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Fertility preservation in women for medical and social reasons: Oocytes vs ovarian tissue

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A B S T R A C T

Approximately 10% of cancers occur in women under 45 years of age. Chemotherapy, radiotherapy, and bone marrow transplantation cure more than 90% of cancer in women, but can result in premature ovarian insufficiency depending on follicular reserve, age, and drugs used. Some benign diseases are also indications for fertility preservation, particularly those requiring chemotherapy (like thalassemia and lupus), recurrent endometriosis, and family history of premature menopause. Social reasons also account for a large proportion of women who wish to postpone pregnancy. This article discusses the two main strategies for fertility preservation, namely oocyte vitrification and ovarian tissue cryopreservation, examining the indications and results of these options. Oocyte cryopreservation is an effective approach, but further studies are needed in cancer patients to ensure the excellent outcomes obtained in women without cancer or in egg donation programs. For prepubertal girls or cases where immediate therapy is required, cryopreservation of ovarian tissue is the only available option.

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Introduction

Indications for different fertility preservation techniques and their outcomes are reviewed in this chapter.

Oocyte vitrification has become the standard approach to preserve fertility in women with benign diseases, those seeking fertility preservation for personal reasons (also called age-related infertility), and women with cancer if treatment can be safely postponed [1,2].

Ovarian tissue cryopreservation is specifically indicated for young girls and women who require immediate cancer treatment [1,3–8].

Fresh tissue transplantation in women with premature ovarian insufficiency (POI) will also be discussed, allowing us to define characteristic differences between fresh and frozen-thawed ovarian tissue reimplantation.

Indications for fertility preservation (Table 1)

a) Malignant diseases

Fertility preservation remains a challenge, particularly in case of breast cancer and hematological malignancies (Hodgkin's lymphoma, non-Hodgkin's lymphoma, and leukemia), which constitute the most frequent indications for fertility preservation [1,4]. Chemotherapy (particularly with cytotoxic alkylating agents), radiotherapy, surgery, or a combination of these treatments can induce POI [1,4,6–10], as the ovaries are very sensitive both to cytotoxic drugs and radiation exposure of 5–10 Gy in the pelvic area [11,12].

The likelihood that POI will develop after therapy is related to the ovarian reserve, which can vary enormously from one individual to the next [6,7]. For this reason, giving a patient or her parents an accurate estimate of the risk of infertility is very difficult, as how a disease will develop cannot be predicted [1].

b) Benign diseases

Benign conditions like autoimmune and hematological diseases sometimes require chemotherapy, radiotherapy, or both, and even bone marrow transplantation in some cases (Table 1), and therefore carry a risk of POI. The presence of bilateral ovarian tumors, or severe or recurrent ovarian endometriosis [13,14], and recurrent ovarian torsion may also impair future fertility. Ovarian endometriomas

Table 1
Indications for fertility preservation.

A) **Malignant diseases** most frequently requiring gonadotoxic chemotherapy and/or radiotherapy or bone marrow transplatation:
- Hematological diseases (leukemia, Hodgkin's lymphoma, and non-Hodgkin's lymphoma)
- Breast cancer
- Sarcoma
- Some pelvic cancers

B) **Benign conditions** for which fertility preservation is indicated:
1. Nononcological systemic diseases requiring chemotherapy, radiotherapy, andor bone marrow transplantation
2. Nonmalignant ovarian diseases:
   - Bilateral ovarian tumors
   - Severe and recurrent ovarian endometriosis
   - Risk of ovarian torsion
3. Risk of premature ovarian insufficiency:
   - Family history
   - Turner syndrome

C) **Social reasons:**
- Age
- Childbearing postponed to later in life

induce local intraovarian inflammation and diminish the ovarian reserve [15] by triggering follicle "burnout," characterized by activated follicle recruitment with subsequent atresia [16]. Moreover, there is increasing evidence that performing cystectomy on endometriomas causes considerable damage to the ovarian reserve [17–20], so fertility preservation should certainly be contemplated in case of recurrence after surgery and in certain conditions like low anti-Müllerian hormone (AMH) levels and age >35 years [21]. Turner syndrome and family history of POI are additional indications for fertility preservation (Table 1) [22,23].

c) Age-related fertility decline

Women are now attempting their first pregnancy later and later in life. They may wish to postpone childbearing for a variety of personal reasons, because of the lack of a stable partner, career choices, or financial issues [24–26].

Embryo and oocyte cryopreservation (Fig. 1)

Embryo cryopreservation is an effective technique, but requires a male partner, which opens the door to all manner of ethical and legal concerns about the fate of orphan embryos if the patient dies or she and her partner separate. On the other hand, cryopreservation of mature oocytes (Fig. 1) preserves a woman's ability to procreate with a chosen partner in the future [27].

Data from a review [26] suggest that the strategy of oocyte vitrification and warming is superior to slow-freezing in terms of clinical outcomes. Laboratories that continue to use slow-freezing should consider transitioning to vitrification techniques for purposes of cryopreservation [26]. Indeed, when fertility preservation is carried out for benign indications or personal reasons, mature oocyte pick-up and vitrification is clearly the highest-yield strategy [25,26] and gives women reproductive autonomy [27]. For women of advanced childbearing age, this technique may be used to extend their fertility potential in view of the known decline in oocyte quality with age [24,25]. Because of increasing interest in fertility preservation, reproductive medicine providers should be aware of success rates and limiting factors of oocyte vitrification to provide patients with accurate information.

a) Oncological indications

There are five key points to bear in mind when fertility preservation by embryo or oocyte cryopreservation is contemplated in women with cancer. First, to allow time for controlled ovarian stimulation (COS), chemotherapy needs to be delayed by at least 10 days, even if random-start protocols are used [3,25,28]. Second, the patient must be postpubertal, as stimulation in the prepubertal period is not very effective due to the absence of response to gonadotropins [3,4,6,7]. Third, specific COS protocols are required depending on the steroid sensitivity of the specific cancer. Fourth, information on oocyte quality in women with cancer is lacking or at least contentious [2,24,29]. Finally, the excellent results obtained in egg donation programs cannot be extrapolated to women who have been treated for cancer [25,27,30,31].

In a first study, Cobo et al. [25] reported outcomes of 120 women who had undergone fertility preservation by means of oocyte vitrification. Among those who were 35 years of age or younger at the time of vitrification, the cumulative live birth rate (CLBR) was 60.5% when 10 oocytes were used (Fig. 2). Among women who were over 35 years of age at the time of the procedure, the CLBR was 29.7% with 10 oocytes, half the rate obtained in the younger group.

In a more recent study [2], Cobo et al. reported the largest series to date, with more than 6000 women and over 8000 fertility preservation cycles, 700 of whom returned to attempt pregnancy. This study allowed the authors to determine the possible impact of underlying malignant disease by comparing results achieved in cancer patients with women in the elective fertility preservation (EFP) group. As in their first study, they evaluated the CLBR according to age at the time of vitrification. In women $\leq$35 years of age, the CLBR per patient was 68.8% and 42.1% in the EFP and the cancer groups, respectively, suggesting that the underlying disease in cancer patients may well impair reproductive

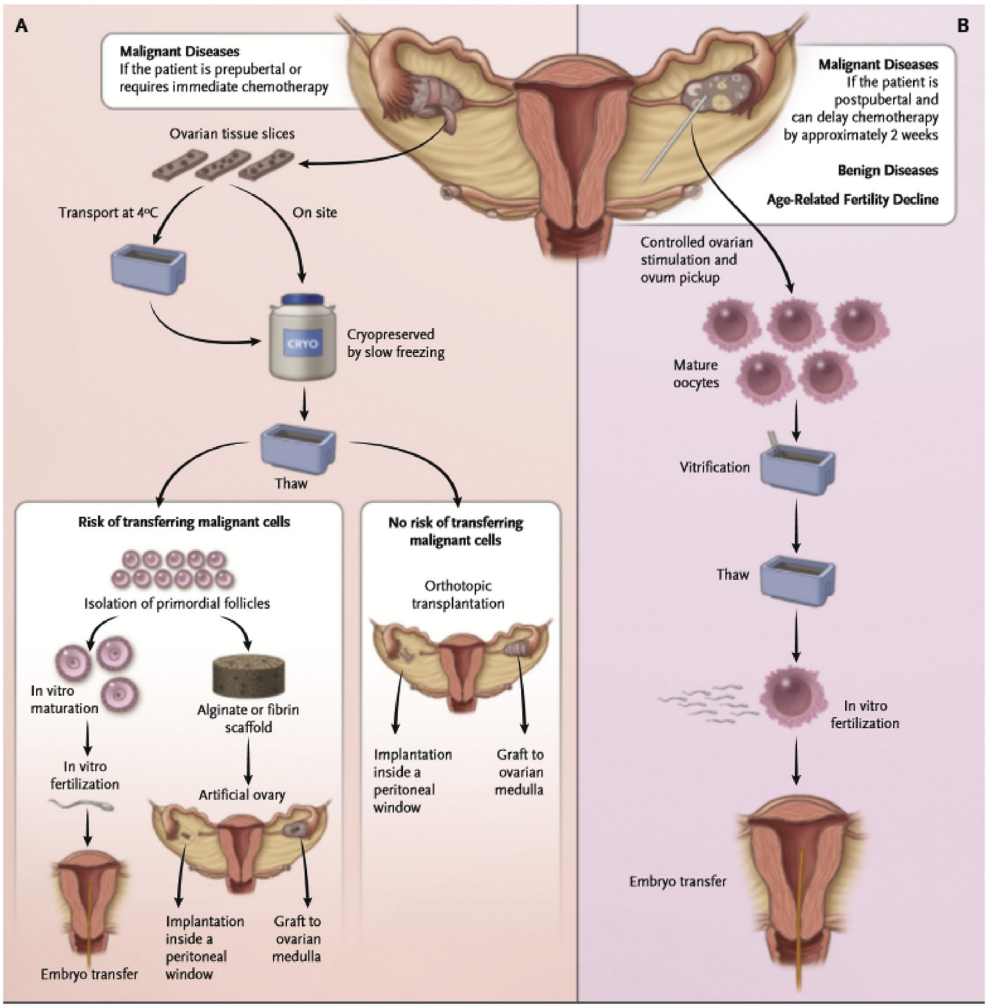

Fig. 1. 1) If the patient is prepubertal or requires immediate chemotherapy. Ovarian tissue is removed in the form of multiple biopsies (or an entire organ) and cut into cortical strips. The tissue is then cryopreserved by slow-freezing on site (or transported to a processing site at a temperature of 4°).

After thawings

• If there is no risk of transmitting malignant cells, the ovarian tissue can be grafted to the ovarian medulla (in the presence of at least one ovary) or reimplanted inside a specially created peritoneal window.

• If there is a risk of transmitting malignant cells, ovarian follicles can be isolated and in vitro-grown to obtain mature oocytes, which can then be fertilized and transferred to the uterine cavity. Isolated follicles may be placed inside a scaffold (alginate or fibrin), creating an "artificial ovary" that can be grafted to the ovarian medulla or peritoneal window.

2) If the patient is postpubertal and can delay chemotherapy by approximately 2 weeks. Mature oocytes are removed after ovarian stimulation and vitrified on site. After thawing, they are inseminated and transferred to the uterine cavity in the form of embryos.

3) The combined technique can also be applied, involving ovarian tissue cryopreservation followed by controlled ovarian stimulation and the vitrification of oocytes. This combined technique theoretically yields a 50%–60% chance of obtaining a live birth.

From Donnez J, Dolmans MM. N Engl J Med 2017; 377:1657–1665.

outcomes. However, other reasons like the use of letrozole in the simulation protocol could not be excluded. The COS protocol itself may also interfere with the number of MII oocytes obtained at pickup for vitrification, and it appears that fewer mature oocytes are retrieved when letrozole is used [32,33].

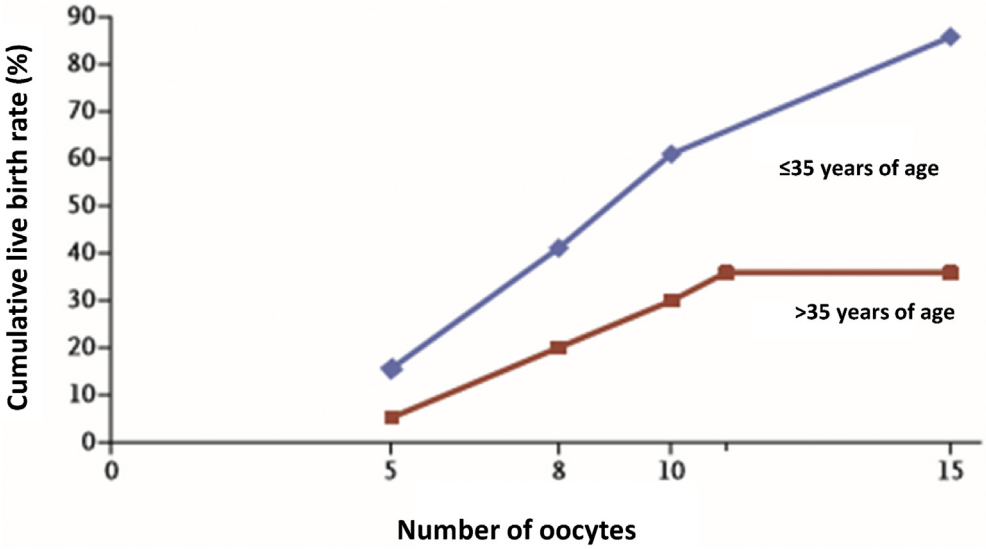

Fig. 2. Cumulative live birth rates with 5–15 vitrified oocytes according to age in an egg donation program (adapted from Cobo et al. [25]).

Moreover, in the cancer group, there are fewer oocytes because there is a limit of IVF attempts. Women undergoing EFP, on the other hand, can repeat attempts with more oocytes.

We have stressed the importance of doctors providing patients with center-specific information about their experience with fertility preservation. Only programs achieving the highest pregnancy rates publish their outcome data, but these results cannot be generalized and extrapolated to centers with less experience in counseling candidates for oocyte cryopreservation.

b) Endometriosis and fertility preservation: the specific issue of endometriosis

Since publication of two papers by Kitajima et al. [15,16], it is clear that endometriosis is one condition that reduces the ovarian reserve, particularly when endometriomas are present. Recent reports by Goodman et al. [34] and Muzii et al. [18] clearly show that AMH is decreased in women with endometriomas, even before surgery. There is no doubt that oxidative stress, iron, and reactive oxygen species also play a role in this decline [35]. Women diagnosed with ovarian endometriosis should be considered potential candidates for fertility preservation [36]. However, as advocated by our group, the first step is protecting the ovarian reserve by competent conservative surgery performed by expert surgeons [37].

A recent conflict of views [37,38] addressed the pros and cons of IVF vs surgery (Fig. 3). It is clear that endometrioma surgery carried out in good conditions yields high pregnancy rates (more than 50%) during the first year postsurgery [39], but the decision to proceed is not an easy one. The scenario is multifaceted and patients may be overwhelmed by the burden of contrasting information [38]. Moreover, physicians may be tempted to guide the decision based on their own values and competences. There is growing evidence that primary emphasis should be placed on giving patients the freedom to choose. Turning to IVF or surgery first should not be the doctor's decision but, wherever possible, the choice of a properly informed patient [37,38]. Nevertheless, as stressed by Velasco Garcia (see in Lessey et al., [38]), the experience of the surgeon is one of the key drivers of success and low rate of complications. In our opinion, fertility preservation options in the case of endometriosis should be seriously discussed in certain conditions, as explained in Fig. 3.

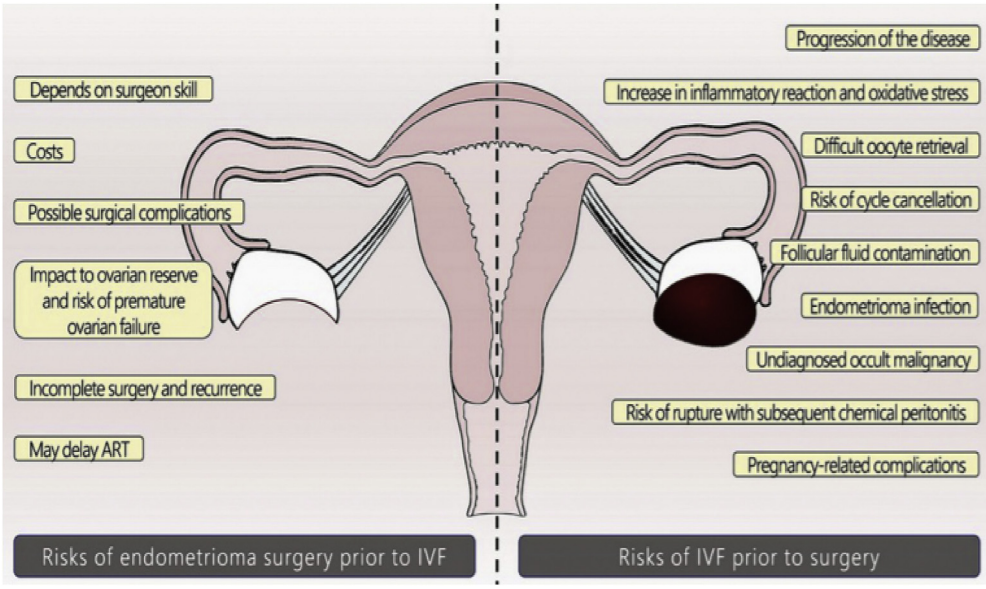

Fig. 3. Endometrioma-related infertility: risks of surgery before IVF versus risks of IVF before surgery.

In a very recent paper, Cobo et al. [36] propose fertility preservation in women with endometriosis as a valid treatment option to help them increase their reproductive chances, and suggest performing surgery after COS and oocyte vitrification in young women. In their large study, they report the results of 485 endometriosis patients who had their oocytes vitrified at a mean age of 35.7 years, and compare these data with the so-called historical control group of EFP patients.

In endometriosis patients, oocyte survival, implantation and pregnancy rates as well as the CLBR were statistically significantly lower (61.9%) than in the EFP group (68.8%) in women less than 35 years at the time of vitrification. One possible explanation is that the quality of oocytes is compromised in women with endometriosis. In the group of women with endometriosis, the CLBR was 28.4% in women aged >35 years. As in all other studies, the age factor is crucial.

The study by Cobo et al. [36] is important because their findings provide key information for counseling purposes.

In conclusion, there are a number of options for the management of endometriosis-related infertility (surgery vs IVF), but fertility preservation should be offered to women with endometriosis, at least those with recurrent disease. We propose an algorithm for fertility preservation in women with endometriosis focusing on the strict indications (Fig. 4): low AMH, age >30 years, bilateral endometrioma, recurrent endometrioma after surgery, endometrioma growing fast, and endometrioma at a young age.

Ovarian tissue cryopreservation (Fig. 1)

In prepubertal girls and women who cannot delay the start of chemotherapy, cryopreservation of ovarian tissue (Fig. 1) is the only option for fertility preservation [1,3]. However, strict selection criteria need to be applied [40].

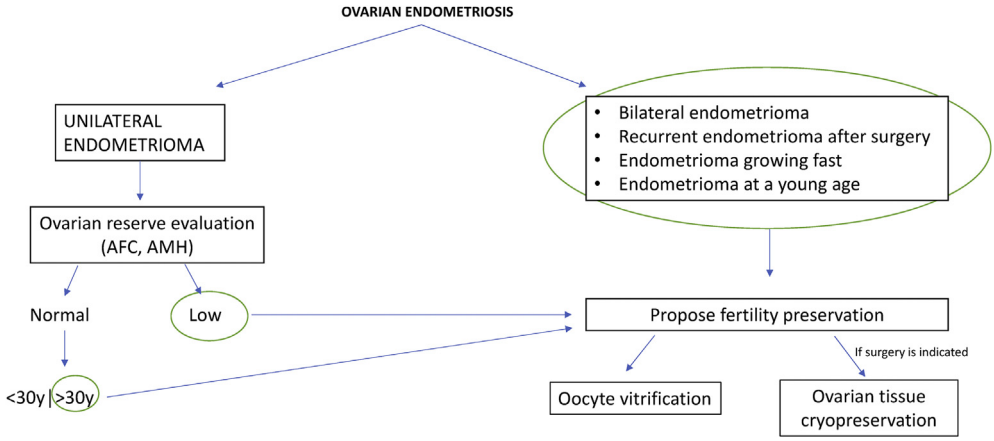

Fig. 4. Algorithm for fertility preservation in women with ovarian endometriosis. The encircled (green) items are indications for fertility preservation in patients with endometrioma: low AMH, age >30 years, bilateral endometrioma, recurrent endometrioma after surgery, endometrioma growing fast, and endometrioma at a young age.

Need for selection criteria

Gonadotoxicity is age-dependent. It is known that first-line cancer treatment does not usually compromise the ovarian reserve by more than 10% in girls under 10 years of age, while those aged 11–12 years show an estimated 30% decline in their ovarian reserve [7,40,41]. There is also a marked association between the intensity of treatment received and the likelihood of POI, even in young girls, but it is impossible to predict exactly who will be affected after aggressive chemotherapy. Alkylating agents are the most toxic. In a review [9], the North American Children's Oncology Group considered the risk of POI to be highest with busulfan administered at a dose of at least 600 mg/m^2 of body surface area, cyclophosphamide at a dose of at least 7.5 g/m^2, and ifosfamide at a dose of at least 60 g/m^2. As we [1,3] and others [40] have stressed, selection criteria are clearly needed, the most important being age <35 years (when the ovarian reserve is still relatively high), a realistic chance of surviving for 5 years, and at least a 50% risk of POI.

Biopsy and cryopreservation

Obtaining multiple biopsy samples from one ovary has not been shown to compromise future hormone production [1], while removal of a single ovary may shorten the time to menopause by 1–2 years [42,43]. The slow-freezing procedure has been widely applied in a clinical setting since 1996 [44,45]. The great majority of centers still favor the slow-freezing technique because more than 95% of live births have been achieved after reimplantation of frozen-thawed ovarian fragments [44–57]. There is also evidence that vitrification of ovarian tissue is not superior to slow-freezing, as some claim, since vitrification has only resulted in two live births to date [58], reported by the team of Suzuki. Moreover, recent research data [59] revealed that in baboons, vitrified ovarian tissue may survive and function for 18 months after reimplantation, but no pregnancies were obtained after several months of regular mating.

Reimplantation of ovarian tissue: pregnancy and live birth rates

Techniques

Described techniques include both orthotopic (pelvic cavity) and heterotopic (outside the pelvic cavity like the forearm or abdominal wall muscle) sites [1,3].

Orthotopic ovarian tissue transplantation. As first described by Donnez et al. [44,47,60], orthotopic transplantation involves grafting ovarian cortical fragments to the exposed medulla of the denuded ovary or a specially created peritoneal site [60]. There are three options depending on individual circumstances:

A. If at least one ovary is present:

The procedure is laparoscopic and starts with decortication of the ovary. A large piece of ovarian cortex is removed with scissors to gain access to the medulla and its vascular network (Fig. 5A–B). Consistent with microsurgical techniques, ovarian cortical pieces are simply placed on the medulla and fixed with Interceed® (Fig. 5C–D).

B. If both ovaries are absent [47,60]:

A peritoneal window may be created in two steps to induce angiogenesis before grafting, as in the case published in 2004 [44], or in one step [47]. The incision for this peritoneal window is made on the anterior leaf of the broad ligament in an area where a vascular network is visible (retroperitoneal vessels) (Fig. 6 A). The fragments are placed inside the window and subsequently covered with Interceed®, the edges of which are fixed with fibrin glue (Fig. 6B–D). In our first case by this technique reported in 2012 [47], the restoration of ovarian function began at 20 weeks and was achieved 24 weeks after transplantation and followed by the first live birth.

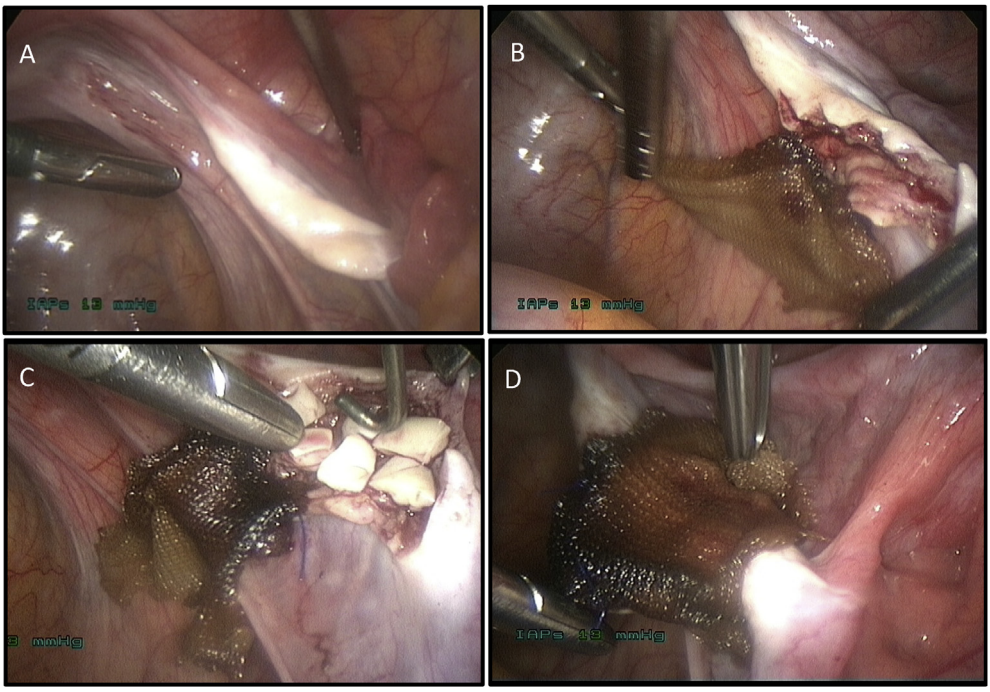

Fig. 5. Orthotopic ovarian tissue transplantation on the ovarian medulla, if at least one ovary is present. The procedure is laparoscopic and starts with the decortication of the ovary. A large piece of ovarian cortex is removed with scissors to gain access to the medulla and its vascular network (Figure 5 A–B). Consistent with microsurgical techniques, ovarian cortical pieces are simply placed on the medulla and fixed with Interceed® (Figure 5 C–D).

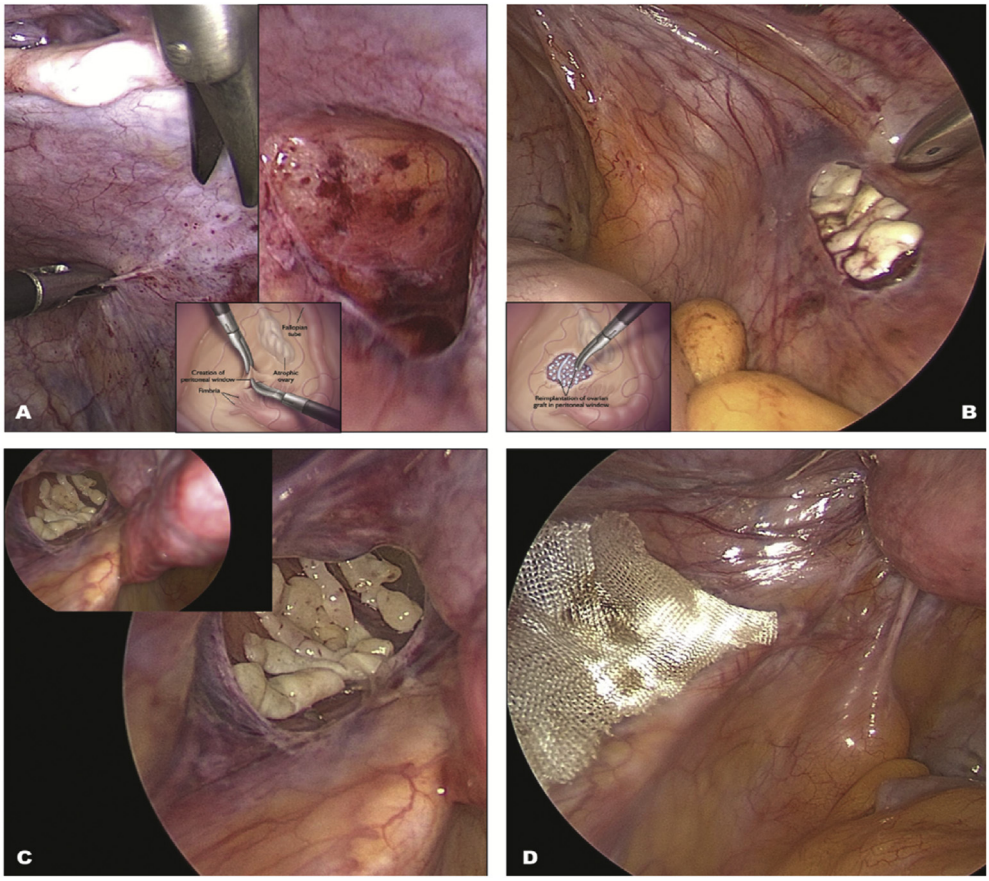

Fig. 6. Orthotopic ovarian tissue transplantation in a peritoneal window, when no ovaries were left. The incision for the peritoneal window is made on the anterior leaf of the broad ligament in an area where a vascular network is visible (retroperitoneal vessels) (Figure 6 A). The fragments are placed inside the window and subsequently covered with Interceed®, the edges of which are fixed with fibrin glue (Figure 6 B–D).

C. Combined technique:

A third option for patients with one or both ovaries still in place is grafting the tissue to two orthotopic sites simultaneously (if there is enough ovarian tissue), namely to the denuded ovary and a peritoneal window [60]. For this type of transplantation, it is of utmost importance to exercise caution in judging the amount of tissue to use, anticipating the potential need for further autografting to the same patient. It is recommended that only one-third of the cryopreserved tissue of each patient be thawed and grafted.

Strategies to improve transplantation outcomes. Early post-transplantation hypoxia remains a challenge because of its negative impact on follicle survival, with follicle loss of >50% often observed during the first few days after grafting [61,62], leading to massive follicle activation and "burn-out" [63–66]. Increasing vascularization in grafted tissue is therefore crucial and efforts are ongoing to improve follicle survival rates to increase the efficiency of ovarian tissue transplantation. One approach involves enhancing graft revascularization by delivering angiogenic and antiapoptotic factors [1,3]. Another seeks to boost neovascularization with adipose tissue-derived stem cells in an experimental model,

instituting a novel two-step transplantation procedure [67]. Using this approach, we very recently demonstrated superior rates of oxygenation and vascularization of ovarian tissue in the early post-grafting period, ultimately resulting in lower apoptosis and higher follicle survival rates [68].

Silber's technique, Meirow's technique, and Andersen's technique. In Silber's technique, the cortex of streak ovaries is resected under magnification, exposing the entire raw surface of the medulla [69]. A section of ovarian cortex is then placed over the raw medulla of each ovary [69–71] and sutured to the medulla with 9/0 nylon interrupted stitches. Meirow uses [72] blunt dissection to create cavities beneath the cortex for each of the strips of thawed ovarian tissue, which are then gently placed inside. Andersen's transplantation procedure [73] involves the grafting of ovarian cortical tissue fragments to subcortical pockets in the remaining follicle-depleted ovary in all patients.

Heterotopic ovarian tissue transplantation. Common sites for heterotopic transplantation are the abdominal wall, forearm, and rectus muscle, among others. Heterotopic transplantation may offer some advantages, including: 1) avoidance of invasive abdominal surgery; 2) effortless monitoring of follicle development and easy retrieval of oocytes; 3) cost-effective technology when repeated transplantations are required; 4) feasibility even in the case of severe pelvic adhesions that preclude orthotopic transplantation; and 5) straightforward removal and/or the replacement of transplanted tissue if necessary. However, results in terms of pregnancy rates are much poorer, with only two pregnancies reported by the team of Gook [74]. It should nevertheless be noted that the fragments, introduced through the abdominal wall in this instance, were placed just beneath the peritoneum, which could be considered a variation of the orthotopic “pelvic cavity” transplantation site.

Results

- Ovarian activity restoration

After reimplantation of ovarian tissue in the pelvic cavity (Fig. 1), ovarian activity is restored in over 95% of cases [46]. Although it is difficult to determine the life span of grafted tissue, the mean duration of ovarian function after transplantation is 4–5 years, but it can persist for up to 7 years [46]. The duration of graft function depends on a range of factors, such as age at cryopreservation, follicle density, and the quality of grafted tissue, to name a few.

- Pregnancy

The first pregnancy issuing from this procedure was reported back in 2004 [44]. Since then, pregnancy and live birth rates have continued to climb steadily, showing an exponential increase. Indeed, as of June 2017, the number of live births had exceeded 130 [1] and that figure has probably reached more than 200 by now [75] (Fig. 7).

In a first study published in 2015, because the number of reimplantations performed worldwide (the true denominator) was not known, data collection was based on patients from five major centers ($n = 111$ patients). Combined results yielded a pregnancy rate of 29% and live birth rate of 23% [57]. These rates were subsequently confirmed in a series of 74 women, with pregnancy and live birth rates of 33% and 25%, respectively [52]. In a very recent paper, data from three major centers (Sheba Medical Center, Israel, Cliniques universitaires Saint Luc, Belgium, and St Louis Infertility Center, USA) involving 60 patients revealed a pregnancy rate of 50% and live birth rate of 41% (Fig. 8) [76].

In our personal series of 23 women undergoing ovarian tissue reimplantation, the live birth rate was 41% (10 out of 22), yielding a total of 15 live births [1]. One woman in our series delivered three times, making her one of two patients worldwide to experience three pregnancies and births resulting from a single ovarian tissue reimplantation procedure.

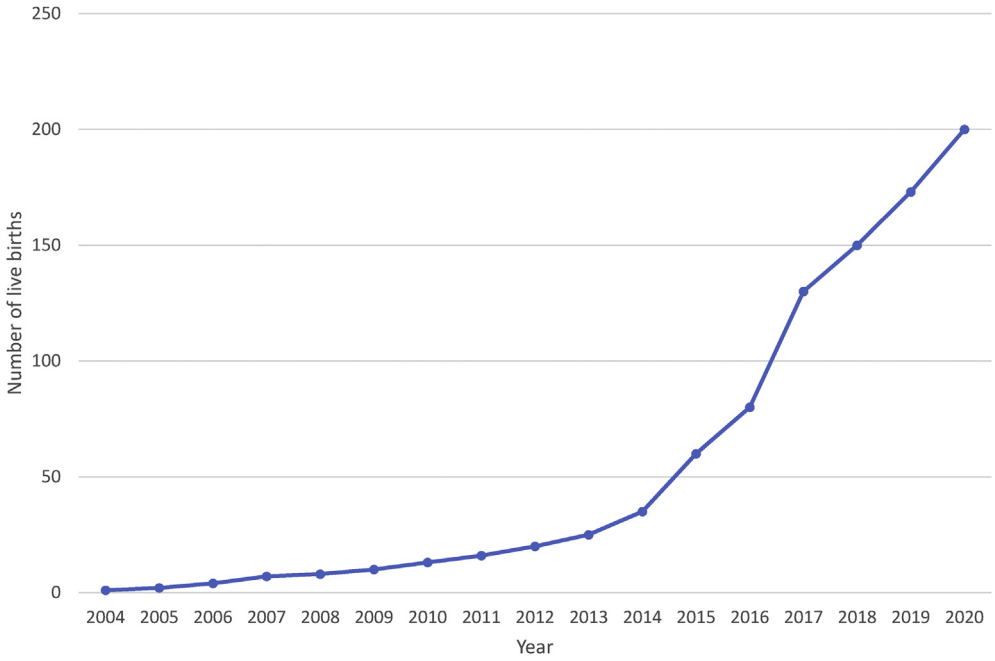

Fig. 7. Since the first pregnancy reported back in 2004, the number of live births has climbed, reaching 130 by 2017 [1] and showing a logarithmic increase over recent years to reach 200 in 2020 [75].

Combined technique: ovarian tissue cryopreservation followed by immediate oocyte vitrification (Fig. 1)

It was recently demonstrated that ovarian tissue cryopreservation, followed immediately by COS and oocyte retrieval (with a view to vitrifying mature oocytes), does not impair oocyte number or quality [77]. By combining oocyte vitrification and ovarian tissue cryopreservation in patients with cancer, a live birth rate of 50%–60% might conceivably be achieved (Fig. 1). The combined technique increases the efficacy of the procedure, thereby giving young cancer patients greater chances of success.

	Sheba Medical Center Tel Aviv	Cliniques Saint Luc Brussels	Infertility Center St Louis
Number of auto-transplantations	32	23	5

Total : 60 patients

At least one pregnancy : 30/60 (50%)

At least one live birth : 25/60 (41.6%)

Fig. 8. Data from three major centers (Sheba Medical Center, Israel, Cliniques universitaires Saint Luc, Belgium, and St Louis Infertility Center, USA) involving 60 patients revealed a pregnancy rate of 50% and live birth rate of 41% [76].

We therefore suggest that this combined technique be offered to postpubertal patients at high risk of POI, as long as chemotherapy can be postponed without jeopardizing cancer treatment, to maximize their chances of conceiving [1,3].

Risk of ovarian metastasis according to cancer

The risk of metastases should be weighed up according to cancer type [78,79] (Table 2). It is considered to be high (>11%) in the case of leukemia, neuroblastoma, and Burkitt lymphoma, and moderate (0.2–11%) in the case of advanced breast cancer, colon cancer, cervical adenocarcinoma, non-Hodgkin's lymphoma, and Ewing sarcoma. The risk is deemed to be very low (<0.2%) in all other pathologies [78,79]. It is nevertheless recommended that in case of any cancer, a tissue fragment be thawed for histological analysis, immunohistochemistry, and polymerase chain reaction (when specific markers are available), before contemplating transplantation.

Fresh ovarian tissue transplantation

There are actually very few indications for fresh ovarian tissue transplantation. One previous instance is monozygotic twins discordant for ovarian failure [70,80], and another is allografting from a related or unrelated subject who has previously donated bone marrow to the patient [81,82]. Any other circumstances would require immunosuppression with its inherent side effects, similar to that of any other solid organ transplant, making it ethically questionable.

a) Transplantation between monozygotic twins

The first successful fresh ovarian tissue transplant in humans occurred in 2005 between identical twins, one of whom was affected by POI and the other who was healthy and fertile. Grafting of ovarian cortex to the medulla of the recipient's ovary was the technique used in this case [70].

In Silber's series, all cases (n = 9) were successful, in that they all restored normal hormone function. Among these 9 patients, 7 conceived, resulting in 14 pregnancies and 11 healthy births [83]. The patients all favored spontaneous pregnancy over IVF and egg donation, and wished to achieve this in a once-only procedure without COS.

In Donnez's series [80], two further live births were documented after allografting of ovarian cortex between monozygotic twins with Turner syndrome (45 XO) and discordant ovarian function.

b) Allografting between two genetically different sisters

The first allograft of ovarian cortex between two genetically different sisters was reported in 2007 [84] and the first series was published in 2010 by Donnez et al. [81]. Three subjects aged 20, 15, and 12 years underwent chemotherapy and total body irradiation prior to bone marrow transplantation, the donor being their HLA-compatible sister in each case. Years later, HLA group analysis revealed complete

Table 2
Risk of ovarian metastasis according to cancer type.

High risk	Moderate risk	Low risk
Leukemia	Breast cancer (infiltrating lobular subtype, stage IV)	Breast cancer (infiltrating ductal subtype, stage I-II)
Neuroblastoma		
Burkitt lymphoma	Colon cancer	Squamous cell carcinoma of the cervix
	Adenocarcinoma of the cervix	Hodgkin's lymphoma
	Non-Hodgkin's lymphoma	Osteogenic carcinoma
	Ewing's sarcoma	Nongenital
		Rhabdomyosarcoma
		Wilms' tumor

chimerism and ovarian allografting was performed, with the tissue donor being the sister who had already donated bone marrow. The technique is shown in Fig. 9, as described by Donnez and Dolmans [1]. No immunosuppressive therapy was administered and no signs of rejection were observed. The restoration of ovarian function occurred in all three cases.

The first live birth to be achieved after ovarian tissue transplantation between two genetically different sisters was reported in 2011 [82]. As this is an acceptable practice in monozygotic twins, there is no apparent reason not to apply it in genetically different sisters when one of the sisters previously received bone marrow from the other, leading to complete chimerism (HLA compatibility) and obviating the need for immunosuppressive treatment [81,82]. This approach allows for natural conception, which could be important on moral, ethical, or religious grounds.

The future

Artificial ovary (Fig. 1)

One alternative to obtaining mature oocytes would be the use of the so-called transplantable artificial ovary. Isolating primordial follicles and transferring them onto a scaffold to replace this native organ would serve to eliminate the risk of transmission of malignant cells [79,85,86]. Recent developments in the isolation technique, involving washing the follicles three times, have proved successful in purging malignant cells [87]. Growing antral follicles were observed after autografting human primordial follicles inside a fibrin scaffold in a mouse model [88].

In vitro development of primordial follicles

A dynamic multistep culture system is required to support each of the transitional stages of follicles [89] (Fig. 1). This multistep approach to in vitro follicle growth must meet the changing needs of the developing oocyte and its surrounding somatic (granulosa) cells to maintain interactions between these cells [89,90]. Challenges, such as the acquisition of meiotic and developmental competence as well as genome imprinting, are numerous.

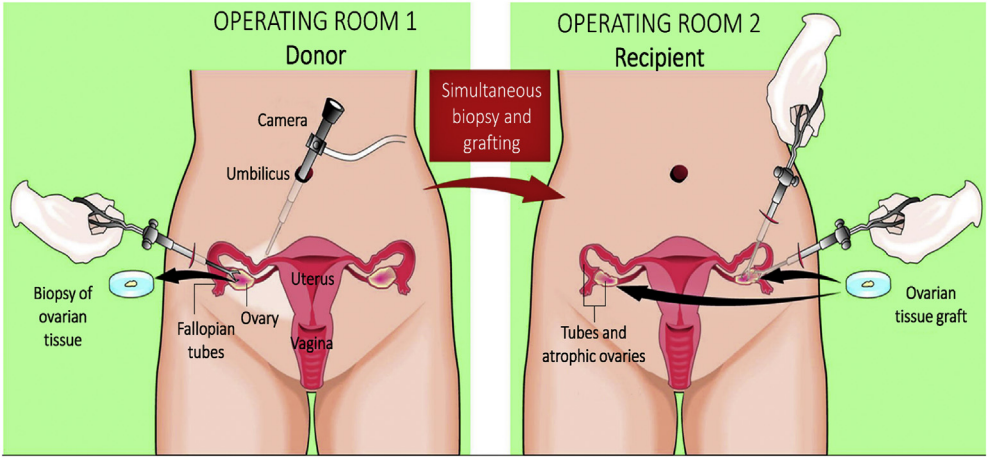

Fig. 9. Fresh ovarian tissue allografting between two genetically different sisters. The two sisters were operated on simultaneously in 2 contiguous operating rooms. Ovarian tissue was laparoscopically removed from the donor's ovary and immediately sutured to the recipient's ovarian medulla.

Ovarian stem cells

The discovery of ovarian stem cells has cast a doubt on the theory that germ cells are no longer produced in female mammals after birth [91]. However, in vitro derivation from ovarian stem cells [92] might be problematic if it interferes with the complex genomic imprinting and epigenetic mechanisms required for the development of fully competent oocytes.

Conclusions

Ensuring safe ovarian tissue transit to allow and extend access to fertility preservation in large countries and low-resource areas is another formidable challenge in this field. While improvements in freezing techniques and strategies to minimize the risks of fertility preservation are still at the research stage in cancer patients, they will in all likelihood be implemented in women with benign diseases (like recurrent endometriosis) in the near future. For nononcological indications, the vitrification of oocytes has emerged as the technique of choice. There is no doubt that the combined technique (ovarian tissue cryopreservation immediately followed by oocyte vitrification) boosts the chances of pregnancy and should at least be contemplated in women with a low ovarian reserve. It was clearly time to move on from experimental studies to more widespread clinical application and this has now been approved; the American Society for Reproductive Medicine no longer considers ovarian tissue cryopreservation an experimental technique [93].

Authors′ roles

MMD and JD equally contributed to the research and interpretation of data discussed in the manuscript and approved the final version.

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Declaration of Competing Interest

JD is a member of the Scientific Advisory Board (SAB) of PregLem S.A. and Obseva. MMD has no conflict of interest to declare.

Practice points

- **Oocyte vitrification** has become the standard approach to preserve fertility in women with benign diseases, those seeking fertility preservation for personal reasons (also called age-related infertility), and women with cancer if treatment can be safely postponed.
- **Ovarian tissue cryopreservation** is specifically indicated for young girls and women who require immediate cancer treatment.
- **Fresh tissue transplantation** in women with POI is a valuable option in specific conditions.
- **Endometriosis** should be considered an indication for fertility preservation: oocytes and ovarian tissue freezing.

Research agenda

1. Fertility preservation in endometriotic patients
2. Oocyte quality in endometriotic patients
3. Strategies to improve revascularization of the ovarian tissue grafts
4. Analysis of oocyte quality and metabolic activity of grafted ovarian tissue
5. Artificial ovary and in vitro culture of primordial follicles

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References

[1] Donnez J, Dolmans MM. Fertility preservation in women. N Engl J Med 2017;377(17):1657–65.
[2] Cobo A, García-Velasco J, Domingo J, Pellicer A, Remohí J. Elective and Onco-fertility preservation: factors related to IVF outcomes. Hum Reprod 2018;33:2222–31.
[3] Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol 2013;9:735–49.
[4] Jadoul P, Dolmans MM, Donnez J. Fertility preservation in girls during childhood: is it feasible, efficient and safe and to whom should it be proposed? Hum Reprod Update 2010;16:617–30.
[5] Anderson RA, Wallace HB. Ovarian hormone, the assessment of the ovarian reserve, and the reproductive outcome of the young patient with cancer. Fertil Steril 2013;99:1469–75.
[6] Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol 2005;6:922.
[7] Wallace WH, Kelsey TW, Anderson RA. Fertility preservation in pre-pubertal girls with cancer: the role of ovarian tissue cryopreservation. Fertil Steril 2016;105:6–12.
[8] Andersen CY, Bollerup AC, Kristensen SG. Defining quality assurance and quality control measures in connection with ovarian tissue cryopreservation and transplantation: a call to action. Hum Reprod 2018;33:1201–4.
[9] Van Dorp W, Mulder RL, Kremer LC, Hudson MM, van den Heuvel-Eibrink MM, van den Berg MH, et al. Recommendations for premature ovarian insufficiency surveillance for female survivors of childhood, adolescent, and young adult cancer: a report from the international late effects of childhood cancer guideline harmonization group in collaboration with the PanCareSurFup consortium. J Clin Oncol 2016;34:3440–50.
[10] Donnez J, Dolmans MM. Preservation of fertility in females with haematological malignancy. Br J Haematol 2011;154:175–84.
[11] Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update 2001;7:535–43.
[12] Wallace WH, Thomson AB, Kelsey TW. The radio sensitivity of the human oocyte. Hum Reprod 2003;18:117–21.
[13] Llarena NC, Falcone T, Flyckt RL. Fertility preservation in women with endometriosis. Clin Med Insights Reprod Health 2019;13. 1179558119873386.
[14] Donnez J. Fertility preservation in women, focusing on cancer, benign diseases and social reasons. Minerva Ginecol 2018;70:385–6.
[15] Kitajima M, Defrère S, Dolmans MM, Colette S, Squifflet J, Van Langendonckt A, et al. Endometriomas as a possible cause of reduced ovarian reserve in women with endometriosis. Fertil Steril 2011;96:685–91.
[16] Kitajima M, Dolmans MM, Donnez O, Masuzaki H, Soares M, Donnez J. Enhanced follicular recruitment and atresia in cortex derived from ovaries with endometriomas. Fertil Steril 2014;101:1031–7.
[17] Raffi F, Metwally M, Amer S. The impact of excision of ovarian endometrioma on ovarian reserve: a systematic review and meta-analysis. J Clin Endocrinol Metab 2012;97:3146–54.
[18] Muzii L, Di Tucci C, Di Feliciantonio M, Galati G, Di Donato V, Musella A, et al. Antimüllerian hormone is reduced in the presence of ovarian endometriomas: a systematic review and meta-analysis. Fertil Steril 2018;110:932–40.
[19] Donnez J, Lousse JC, Jadoul P, Donnez O, Squifflet J. Laparoscopic management of endometriomas using a combined technique of excisional (cystectomy) and ablative surgery. Fertil Steril 2010;94:28–32.
[20] Jadoul P, Kitajima M, Donnez O, Squifflet J, Donnez J. Surgical treatment of ovarian endometriomas: state of the art? Fertil Steril 2012;98:556–63.
[21] Donnez J, Squifflet J, Jadoul P, Lousse JC, Dolmans MM, Donnez O. Fertility preservation in women with ovarian endometriosis. Front Biosci (Elite Ed). 2012;4:1654–62.
[22] Nelson LM. Primary ovarian insufficiency. N Engl J Med 2009;360:606–614. 24.
[23] Caburet S, Zavadakova P, Ben-Neriah Z, Bouhali K, Dipietromaria A, Charon C, et al. Genome-wide linkage in a highly consanguineous pedigree reveals two novel loci on chromosome 7 for non-syndromic familial premature ovarian failure. PLoS One 2012;7(3):e33412.
[24] Cobo A, Garcia-Velasco JA, Domingo J, Remohí 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.

[25] Cobo A, García-Velasco JA, Coello A, Domingo J, Pellicer A, Remohí J. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril 2016;105:755–64.
[26] Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update 2017;23:139–55.
[27] Rienzi L, Ubaldi FM. Oocyte versus embryo cryopreservation for fertility preservation in cancer patients: guaranteeing a women's autonomy. J Assist Reprod Genet 2015;32:1195–6.
[28] Cakmak H, Rosen MP. Random-start ovarian stimulation in patients with cancer. Curr Opin Obstet Gynecol 2015;27:215–21.
[29] Decanter C, Robin G, Mailliez A, Sigala J, Morschhauser F, Ramdane N, et al. Prospective assessment of follicular growth and the oocyte cohort after ovarian stimulation for fertility preservation in 90 cancer patients versus 180 matched controls. Reprod Biomed Online 2018;36:543–51.
[30] Stoop D. Oocyte vitrification for elective fertility preservation: lessons for patient counseling. Fertil Steril 2016;105:603–4.
[31] Cobo A, Garrido N, Pellicer 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.
[32] Domingo J, Guillén V, Ayllón Y, Martínez M, Muñoz E, Pellicer A, et al. Ovarian response to controlled ovarian hyperstimulation in cancer patients is diminished even before oncological treatment. Fertil Steril 2012;97:930–4.
[33] Oktay K, Hourvitz A, Sahin G, Oktem O, Safro B, Cil A, et al. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J Clin Endocrinol Metab 2006;91:3885–90.
[34] Goodman LR, Goldberg JM, Flyckt RL, Gupta M, Harwalker J, Falcone T. Effect of surgery on ovarian reserve in women with endometriomas, endometriosis and controls. Am J Obstet Gynecol 2016;215:589.e1–6.
[35] Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. Fertil Steril 2002;77:861–70.
[36] Cobo A, Giles J, Paolelli S, Pellicer A, Remohí J, García-Velasco JA. Oocyte vitrification for fertility preservation in women with endometriosis: an observational study. Fertil Steril 2020;113:836–44.
[37] Donnez J. Women with endometrioma-related infertility face a dilemma when choosing the appropriate therapy: surgery or in vitro fertilization. Fertil Steril 2018;110:1216–7.
[38] Lessey BA, Gordts S, Donnez O, Somigliana E, Chapron C, Garcia-Velasco JA, et al. Ovarian endometriosis and infertility: in vitro fertilization (IVF) or surgery as the first approach? Fertil Steril 2018;110:1218–26.
[39] Donnez J, Nisolle M, Gillet N, Smets M, Bassil S, Casanas-Roux F. Large ovarian endometriomas. Hum Reprod 1996;11:641–6.
[40] Wallace WH, Smith AG, Kelsey TW, Edgar AE, Anderson RA. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. Lancet Oncol 2014;15:1129–36.
[41] El Issaoui M, Giorgione V, Mamsen LS, Rechnitzer C, Birkebæk N, Clausen N, et al. Effect of first line cancer treatment on the ovarian reserve and follicular density in girls under the age of 18 years. Fertil Steril 2016;106:1757–1762.e1.
[42] Bjelland EK, Wilkosz P, Tanbo TG, Eskild A. Is unilateral oophorectomy associated with age at menopause? A population study (the HUNT2 Survey). Hum Reprod 2014;29:835–41.
[43] Wilkosz P, Greggains GD, Tanbo TG, Fedorcsak P. Female reproductive decline is determined by remaining ovarian reserve and age. PLoS One 2014;13(9):e108343.
[44] Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;364:2020.
[45] 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.
[46] Donnez J, Dolmans MM. 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.
[47] 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.
[48] Andersen CY, Rosendahl M, Byskov AG, Loft A, Ottosen C, Dueholm M, et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. Hum Reprod 2008;23:2266–72.
[49] Donnez J, Martinez-Madrid B, Jadoul P, Van Langendonckt A, Demylle D, Dolmans MM. Ovarian tissue cryopreservation and transplantation: a review. Hum Reprod Update 2006;12:519–35.
[50] Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005;353:318–21.
[51] Donnez J, Dolmans MM, Pellicer A, Diaz-García C, Ernst E, Macklon KT, et al. Fertility preservation for age-related fertility decline. Lancet 2015;385:506–7.
[52] Van der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krüssel J, et al. FertiPROTEKT network. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. Hum Reprod 2016;31:2031–41.
[53] Meirow D, Ra'anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertil Steril 2016;106:467–74.
[54] Dunlop CE, Brady BM, McLaughlin M, Telfer EE, White J, Cowie F, et al. Re-implantation of cryopreserved ovarian cortex resulting in restoration of ovarian function, natural conception and successful pregnancy after haematopoietic stem cell transplantation for Wilms tumour. J Assist Reprod Genet 2016;33:1615–20.
[55] Rodriguez-Wallberg KA, Tanbo T, Tinkanen H, Thurin-Kjellberg A, Nedstrand E, Kitlinski ML, et al. Ovarian tissue cryopreservation and transplantation among alternatives for fertility preservation in the Nordic countries - compilation of 20 years of multicenter experience. Acta Obstet Gynecol Scand 2016;95:1015–26.

[56] Jensen AK, Macklon KT, Fedder J, Ernst E, Humaidan P, Andersen CY. 86 successful births and 9 ongoing pregnancies worldwide in women transplanted with frozen-thawed ovarian tissue: focus on birth and perinatal outcome in 40 of these children. J Assist Reprod Genet 2017;34:325–36.
[57] Donnez J, Dolmans MM, Diaz C, Pellicer A. Ovarian cortex transplantation: time to move on from experimental studies to open clinical application. Fertil Steril 2015;104:1097–8.
[58] Suzuki N. Ovarian tissue cryopreservation using vitrification and/or in vitro activated technology. Hum Reprod 2015;30:2461–2.
[59] Amorim CA, Donnez J, Dehoux JP, Scalercio SR, Squifflet J, Dolmans MM. Long-term follow-up of vitrified and autografted baboon (Papio anubis) ovarian tissue. Hum Reprod 2019;34:323–34.
[60] Donnez J, Manavella DD, Dolmans MM. Techniques for ovarian tissue transplantation and results. Minerva Ginecol 2018;70:424–31.
[61] Van Eyck AS, Bouzin C, Feron O, Romeu L, Van Langendonckt A, Donnez J, et al. Both host and graft vessels contribute to revascularization of xenografted human ovarian tissue in a murine model. Fertil Steril 2010;93:1676–85.
[62] Van Eyck AS, Jordan BF, Gallez B, Heilier JF, Van Langendonckt A, Donnez J. Electron paramagnetic resonance as a tool to evaluate human ovarian tissue reoxygenation after xenografting. Fertil Steril 2009;92:374–81.
[63] Dolmans MM, Martinez-Madrid B, Gadisseux E, Guiot Y, Yuan WY, Torre A, et al. Short-term transplantation of isolated human ovarian follicles and cortical tissue into nude mice. Reproduction 2007;134:253–62.
[64] Masciangelo R, Hossay C, Chiti MC, Manavella DD, Amorim CA, Donnez J, et al. Role of the PI3K and Hippo pathways in follicle activation after grafting of human ovarian tissue. J Assist Reprod Genet 2020;37:101–8.
[65] Masciangelo R, Hossay C, Donnez J, Dolmans MM. Does the Akt pathway play a role in follicle activation after grafting of human ovarian tissue? Reprod Biomed Online 2019;39:196–8.
[66] Gavish Z, Peer G, Roness H, Cohen Y, Meirow D. Follicle activation and 'burn-out' contribute to post-transplantation follicle loss in ovarian tissue grafts: the effect of graft thickness. Hum Reprod 2014;29:989–96.
[67] Manavella DD, Cacciottola L, Desmet CM, Jordan BF, Donnez J, Amorim CA, et al. Adipose tissue-derived stem cells in a fibrin implant enhance neovascularization in a peritoneal grafting site: a potential way to improve ovarian tissue transplantation. Hum Reprod 2018;33:270–9.
[68] Manavella DD, Cacciottola L, Pommé S, Desmet CM, Jordan BF, Donnez J, et al. Two-step transplantation with adipose tissue-derived stem cells increases follicle survival by enhancing vascularization in xenografted frozen-thawed human ovarian tissue. Hum Reprod 2018;33:1107–16.
[69] Silber SJ, DeRosa M, Pineda J, Lenahan K, Greia D, Gorman K, et al. A series of monozygotic twins discordant for ovarian failure: ovary transplantation (cortical versus microvascular) and cryopreservation. Hum Reprod 2008;23:1531–7.
[70] Silber SJ, Lenahan KM, Levine DJ, Pineda JA, Gorman KS, Friez MJ, et al. Ovarian transplantation between monozygotic twins discordant for premature ovarian failure. N Engl J Med 2005;353:58–63.
[71] Silber S. Ovarian tissue cryopreservation and transplantation: scientific implications. J Assist Reprod Genet 2016;33:1595–603.
[72] Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005;353:318–21.
[73] Gellert SE, Pors SE, Kristensen SG, Bay-BjØrn AM, Ernst E, Yding Andersen C. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer reviewed papers and on the Danish cohort. J Assit Reprod Genet 2018;35:516–70.
[74] 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.
[75] Dolmans MM, Falcone T, Patrizio P. Reaching unreachable conclusions. Fertil Steril 2020;114:279–80.
[76] Shapira M, Dolmans MM, Silber S, Meirow D. Evaluation of ovarian tissue transplantation- results from three clinical centers. Fertil Steril 2020;114:388–97.
[77] Dolmans MM, Marotta ML, Pirard C, Donnez J, Donnez O. Ovarian tissue cryopreservation followed by controlled ovarian stimulation and pick-up of mature oocytes does not impair the number or quality of retrieved oocytes. J Ovarian Res 2014;26(7):80.
[78] 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.
[79] Dolmans MM, Amorim CA. Fertility preservation: construction and use of artificial ovaries. Reproduction 2019;158:F15–25.
[80] Donnez J, Dolmans MM, Squifflet J, Kerbrat G, Jadoul P. Live birth after allografting of ovarian cortex between monozygotic twins with Turner syndrome (45,XO/46,XX mosaicism) and discordant ovarian function. Fertil Steril 2011;96:1407–11.
[81] Donnez J, Squifflet J, Pirard C, Jadoul P, Dolmans MM. Restoration of ovarian function after allografting of ovarian cortex between genetically non-identical sisters. Hum Reprod 2010;25:2489–95.
[82] Donnez J, Squifflet J, Pirard C, Demylle D, Delbaere A, Armenio L, et al. Live birth after allografting of ovarian cortex between genetically non-identical sisters. Hum Reprod 2011;26:1384–8.
[83] Silber S. How ovarian transplantation works and how resting follicle recruitment occurs: a review of results reported from one center. Womens Health 2016;12:217–27.
[84] Donnez J, Dolmans MM, Pirard C, Van Langendonckt A, Demylle D, Jadoul P, et al. Allograft of ovarian cortex between two genetically non-identical sisters: case report. Hum Reprod 2007;22:2653–9.
[85] Luyckx V, Dolmans MM, 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.
[86] Chiti MC, Dolmans MM, Orellana O, Soares M, Paulini F, Donnez J, et al. Influence of follicle stage on artificial ovary outcome using fibrin as a matrix. Hum Reprod 2016;31:2898.
[87] Soares M, Saussoy P, Maskens M, Reul H, Amorim CA, Donnez J, et al. Eliminating malignant cells from cryopreserved ovarian tissue is possible in leukemia patients. Br J Haematol 2017;178:231–9.

[88] Paulini F, Vilela JM, Chiti MC, Donnez J, Jadoul P, Dolmans MM, et al. Survival and growth of human preantral follicles after cryopreservation of ovarian tissue, follicle isolation and short-term xenografting. Reprod Biomed Online 2016;33:425–32.
[89] Telfer EE, Zelinski MB. Ovarian follicle culture: advances and challenges for human and nonhuman primates. Fertil Steril 2013;99:1523–33.
[90] Xiao S, Zhang J, Romero MM, Smith KN, Shea LD, Wooddruff TK. In vitro follicle growth supports human oocyte meiotic maturation. Sci Rep 2015;5:17323.
[91] Truman AM, Tilly JL, Woods DC. Ovarian regeneration: the potential for stem cell contribution in the postnatal ovary to sustained endocrine function. Mol Cell Endocrinol 2017;445:74–84.
[92] Morohaku K, Tanimoto R, Sasaki K, Kawahara-Miki R, Kono T, Hayashi K, et al. Complete in vitro generation of fertile oocytes from mouse primordial germ cells. Proc Natl Acad Sci U S A 2016;113:9021–6.
[93] Practice Committee of the American Society for Reproductive Medicine. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril 2019;112:1022–33.