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REVIEW

Medical techniques of fertility preservation in the male and female

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Summary Therapeutic advances in many medical fields have led to the need to consider patient quality of life after curative medico-surgical treatments for malignancy. Thus, it has become a major issue for young patients to preserve the ability to become “genetic” parents, with their own gametes.

While the preservation of male fertility has been an established technique for more than 30 years, it is only in the last decade that progress in cryopreservation techniques has allowed surgeons to offer successful oocyte and ovarian tissue cryobanking. However, in addition to the still experimental nature of some fertility preservation techniques, this practice also raises many ethical and moral questions.

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The progress made in recent years in the treatment of cancers in young patients has allowed a marked increase in cure rates and the life expectancy of men and women who present with malignant disease. However, the oncologic treatments employed often have gonadotoxicity that will negatively impact the survivors’ fertility. Recent advances in cryopreservation now make it possible to consider fertility preservation (FP) techniques for both men and women. These aim to offer the best possible quality of life following treatment for cancer, in particular by giving patients the

maximum chance of achieving “genetic” parenthood with their own gametes.

FP is governed by the Bioethics Law of 2004. Article L. 2141-11 of the Public Health Code, amended by Law 2011-814 of July 7, 2011. This provides that “any person may benefit from the collection and the preservation of their gametes or germinal tissue, with a view to subsequent provision of assisted reproductive technology (ART), or for the preservation and restoration of their fertility, when care is likely to impair fertility, or when fertility is likely to be prematurely altered:-

Thus, all patients who are scheduled to undergo treatment or surgery that may entail a risk of infertility, should be referred to a specialized center to receive information on the risks of treatment-related gonadotoxicity, on the possibilities of FP, and on the possibilities of employing FP techniques. For both men and women, these steps

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are carried out in the CECOS (Centers for the Study and Conservation of ova and spermatazoa).

Male fertility preservation

Male FP has been practiced for more than three decades, thanks to the efficiency of sperm freezing. Advances in ART, including *In Vitro* Fertilization (IVF), particularly using Intracytoplasmic Sperm Injection (ICSI), have enabled the use of frozen spermatozoa even when *in vivo* spermatogenic parameters are highly impaired.

Spermatogenesis takes place in the testicles in the seminiferous tubules and allows the formation of spermatozoa from germinal stem cells. This process begins at puberty, continues throughout life, and depends on hormonal production and a specific intratesticular microenvironment.

Indications of male fertility preservation

Indications:

- Medical treatment that may temporarily or permanently alter spermatogenesis (chemotherapy, radiotherapy);
- Surgery that can alter normal ejaculation (prostatic surgery, urethral surgery, proctectomy, some lymph node dissections);
- Before vasectomy;
- In the course of IVF, when the availability of frozen spermatozoa optimizes the management and increases the chances of success.

Male fertility preservation in adult and pubertal adolescent males

Male FP relies on the freezing of ejaculated spermatozoa, collected after masturbation at the laboratory. Compliance with three to five days of ejaculatory abstinence is recommended, but not essential, and should not delay FP, especially in urgent indications of gonadotoxic treatment. Sperm freezing must be done before the initiation of any treatment to avoid genetic alterations and a decrease in both quantity, and quality of spermatozoa.

The semen parameters are analyzed on a sample of ejaculate according to the criteria of the World Health Organization (WHO) [1]. Sperm is diluted in a cryoprotective medium and then conditioned in vials that will be frozen and stored in liquid nitrogen at -196°C . Ideally, two or three sperm collections are proposed in order to accumulate a sufficient number of vials (15–20 on average). It has been well established that frozen sperm can be preserved for more than 20 years without impairing the sperm fertilization potential [2].

The freezing of spermatozoa is possible with good results from the onset of puberty, *i.e.*, from 11 to 14 years depending on the degree of psychosexual maturation [3]. Sperm production remains continuous throughout life with no theoretical upper age limit. Nevertheless, this raises a certain number of ethical problems, since recent data have revealed particular psychological consequences in children conceived by men over the age of 60 [4,5], and this situation may lead to the birth of children who will become prematurely orphaned by the death of their father.

In certain situations, the freezing of ejaculated spermatozoa can be difficult or impossible. Collection failures occur in about 5% of cases, favored by poor general health and/or stress [6]. If time permits, another effort at sperm

collection is proposed before the start of treatment. Spermatic parameters can be extremely poor in some patients with severe oligoasthenoteratozoospermia or azoospermia, making any attempt of sperm autopservation useless. These situations are often due to the primary pathology requiring FP. In patients with retrograde ejaculation refractory to medical treatment, spermatozoa can be recovered from the previously alkalized urine [7] and then frozen. In case of neurological erectile disorders, some teams propose the use of vibratory penile stimulation. Endorectal electrostimulation under general anesthesia or spinal anesthesia is another more invasive possibility, reserved for certain specialized centers; its results are not very convincing in terms of the quality of sperm obtained [8]. As a last resort, when collection is impossible or when the patient has azoospermia, surgical harvesting of a sample of epididymal or testicular spermatozoa may be considered for freezing [9].

Preserving fertility in the prepubertal boy

In the particular case of the prepubertal child, who does not yet have spermatozoa, but whose seminiferous tubules contain spermatogonia stem cells, it is possible to consider freezing testicular tissue [10,11]. Although still experimental, several promising strategies for reusing these frozen cells have been considered, *including in vitro* maturation or transplantation.

Female fertility preservation

Female FP is an area of recent medical innovation, which owes its rapid growth to the emergence of new oocyte collection techniques, as well as to the improvement of techniques of egg cryopreservation. Initially developed to preserve the subsequent fertility of women with cancer who would receive gonadotoxic treatment such as chemotherapy, pelvic radiotherapy, or ovarian surgery, the indications have more recently been extended to all medical or surgical situations that may lead to premature decrease of the ovarian reserve. Various techniques have therefore been developed to try to respond to a possible desire for subsequent "genetic" maternity for these patients. The choice of technique must take into account the patient age, pubertal status, ovarian reserve, the underlying pathology, the possible gonadotoxicity of the treatments, and the amount of time available before starting treatment.

Oocyte or embryo freezing

Embryo cryopreservation has been feasible for 30 years and is routinely used in IVF centers. This technique has long been the only one considered as non-experimental for female FP. Since January 2013, oocyte freezing can also be proposed as a technique of choice [12], because of steadily improving results [13]. The vitrification technique has recently supplanted slow freezing for both embryos and oocytes. It consists of a very rapid lowering of temperature thanks to the use of cryoprotective agents at high concentrations, thus avoiding the formation of ice crystals. This makes it possible to obtain excellent survival rates during thawing [14]. Outside the context of oncofertility, and when the oocyte freezing was carried out before the age of 38, the vitrification of eight oocytes would lead to a 46% chance of initiating a pregnancy [15] and cryopreservation of between 15 and

20 oocytes would allow a 70–80% chance of achieving a live birth [16]. At present, no obstetrical or perinatal excess risk has been demonstrated after the use of oocyte vitrification [17].

While oocyte freezing is now proven effective, the frozen embryo technique is widely available and well mastered in all IVF centers, and long-term data are generally better known. The question of whether to freeze embryos or oocytes can sometimes arise for a woman who is already in a relationship. It must be emphasized that embryonic cryopreservation actually involves preserving the fertility of both parents, and that the thawing of embryos can only be considered with the joint consent of both parents. Oocyte cryopreservation has the immense advantage of guaranteeing women's reproductive autonomy, allowing for flexibility of choice during their re-use, and should therefore be preferred.

There are two methods of oocyte collection: after ovarian hormonal stimulation, or without hormonal administration with planned *in vitro* maturation. These two techniques are generally only offered to women less than 40 years of age because of the poor results expected beyond this age.

Recovery of mature oocytes after ovarian hormonal stimulation

Hormonal ovarian stimulation allows the production of mature oocytes. The modalities of this stimulation are generally identical to those used for infertile couples. The aim is to bring several antral follicles (about 5 mm in diameter) to the pre-ovulatory stage (16–22 mm in diameter). Thus, FSH (follicle stimulating hormone) activity is combined with blocking of ovulation by GnRH (gonadotropin releasing hormone) antagonists to enlarge and mature antral follicles while preventing premature ovulation. When a sufficient number of follicles have reached the pre-ovulatory stage, LH (luteinizing hormone) is administered in order to induce the

last stages of follicular and oocyte maturation. The use of a GnRH agonist for triggering is recommended in FP in order to reduce the risk of ovarian hyperstimulation syndrome [18]. Oocyte retrieval is performed by ultrasound-guided transvaginal puncture, under local or general anesthesia, 36 h after the onset of ovulation. Oocytes, matured *in vivo* by the administration of exogenous FSH, are collected and then vitrified and/or fertilized for embryonic freezing. Ovarian stimulation should be strong enough to obtain a maximum of mature oocytes while avoiding complications that may delay the initiation of cancer therapy, especially ovarian hyperstimulation syndrome.

The optimal dose of gonadotropins is chosen according to markers of ovarian follicular status: AMH (Anti-Müllerian Hormone) and ultrasound assessment of antral follicle count (AFC), while taking into account a possible risk of poor response due to the primary pathology indicating FP [19]. Conventionally, ovarian stimulation is administered for 12–15 days and is initiated in the early follicular phase (2nd–3rd day of the cycle) (Fig. 1). However, it has been shown that stimulation can also be initiated outside the follicular phase (Fig. 2). Indeed, several waves of follicular recruitment take place during a menstrual cycle with the regular appearance of new FSH-sensitive follicles [20]. The number of oocytes recovered is similar for stimulations initiated in the follicular or luteal phase [21], but a change in the duration of stimulation can be observed [22]. The oocyte quality is also not negatively modified by these “random start protocols” insofar as the oocyte is devoid of progesterone receptors. For pathologies that are hormone-dependent, particularly for breast cancer, special protocols have also been developed to control stimulation-induced supraphysiological levels of estradiol. Letrozole, an aromatase inhibitor, when used in parallel with FSH, allows a significant decrease in serum estradiol levels during ovarian stimulation, with an equivalent number of oocytes harvested compared to the standard protocol [23]. In patients with breast cancer, pregnancy rates are equivalent to those

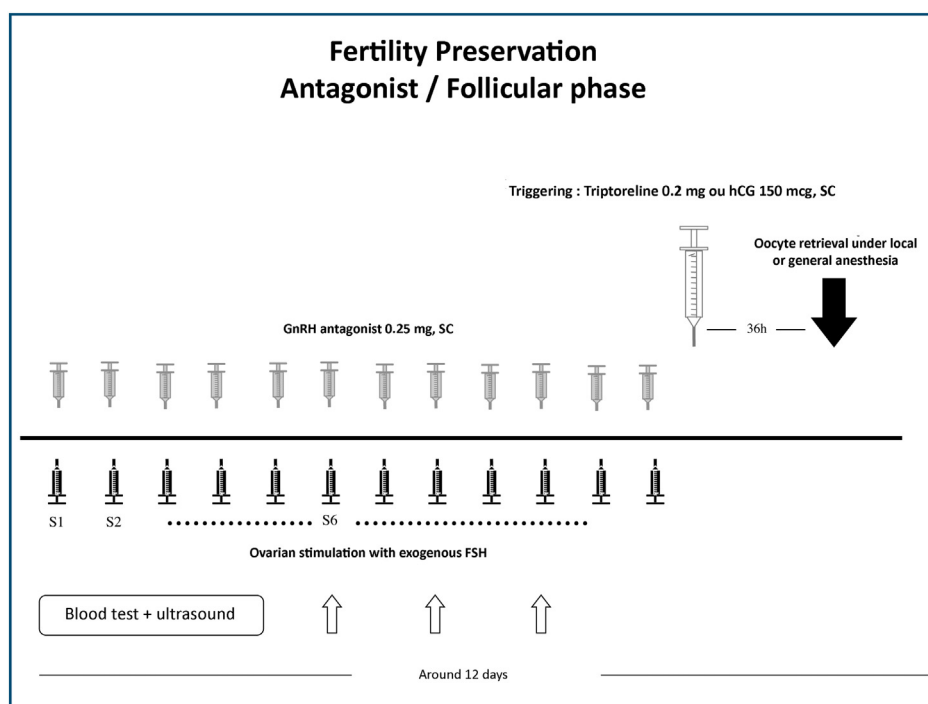


Figure 1. Schema of ovarian stimulation in the early follicular phase.

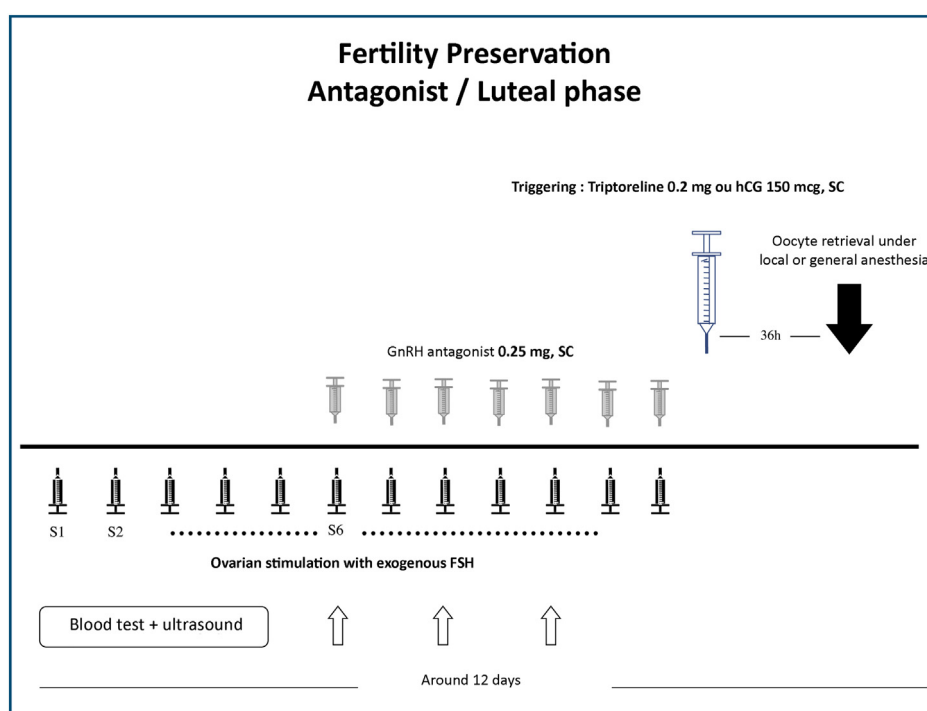


Figure 2. Schema for ovarian stimulation in the luteal phase.

reported in infertile women who have undergone ovarian stimulation without aromatase inhibition [24]. Breast cancer recurrence rates were no higher in women who used these ovarian stimulation protocols for FP [25]. In patients with decreased ovarian reserve, ovarian stimulation, even at high doses, will not allow multiple mature oocytes to be harvested. Oocyte retrieval of the dominant follicle in a so-called “semi-natural cycle” can then be proposed to them [26]. This protocol makes it possible to obtain one or even two mature oocytes(s) per cycle. While it is not very demanding, it has the advantage that it can be repeated over time, thus integrating into an oocyte accumulation strategy. This protocol is not indicated in an emergency context because of the too small number of oocytes that can be collected on a single cycle.

Oocyte harvesting for *in vitro* maturation (IVM)

Embryo or oocyte vitrification is currently possible from oocytes retrieved at an immature stage and then matured *in vitro*. *In vitro* maturation (IVM) consists in collecting cumulo-oocyte complexes via ultrasound-guided transvaginal puncture of small antral follicles. The oocytes, at germinative vesicle stage, are matured in a medium containing FSH, LH and albumin. After 24–48 h, the matured oocytes (in metaphase of the second *in vitro* meiotic division) are then vitrified or fertilized.

Initially developed for patients with polycystic ovary syndrome who are at increased risk of ovarian hyperstimulation syndrome, IVM has seen its indications expand for use in patients with mutations of the FSH receptor and finally to patients whose fertility is threatened by oncologic treatment [27].

This technique has several advantages:

- It can be performed urgently without prior treatment, whatever the phase of the menstrual cycle without

impacting the number of oocytes that can be collected [28];

- It does not require prior ovarian stimulation and therefore does not induce supra-physiological elevation of serum estradiol levels.

The average number of mature oocytes obtained after IVM varies between 6 and 12 [29], depending on the initial AFC. These numbers are similar to the number of vitrified oocytes obtained after ovarian stimulation with a protocol using aromatase inhibitors [25]. Puncture can be tricky because of the small size of the follicles to be harvested (between 2 and 10 mm), the mobile nature of an unstimulated ovary, and the proximity of the pelvic vessels (Fig. 3). In addition, the number of oocytes retrieved is sometimes low compared to the initial AFC. Between 50 and 65% of oocytes reach a mature stage [30]. However, the potential of IVM oocytes is likely to be lower than that of gametes collected after exogenous gonadotropin stimulation [31]. Although the data on the approximately 5000 children born with the help of the IVM technique are reassuring [32], this technique is still considered experimental by the American Society for Reproductive Medicine [33].

Cryoconservation of ovarian tissue

The objective of ovarian tissue cryopreservation is to preserve the primordial follicles that reside in the ovarian cortex. Under general anesthesia, an entire ovary or a flap of ovarian cortex is removed by laparoscopy. In the laboratory, the ovarian cortex is isolated from the medulla and then frozen as fragments of about 1 cm² (Fig. 4) by slow freezing to retain a large number of follicles. When the time comes for re-use, the fragments are thawed and then grafted avascularly in an orthotopic site (ovarian fossa, peritoneal cavity, or remaining ovary) or, less commonly, in a heterotopic site (forearm, abdominal wall). Only the primordial and primary follicles are resistant to freeze-thaw processes, but signif-

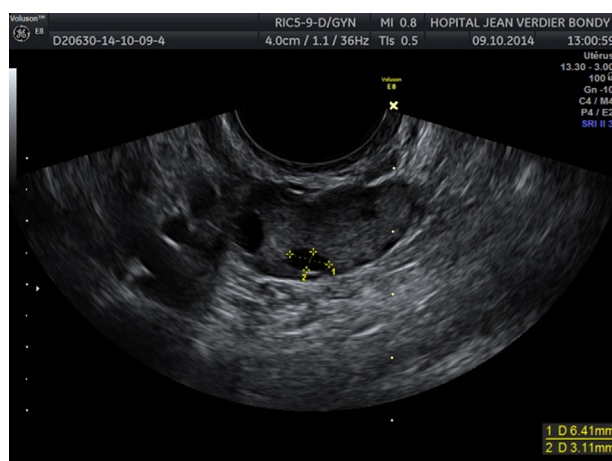


Figure 3. Needle aspiration of oocytes for *in vitro* maturation through an echo-guided transvaginal approach.

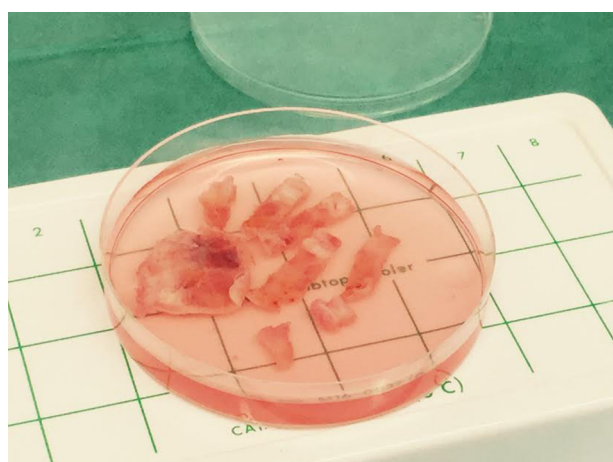


Figure 4. Fragments of ovarian cortex before freezing.

icant follicular loss may nevertheless occur during these stages, as well as during the transplant itself, due to ovarian tissue hypoxia related to delay in neovascularization [34].

The first live birth after frozen ovarian tissue transplant was reported in 2004 in a patient who had undergone multidrug chemotherapy for stage IV Hodgkin lymphoma [35]. In 2012, the same team published the first live birth after frozen ovarian tissue graft in a patient with bilateral salpingo-oophorectomy, leaving no doubt as to the efficacy of this method [36].

This technique also permits a return of endocrine ovarian function, albeit temporary, not exceeding a few months or years [37]. It is also of interest for pre-pubertal girls [38]. The first successful induction of puberty after transplantation of cryopreserved ovarian tissue in childhood was reported in 2012 [39]. In 2015, the first natural childbirth after orthotopic transplantation of ovarian tissue harvested in childhood was obtained [40].

Like IVM, ovarian tissue harvesting and freezing can be performed without delay in urgent situations, especially when there is urgent need to start a highly gonadotoxic chemotherapy. It may be associated with IVM performed at the same intervention, regardless of whether the patients is pubertal or not [41].

This technique is not recommended for patients older than 37 because of the uncertainty of its effectiveness, in relation to physiologically impaired ovarian reserve and the high percentage of follicular loss during transplantation

[42]. The majority of the teams harvest a single ovary, in order to leave the possibility of a natural pregnancy thanks to the remaining ovary if the gonadotoxic treatments have not been totally sterilizing.

However, for certain types of cancer, the re-use of ovarian fragments is not recommended, or is even contraindicated, because of a risk of implantation of malignant cells in the ovary, with the theoretical risk of reintroducing the initial pathology during the transplant [43]. However, alternatives to transplantation, such as *in vitro* folliculogenesis or artificial ovaries, are being developed to optimize the safe re-use of follicles contained in the ovarian fragments removed.

While the grafting of frozen ovarian tissue fragments is being performed more and more frequently [44], this technique is still considered experimental. At present, 86 births have been obtained worldwide after thawing and orthotopic grafting, with or without ART [45].

GnRH agonists

Administration of GnRH agonists with the aim of preserving fertility, while the patient is undergoing gonadotoxic therapy, is very controversial. Indeed, the various published studies present contradictory results on the beneficial role of GnRH agonists on post-chemotherapy ovarian function [46–48].

An analysis of the literature is difficult and there are very few data concerning pregnancies after cancer in relation to whether the patient was treated with a GnRH agonist or not. A recent meta-analysis concluded that it is likely to be ineffective [49].

Ovarian transposition

Ovarian transposition is proposed for patients who will undergo abdominal or pelvic radiotherapy and whose ovaries lie in the proposed irradiation field. Laparoscopic ovarian transposition aims to displace the ovaries from the irradiation areas, thereby protecting them from the gonadotoxic effects of radiation. The blood vessels that supply the ovaries are displaced in such a way as to help maintain the viability and endocrine activity of the ovaries. Since the normal anatomic relationship between the ovaries, the fallopian tubes and the uterus no longer exists, natural pregnancies after this intervention are relatively rare, especially since ovarian function is often impaired by decreased vascularity and some degree of radiation damage. Ovarian transposition remains primarily a technique for preserving ovarian endocrine function more than as a method to preserve fertility. It should therefore be combined with other alternatives such as cryopreservation of ovarian cortex or oocytes. Finally, one should bear in mind that pelvic radiation also alters uterine function that may interfere with normal obstetric prognosis, even when gametes have been harvested in anticipation of FP [50].

Conclusion

Fertility preservation has become fundamental in the multidisciplinary management of young patients whose surgical or chemotherapeutic treatment could alter their reproductive function. While the techniques of FP have been relatively well defined in males, FP techniques in women have only recently emerged due to the complexity of

folliculogenesis, its low numerical yield, as well as the accessibility to the ovaries to invasive techniques. The technique of ovarian stimulation followed by egg cryopreservation should always be privileged.

Key points:

- Any patient receiving a potentially gonadotoxic treatment should be referred to a center for oncofertility consultation.
- The preservation of male fertility is essentially based on the cryopreservation of ejaculated spermatozoa.
- In the preservation of female fertility, ovarian stimulation is the reference technique, to be preferred whenever possible.
- Vitrification is an effective and validated technique.
- The freezing of ovarian fragments and *in vitro* maturation of oocytes are still considered experimental techniques.

Disclosure of interest

The authors declare that they have no competing interest.

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