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

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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. (Fertil Steril® 2017;■:■–■. ©2017 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)

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

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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 Testicular damage (22) Gender reassignment procedures (23) Severe body trauma requiring surgical intervention	Klinefelter's syndrome (13)

Note: POI = premature ovarian insufficiency.

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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 electro-ejaculation. 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
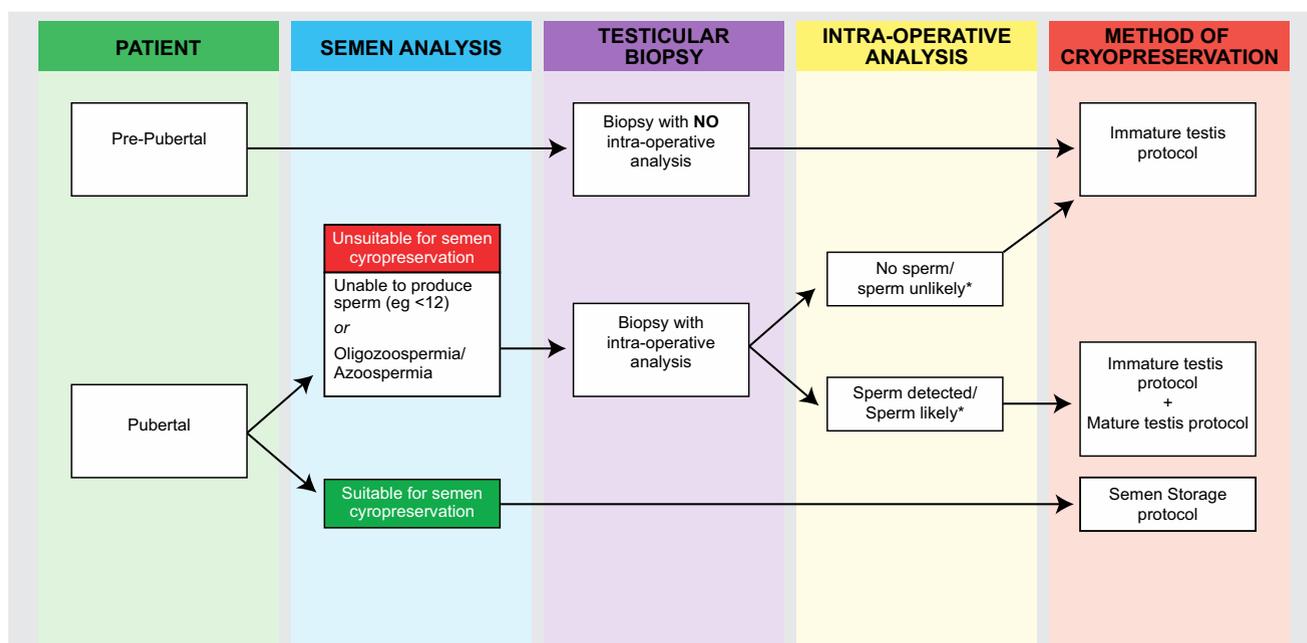
Algorithm for the cryopreservation of testicular tissue/sperm in pre-pubertal and adolescent patients at high risk of infertility. Clinical assessment for puberty should be carried out by a clinician with experience in pubertal assessment. It must be stressed that no clinical parameter can accurately 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).

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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 ≤ 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 ± 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.

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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 [phosphatidylinosito 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
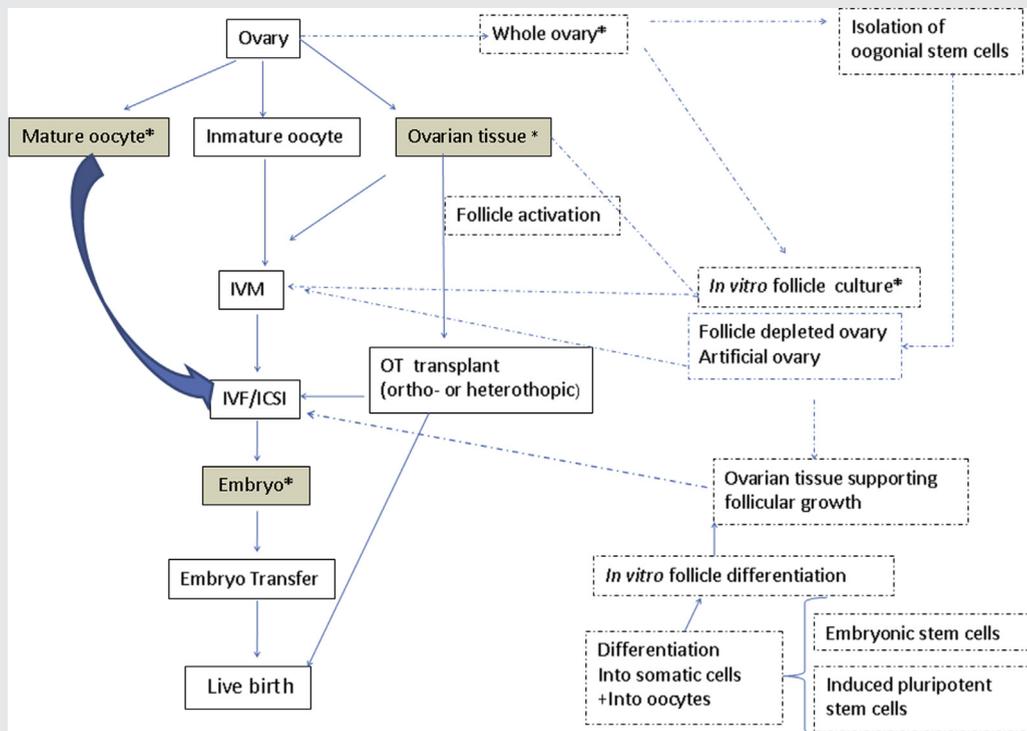
Fertility preservation techniques in women. Experimental procedures are indicated in discontinuous boxes, while established ones (i.e. those proven to restore fertility, with live births reported) are indicated in shaded boxes. Vitrified-thawed oocytes can be fertilized by IVF/ICSI for embryo transfer. 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.

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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.

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.

CONFLICT OF INTEREST
None.

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

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