal tissue thus better mimicking the

FIGURE 2



Fertility preservation techniques in women. Experimental procedures are indicated in discontinuous boxes, while established ones (i.e. those proven to restore fertility, with live births reported) are indicated in shaded boxes. Vitrified-thawed oocytes can be fertilized by IVF/ICSI for embryo transfer. Immature oocytes can be matured in vitro (IVM) for IVF/ICSI. Research is undergoing on the potential use of oogonial stem cells to repopulate follicle-depleted ovaries or differentiating follicle somatic cells and oocytes from embryonic stem cells or induced pluripotent stem cells to assemble de novo follicles for transplantation or IVM and IVF/ICSI. *Cryopreserved. OT = ovarian tissue.

Martinez. Update on fertility preservation. Fertil Steril 2017.

in vivo ovarian growth environment. Several multi-step culture systems have succeeded in culturing human follicles (84, 88–91). Critically, all IVG systems used for fertility restoration for FP patients must start with the in situ culture of primordial follicles from cryopreserved tissue. The recent production of meiotically competent metaphase II non-human primate and human oocytes following IVG of freshly isolated secondary follicles (92, 93) is encouraging. However, whether mature human oocytes can be obtained from primordial follicles grown from cryopreserved ovarian tissue using these culture strategies or whether the oocytes so derived are competent to complete cytoplasmic and nuclear maturation in a timely manner is yet to be confirmed. It also remains to be confirmed whether their genomic imprint establishment and maintenance is normal (94) and whether metaphase II gametes so produced are healthy and able to undergo fertilization and support normal early embryonic development until the embryonic genome is activated. Considerable further research effort is therefore needed to confirm the safety and efficacy of oocytes derived following extended IVG and the maturation of human oocytes from cryopreserved tissue before this technology can be used to restore fertility in FP patients.

Artificial ovaries. An alternative to the in vitro culture of primordial follicles is their development into an engineered 'artificial ovary', consisting of isolated preantral follicles along with other ovarian cells assembled in a structure- 3D matrix, or scaffold, which allow follicles to grow and develop in an ovarian-like environment (95, 96). Ovarian cells from fresh medullary tissue have been suggested as the best source of isolated stromal cells for the artificial ovary (97). Once transplanted to the patient, this artificial ovary would potentially restore fertility and endocrine function (37). Following this procedure (98), reported the production of estradiol in vitro by primary ovarian cells of mice seeds into a previously decellularized ovary. Moreover, when transplanted into ovariectomized mice, grafts from these cells were able to initiate puberty.

New fertoprotective agents. The most recent theory of chemotherapy-induced follicle loss suggests that, simultaneously with large follicle apoptosis, chemotherapy also triggers activation of dormant follicle growth. Current research focuses thus on both agents with anti-apoptotic properties (imatinib, sphingosine-1-phosphate, thyroid hormone T3, granulocyte colony- stimulating factor and tamoxifen) that have been shown to reduce follicle loss in animal models (37), and on agents that also prevent follicle activation such as AS101, an immune modulator that acts on the PI3K/PTEN/AKT follicle activation pathway (99), and

anti-Mullerian hormone (100). Clinical applicability of these agents depends not only on their fertoprotective capacity, but also on their potential interaction with cancer treatments.

Men

In a similar way, the risk of reintroducing malignant cells via the graft might be overcome by in vitro spermatogenesis. As per follicle culture, SSCs (whole testicular biopsy or isolated SSCs) are cultured in 3D systems that resemble the in vivo situation (7). Recently, Nickkholgh et al. have reported the genetic stability of a long-term culture of human SSCs from two prostate cancer patients and although changes in the methylation status were observed, the consequences of these epigenetic changes on the functionality of the sperm of the health of the offspring are unknown (101). The fertilizing ability of in vitro-cultured sperm is to be established before assessing the clinical value of this technique. Moreover, fertility restoration strategies by autotransplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans (7).

Both Sexes

Artificial gametes. To date, infertile patients lacking functional oocytes or sperm cannot benefit from currently available ART unless donor gametes are used. The use of primordial germ cells (PGC) present in the gonads, such as SSC, is a promising approach for treating infertility. However, the population of these cells is scarce and decreases with age. Pluripotent stem cells (PSC), such as embryonic stem cells or induced PSC, constitute other potential sources of gametes (102).

Hayashi and Saitou (103) derived functional PGCs from PSC potentially able to generate functional oocytes and sperm (102). Recently (104), have reported the generation of haploid mouse spermatid-like cells able to produce viable and fertile offspring. Notwithstanding these advances, a recent critical review of available evidence in humans and animal models carried out by the ESHRE Special Interest Group in Stem Cells concluded that 'to date there are no proven stem cell-based means to improve reproductive function, either by producing functional gametes in vitro, or stimulating the resident stem cell population in the ovary to elicit de novo oocyte production' (102).

SUMMARY

The Expert Working Group made the following recommendations:

- Several oncological and non-oncological diseases may affect current or future fertility, either due to the disease itself or to gonadotoxic treatment, and need an adequate FP approach. These patients should be counselled regarding potential fertility loss and should be referred to fertility specialists to discuss options for FP and current results.
- Women wishing to postpone maternity and transgender individuals before starting hormone therapy or undergoing surgery to remove/alter their reproductive organs, should also be counselled accordingly.
- Embryo and oocyte cryopreservation are first-line FP methods in post-pubertal women. Metaphase II oocyte cryopreservation (vitrification) is the preferred option.
- Cumulative evidence of restoration of ovarian function and spontaneous pregnancies after ART following orthotopic transplantation of cryopreserved ovarian tissue supports its future consideration as an open clinical application.
- Semen cryopreservation is the only established FP technique in men.
- Testicular tissue cryopreservation should be recommended in pre- pubertal boys even though fertility restoration strategies by autotransplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans.
- The establishment of international registries on the short- and long- term outcomes of FP techniques is strongly recommended.

ACKNOWLEDGMENTS

We would like to gratefully acknowledge all the participants in the meeting, and especially to the ASRM, ESHRE and ISFP for supporting the project. We also thank Beatriz Viejo Ph.D. for editorial assistance in the preparation of this report.

AUTHORS' ROLES

F.M. drafted the article. C.Y.A., C.G., M.M.D., F.M., D.M., P.P., H.P., M.R., P.deS., A.V., H.W. drafted and revised the article; C.Y.A., P.N.B.; R.B., A.C., J.D., M.M.D., H.E., A.F., C.G., M.G., S.K., F.M., D.M., P.P., A. P., H.P., M.R., P.deS., A.V., H.W. reviewed and discussed the evidence. All authors approved the final version of the manuscript.

FUNDING

None.

CONFLICT OF INTEREST

None.

APPENDIX

ISFP–ESHRE–ASRM Expert Working Group

List of Participants

Claus Yding Andersen; Copenhagen University Hospital Rigshospitalet, Denmark. yding@rh.dk

PN Barri; Servicio de Medicina de la Reproducción, Hospital Universitario Dexeus, Barcelona, Spain. perbar@dexeus.com

Robert Brannigan; Feinberg School of Medicine, Northwester University, Chicago, USA. rebrannigan@gmail.com

A Cobo; Instituto Valenciano de Infertilidad, Valencia, Spain. ana.cobo@ivi.es

Jacques Donnez; Society for Research into Infertility (SRI), Brussels, Belgium. jacques.donnez@gmail.com

Marie Madeleine Dolmans; Gynaecology Department, Cliniques Universitaires Saint-Luc; Pôle de Gynécologie, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium. mmdolmans@gmail.com/Marie-madeleine.dolmans@uclouvain.be

J.L.H. (Hans) Evers; *Human Reproduction* Editor-in-Chief (ESHRE). Jh.evers@gmail.com

AnisFeki; Department of Obstetrics and Gynecology, Hôpital Cantonal, Fribourg, Switzerland. Anis.Feki@h-fr.ch

MariëtteGoddijn; Center for Reproductive Medicine, Department of Obstetrics and Gynecology, University of Amsterdam, Amsterdam, Netherlands.m.goddijn@amc.uva.nl

Clarisa Gracia; School of Medicine, University of Pennsylvania, Pennsylvania, USA. Cgracia@uphs.upenn.edu

Sam Kim; Department of Obstetrics and Gynecology, University Kansas School of Medicine, Kansas, USA. skim2@kumc.edu

Francisca Martinez, Servicio de Medicina de la Reproducción, Hospital Universitario Dexeus, Barcelona, Spain. pacmar@dexeus.com

Dror Meirow; Sheba Medical Center, Tel-Hashomer, Israel. Meirow@post.tau.ac.il

Pasquale Patrizio; Yale University Fertility Center, New Haven, CT 06511, USA. pasquale.patrizio@yale.edu

Antonio Pellicer; *Fertility and Sterility* Editor-In-Chief, American Society for Reproductive Medicine (ASRM). apellicer@ivi.es

Helen Picton; Division of Reproduction and Early Development, Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, UK LS2 9JT. H.M.Picton@leeds.ac.uk

Mitchel Rosen; University of California, San Francisco, USA. Mitchell.Rosen@ucsf.edu

Petra de Sutter; Department of Reproductive Medicine, University of Gent, Gent, Belgium Petra.DeSutter@uzgent.be

Anna Veiga; Servicio de Medicina de la Reproducción, Hospital Universitario Dexeus, Barcelona Spain. anavei@dexeus.com

HamishWallace; Royal Hospital for Sick Children, Edinburgh, Scotland. Hamish.wallace@nhs.net

REFERENCES

1. Ethics Committee of ASRM. Fertility preservation and reproduction in patients facing gonadotoxic therapies: a committee opinion. Fertil Steril 2013;100:1224–31.
2. ISFP Practice Committee, Kim SS, Donnez J, Barri P, Pellicer A, Patrizio P, et al. Recommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. J Assist Reprod Genet 2012;29:465–8.
3. Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim HA Jr, Peccatori FA, et al. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med 2016;14:1.
4. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500–10.
5. Mahajan N. Fertility preservation in female cancer patients: an overview. J Hum Reprod Sci 2015;8:3–13.
6. Oktay K, Rodriguez-Wallberg K. Fertility preservation during cancer treatment: clinical guidelines. Cancer Manag Res 2014;6:105–17.
7. Picton HM, Wyns C, Anderson RA, Goossens E, Jahnukainen K, Kliesch S, et al. A European perspective on testicular tissue cryopreservation for fertility preservation in prepubertal and adolescent boys. Hum Reprod 2015;30:2463–75.
8. Phillips SM, Padgett LS, Leisenring WM, Stratton KK, Bishop K, Krull KR, et al. Survivors of childhood cancer in the United States: prevalence and burden of morbidity. Cancer Epidemiol Biomarkers Prev 2015;24:653–63.
9. Meistrich ML. Effects of chemotherapy and radiotherapy on spermatogenesis in humans. Fertil Steril 2013;100:1180–6.
10. Levine J. Preserving fertility in children and adolescents with cancer. Children 2014;1:166–85.
11. Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol 2013;9:735–49.
12. Chow EJ, Stratton KL, Leisenring WM, Oeffinger KC, Sklar CA, Donaldson, et al. 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 2016;17:567–76.
13. Bedaiwy MA, Botros R. Fertility Preservation. Advances and Controversies. Daryaganj, New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd; 2014.
14. Joshi S, Savani BN, Chow EJ, Gilleece MH, Halter J, Jacobsohn DA, et al. Clinical guide to fertility preservation in hematopoietic cell transplant recipients. Bone Marrow Transplant 2014;49:477–84.
15. ESHRE POI Guideline Development Group. Guideline on the management of premature ovarian insufficiency. European Society of Human Reproduction and Embryology. Available at: https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Management-of-premature-ovarian-insufficiency.aspx; 2015. Accessed July 2, 2016.
16. Donnez J, Kim SS. Principles and Practice of Fertility Preservation. New York, US: Cambridge University Press; 2011.
17. Harward LE, Mitchell K, Pieper C, Copland S, Criscione-Schreiber LG, Clowse ME. The impact of cyclophosphamide on menstruation and pregnancy in women with rheumatologic disease. Lupus 2013;22:81–6.
18. Gleicher N, Yu Y, Himaya E, Barad DH, Weghofer A, Wu Y-G, et al. Early decline in functional ovarian reserve in young women with low (CGGn < 26) FMR1 gene alleles. Transl Res 2015;166:502–507.e1–2.
19. Fridovich-Keil JL, Gubbels CS, Spencer JB, Sanders RD, Land JA, Rubio-Gozalbo E. Ovarian function in girls and women with GALT-deficiency galactosemia. J Inherit Metab Dis 2011;34:357–66.
20. Roussou P, Tsagarakis NJ, Kountouras D, Livadas S, Diamanti-Kandarakis E. Beta-thalassemia major and female fertility: the role of iron and iron-induced oxidative stress. Anemia 2013, Article ID 617204.
21. Somigliana E, Viganò P, Filippi F, Papaleo E, Benaglia L, Candiani M, et al. Fertility preservation in women with endometriosis: for all, for some, for none? Hum Reprod 2015;30:1280–6.
22. Stahl PJ, Stember DS, Hsiao W, Schlegel PN. Indications and strategies for fertility preservation in men. Clin Obstet Gynecol 2010;53:815–27.
23. Darney PD. Hormonal contraception. In: Kronenberg HM, Melmer S, Polonsky KS, Larsen PR, editors. Williams Textbook of Endocrinology. Philadelphia, PA: Saunders/Elsevier; 2008:615–44.
24. Marder W, Fisseha S, Ganser MA, Somers EC. Ovarian damage during chemotherapy in autoimmune diseases: broad health implications beyond fertility. Clin Med Insights Reprod Health 2012;2012:9–18.

25. Suehiro RM, Borba EF, Bonfa E, Okay TS, Cocuzza M, Soares PM, et al. Testicular Sertoli cell function in male systemic lupus erythematosus. Rheumatology (Oxford) 2008;47:1692–7.
26. Lau NM, Huang JY, MacDonald S, Elizur S, Gidoni Y, Holzer H, et al. Feasibility of fertility preservation in young females with Turner syndrome. Reprod Biomed Online 2009;18:290–5.
27. Practice Committee of the ASRM. Endometriosis and infertility: a committee opinion. Fertil Steril 2012;98:591–8.
28. Bedoschi G, Turan V, Oktay K. Fertility preservation options in women with endometriosis. Minerva Ginecol 2013;65:99–103.
29. Woodruff DY, Horwitz G, Weigel J, Nangia AK. Fertility preservation following torsion and severe ischemic injury of a solitary testis. Fertil Steril 2010;94:352.e4–5.
30. Coleman E, Bockting W, Botzer M, Cohen-Kettenis P, DeCuypere G, Feldman J, et al. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. Int J Transgenderism 2012;13:165–232.
31. Hembree WC, Cohen-Kettenis P, Delemarre-van de Waal HA, Gooren LJ, Meyer WJ, Spack NP, et al. Endocrine treatment of transsexual persons: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2009;94:3132–54.
32. De Wert G, Dondorp W, Shenfield F, Barri P, Devroey P, Diedrich K, et al. ESHRE Task Force on Ethics and Law 23: medically assisted reproduction in singles, lesbian and gay couples, and transsexual peopledagger. Hum Reprod 2014;29:1859–65.
33. The ACOG Committee on Gynecologic Practice. Practice Committee of the American Society for Reproductive Medicine. Female age-related fertility decline. Committee Opinion No. 589. Obstet Gynecol 2014;123:719–21.
34. Stoop D, van der Veen F, Deneyer M, Nekkebroeck J, Tournaye H. Oocyte banking for anticipated gamete exhaustion (AGE) is a preventive intervention, neither social nor nonmedical. Reprod Biomed Online 2014;28:548–51.
35. Bedoschi G, Oktay K. Current approach to fertility preservation by embryo cryopreservation. Fertil Steril 2013;99:1496–502.
36. Cobo A, Garcia-Velasco JA, Domingo J, Remohi J, Pellicer A. Is vitrification of oocytes useful for fertility preservation for age-related fertility decline and in cancer patients? Fertil Steril 2013;99:1485–95.
37. Kim SY, Kim SK, Lee JR, Woodruff TK. Toward precision medicine for preserving fertility in cancer patients: existing and emerging fertility preservation options for women. J Gynecol Onco 2016;27:e22.
38. Dahhan T, Dancet EA, Miedema DV, van der Veen F, Goddijn M. Reproductive choices and outcomes after freezing oocytes for medical reasons: a follow-up study. Hum Reprod 2014;29:1925–30.
39. Oktay K, Buyuk E, Rodriguez-Wallberg KA, Sahin G. In vitro maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation. Reprod Biomed Online 2010;20:634–8.
40. Donnez J, Jadoul P, Pirard C, Hutchings G, Demylle D, Squifflet J, et al. Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. Fertil Steril 2012;98:720–5.
41. Donnez J, Dolmans MM, Martinez-Madrid B, Demylle D, Van Langendonckt A. The role of cryopreservation for women prior to treatment of malignancy. Curr Opin Obstet Gynecol 2005;17:333–8.
42. Campbell BK, Hernandez-Medrano J, Onions V, Pincott-Allen C, Aljaser F, Fisher J, et al. Restoration of ovarian function and natural fertility following the cryopreservation and autotransplantation of whole adult sheep ovaries. Hum Reprod 2014;29:1749–63.
43. Onions VJ, Webb R, Pincott-Allen C, Picton HM, Campbell BK. The effects of whole ovarian perfusion and cryopreservation on endothelial cell-related gene expression in the ovarian medulla and pedicle. Mol Hum Reprod 2013;19:205–15.
44. Bedaiwy MA, Hussein MR, Biscotti C, Falcone T. Cryopreservation of intact human ovary with its vascular pedicle. Hum Reprod 2006;21:3258–69.
45. Martínez M, Rabadan S, Domingo J, Cobo A, Pellicer A, Garcia-Velasco JA. Obstetric outcome after oocyte vitrification and warming for fertility preservation in women with cancer. Reprod Biomed Online 2014;29:722–8.
46. Meirow D, Dor J, Kaufman B, Shrim A, Rabinovici J, Schiff E, et al. Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms of ovarian injury. Hum Reprod 2007;22:1626–33.
47. 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;91:694–7.
48. Del Mastro L, Boni L, Michelotti A, Gamucci T, Olmeo N, Gori S, 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;306:269–76.
49. Demeestere I, Brice P, Peccatori FA, Kentos A, Gaillard I, Zachee P, et al. Gonadotropin-releasing hormone agonist for the prevention of chemotherapy-induced ovarian failure in patients with lymphoma: 1-year follow-up of a prospective randomized trial. J Clin Oncol 2013;31:903–9.
50. Elgindy EA, El-Haieg DO, Khorshid OM, Ismail EI, Abdelgawad M, Sallam HN, et al. Gonadatrophin suppression to prevent chemotherapy-induced ovarian damage: a randomized controlled trial. Obstet Gynecol 2013;121:78–86.
51. Gerber B, Minckwitz GV, Stehle H, Reimer T, Felberbaum R, Maass N, et al. Effect of luteinizing hormone-releasing hormone agonist on ovarian function after modern adjuvant breast cancer chemotherapy: the GBG 37 ZORO study. JCO 2011;29:2334–41.
52. Moore HC, Unger JM, Phillips KA, Boyle F, Hitre E, Porter D, et al. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy. N Engl J Med 2015;372:923–32.
53. Munster PN, Moore AP, Ismail-Khan R, Cox CE, Lacevic M, Gross-King M, et al. 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;30:533–8.
54. Sverrisdottir A, Nystedt M, Johansson H, Fornander T. Adjuvant goserelin and ovarian preservation in chemotherapy treated patients with early breast cancer: results from a randomized trial. Breast Cancer Res Treat 2009;117:561–7.
55. Del Mastro L, Ceppi M, Poggio F, Bighin C, Peccatori F, Demeestere I, et al. 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 2014;40:675–83.
56. Lambertini M, Ceppi M, Poggio F, Peccatori FA, Azim HA, Ugolini D, et al. 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 2015;26:2408–19.
57. Demeestere I, Brice P, Peccatori FA, Kentos A, Dupuis J, Zachee P, et al. 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 2016 [Epub ahead of print].
58. Dolmans MM, Hollanders de Ouderaen S, Demylle D, Pirard C. Utilization rates and results of long-term embryo cryopreservation before gonadotoxic treatment. J Assist Reprod Genet 2015;32:1233–7.
59. Oktay K, Turan V, Bedoschi G, Pacheco FS, Moy F. Fertility preservation success subsequent to concurrent aromatase inhibitor treatment and ovarian stimulation in women with breast cancer. J Clin Oncol 2015;33:2424–9.
60. Cobo A, Garrido N, Pellicer A, Remohí J. Six years' experience in ovum donation using vitrified oocytes: report of cumulative outcomes, impact of storage time, and development of a predictive model for oocyte survival rate. Fertil Steril 2015;104:1426–34.e8.
61. Cobo A, García-Velasco JA, Coello A, Domingo J, Pellicer A, Remohí J. Oocytes vitrification as an efficient option for elective fertility preservation. Fertil Steril 2016;105:755–64.
62. Donnez J, Dolmans M-M, Diaz C, Pellicer A. Ovarian cortex transplantation: time to move on from experimental studies to open clinical application. Fertil Steril 2015;104:1097–8.

63. Cil AP, Bang H, Oktay K. Age-specific probability of live birth with oocyte cryopreservation: an individual patient data meta-analysis. Fertil Steril 2013;100:492–9.e3.
64. Stoop D. Oocyte vitrification for elective fertility preservation: lessons for patient counseling. Fertil Steril 2016;105:603–4.
65. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod 2010; 25:66–73.
66. Solé M, Santaló J, Boada M, Clua E, Rodríguez I, Martínez F, et al. How does vitrification affect oocyte viability in oocyte donation cycles? A prospective study to compare outcomes achieved with fresh versus vitrified sibling oocytes. Hum Reprod 2013;28:2087–92.
67. Alvarez M, Solé M, Devesa M, Fábregas R, Boada M, Tur R, et al. Live birth using vitrified–warmed oocytes in invasive ovarian cancer: case report and literature review. Reprod Biomed Online 2014;28:663–8.
68. Demeestere I, Simon P, Dedeken L, Moffa F, Tsépélidis S, Brachet C, et al. Live birth after autograft of ovarian tissue cryopreserved during childhood. Hum Reprod 2015;30:2107–9.
69. Donnez J, Dolmans M-M. Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. J Assist Reprod Genet 2015;32:1167–70.
70. Jensen AK, Kristensen SG, Macklon KT, Jeppesen JV, Fedder J, Ernst E, et al. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. Hum Reprod 2015;30:2838–45.
71. Oktay K, Bedoschi G, Pacheco F, Turan V, Emirdar V. First pregnancies, live birth, and in vitro fertilization outcomes after transplantation of frozen-banked ovarian tissue with a human extracellular matrix scaffold using robot-assisted minimally invasive surgery. Am J Obstet Gynecol 2016; 214:94.e1–9.
72. Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, et al. First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. Hum Reprod 2013;28:2996–9.
73. Andersen CY. Success and challenges in fertility preservation after ovarian tissue grafting. Lancet 2015;385:1947–8.
74. Garcia A, Herrero MB, Holzer H, Tulandi T, Chan P. Assisted reproductive outcomes of male cancer survivors. J Cancer Surviv 2015;9:208–14.
75. Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. Endocr Rev 2015;36:1–24.
76. Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. Proc Natl Acad Sci U S A 2013;110:17474–9.
77. Novella-Maestre E, Herraiz S, Rodriguez-Iglesias B, Diaz-Garcia C, Pellicer A. Short-term PTEN inhibition improves in vitro activation of primordial follicles, preserves follicular viability, and restores AMH levels in cryopreserved ovarian tissue from cancer patients. PLoS ONE 2015;10: e0127786.
78. Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, et al. A review of 15 years of ovarian tissue bank activities. J Assist Reprod Genet 2013;30:305–14.
79. Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. Fertil Steril 2013;99:1514–22.
80. Ernst EH, Offersen BV, Andersen CY, Ernst E. Legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue due to cancer recurrence. J Assist Reprod Genet 2013;30:975–8.
81. Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: a review of the literature. J Assist Reprod Genet 2013;30:11–24.
82. Sorensen SD, Greve T, Wielenga VT, Wallace WH, Andersen CY. Safety considerations for transplanting cryopreserved ovarian tissue to restore fertility in female patients who have recovered from Ewing's sarcoma. Future Oncol 2014;10:277–83.
83. Yding Andersen C, Ernst E, Baerentzen S, Birkebaek NH, Clausen N. No malignancy detected in surplus ovarian tissue from a former Ewing sarcoma patient who experienced relapse four years after being grafted with frozen/thawed ovarian tissue. J Assist Reprod Genet 2014;31:1567–8.
84. Picton HM, Harris SE, Muruvi W, Chambers EL. The in vitro growth and maturation of follicles. Reproduction 2008;136:703–15.
85. Smitz J, Dolmans MM, Donnez J, Fortune JE, Hovatta O, Jewgenow K, et al. Current achievements and future research directions in ovarian tissue culture, in vitro follicle development and transplantation: implications for fertility preservation. Hum Reprod Update 2010;16:395–414.
86. Telfer EE, Zelinski MB. Ovarian follicle culture: advances and challenges for human and nonhuman primates. Fertil Steril 2013;99:1523–33.
87. Anderson RA, McLaughlin M, Wallace WH, Albertini DF, Telfer EE. The immature human ovary shows loss of abnormal follicles and increasing follicle developmental competence through childhood and adolescence. Hum Reprod 2014;29:97–106.
88. Barrett SL, Shea LD, Woodruff TK. Noninvasive index of cryorecovery and growth potential for human follicles in vitro. Biol Reprod 2010; 82:1180–9.
89. McLaughlin M, Telfer EE. Oocyte development in bovine primordial follicles is promoted by activin and FSH within a two-step serum-free culture system. Reproduction 2010;139:971–8.
90. Newton H, Picton H, Gosden RG. In vitro growth of oocyte-granulosa cell complexes isolated from cryopreserved ovine tissue. J Reprod Fertil 1999; 115:141–50.
91. Skory RM, Xu Y, Shea LD, Woodruff TK. Engineering the ovarian cycle using in vitro follicle culture. Hum Reprod 2015;30:1386–95.
92. Xiao S, Zhang J, Romero MM, Smith KN, Shea LD, Woodruff TK. In vitro follicle growth supports human oocyte meiotic maturation. Sci Rep 2015;5: 17323.
93. Xu M, Fazleabas AT, Shikanov A, Jackson E, Barrett SL, Hirshfeld-Cytron J, et al. In vitro oocyte maturation and preantral follicle culture from the luteal-phase baboon ovary produce mature oocytes. Biol Reprod 2011; 84:689–97.
94. Anckaert E, De Rycke M, Smitz J. Culture of oocytes and risk of imprinting defects. Hum Reprod Update 2013;19:52–66.
95. Luyckx V, Dolmans M-M, Vanacker J, Legat C, Fortuño Moya C, Donnez J, et al. A new step toward the artificial ovary: survival and proliferation of isolated murine follicles after autologous transplantation in a fibrin scaffold. Fertil Steril 2014;101:1149–56.
96. Vanacker J, Dolmans MM, Luyckx V, Donnez J, Amorim CA. First transplantation of isolated murine follicles in alginate. Regen Med 2014;9:609–19.
97. Soares M, Sahrari K, Chiti MC, Amorim CA, Ambroise J, Donnez J, et al. The best source of isolated stromal cells for the artificial ovary: medulla or cortex, cryopreserved or fresh? Hum Reprod 2015;30:1589–98.
98. Laronda MM, Jakus AE, Whelan KA, Wertheim JA, Shah RN, Woodruff TK. Initiation of puberty in mice following decellularized ovary transplant. Biomaterials 2015;50:20–9.
99. Kalich-Philosoph L, Roness H, Carmely A, Fishel-Bartal M, Ligumsky H, Paglin S, et al. Cyclophosphamide triggers follicle activation and 'burnout'; AS101 prevents follicle loss and preserves fertility. Sci Transl Med 2013;5: 185ra62.
100. Roness H, Kashi O, Meirow D. Prevention of chemotherapy-induced ovarian damage. Fertil Steril 2016;105:20–9.
101. Nickkholgh B, Mizrak SC, van Daalen SK, Korver CM, Sadri-Ardekani H, Repping S, et al. Genetic and epigenetic stability of human spermatogonial stem cells during long-term culture. Fertil Steril 2014;102:1700–7.e1.
102. Vassena R, Eguizabal C, Heindryckx B, Sermon K, Simon C, van Pelt AM, et al. Stem cells in reproductive medicine: ready for the patient? Hum Reprod 2015;30:2014–21.
103. Hayashi K, Saitou M. Stepwise differentiation from naïve state pluripotent stem cells to functional primordial germ cells through an epiblast-like state. Methods Mol Biol 2013;1074:175–83.
104. Zhou Q, Wang M, Yuan Y, Wang X, Fu R, Wan H, et al. Complete meiosis from embryonic stem cell-derived germ cells in vitro. Cell Stem Cell 2016; 18:330–40.