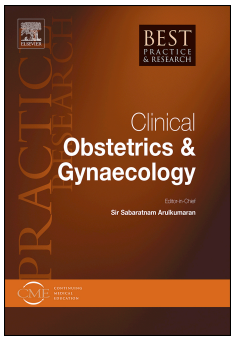


Fertility Preservation In Women For Medical And Social Reasons: Oocytes Vs Ovarian Tissue

Marie-Madeleine Dolmans, MD, PhD, Jacques Donnez, MD, PhD

PII: S1521-6934(20)30122-X

DOI: https://doi.org/10.1016/j.bpobgyn.2020.06.011

Reference: YBEOG 2073

To appear in: *Best Practice & Research Clinical Obstetrics & Gynaecology*

Received Date: 30 April 2020

Accepted Date: 13 June 2020

Please cite this article as: Dolmans M-M, Donnez J, Fertility Preservation In Women For Medical And Social Reasons: Oocytes Vs Ovarian Tissue , *Best Practice & Research Clinical Obstetrics & Gynaecology*, https://doi.org/10.1016/j.bpobgyn.2020.06.011.

FERTILITY PRESERVATION IN WOMEN FOR MEDICAL AND SOCIAL REASONS:

OOCYTES VS OVARIAN TISSUE

Marie-Madeleine Dolmans[a,b], MD, PhD, Jacques Donnez [c], MD, PhD

[a] Pôle de Gynécologie, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, 1200 Brussels, Belgium

[b] Gynecology Department, Cliniques Universitaires Saint Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium

E-mail: marie-madeleine.dolmans@uclouvain.be

[c] Professor EM, Université Catholique de Louvain,

Director, Société de Recherche pour l'Infertilité (SRI), 143 Avenue Grandchamp, 1150 Brussels, Belgium

E-mail: jacques.donnez@gmail.com

Corresponding author:

Jacques Donnez, MD, PhD

Professor EM, Catholic University of Louvain

Director, Société de Recherche pour l'Infertilité (SRI)

143 Avenue Grandchamp, B-1150 Brussels, Belgium

E-mail: jacques.donnez@gmail.com

Abstract

Approximately 10% of cancers occur in women under 45 years of age. Chemotherapy, radiotherapy and bone marrow transplantation cure more than 90% of cancer 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.

Keywords: Ovarian tissue, oocytes, transplantation, vitrification, fertility preservation, freezing.

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 (especially 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 hemotological 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 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 (Figure 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). Due to increasing interest in fertility preservation, reproductive medicine providers should be aware of success rates and limiting factors of oocyte vitrification in order to provide patients with accurate information.

a) <u>Oncological indications</u>

There are five key points to bear in mind when fertility preservation by embryo or oocyte cryopreservation is contemplated in women with cancer. First, in order 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 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 cumulative live birth rate 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 cumulative live birth rate (CLBR) according to age at the time of vitrification. In women ≤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 outcomes. However, other reasons like 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 pick-up for vitrification, and it appears that fewer mature oocytes are retrieved when letrozole is used (32,33). Moreover, in 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) <u>Endometriosis and fertility preservation: the specific issue of endometriosis</u>

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 (ROS) 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 (Figure 3). It is clear that endometrioma surgery carried out in good conditions yields high pregnancy rates (more than 50%) during the first year post-surgery (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 case of endometriosis should be seriously discussed in certain conditions, as explained in Figure 3.

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%) then 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 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 (Figure 4): low AMH, age > 30 years, bilateral endometrioma, recurrent endometrioma after surgery, endometrioma growing fast and endometrioma at a young age.

OVARIAN TISSUE CRYOPRESERVATION (Figure 1)

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

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 $7.5g/m^2$ 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 to 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 to 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 (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).

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) (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). In our first case by this technique reported in 2012 (47), restoration of ovarian function began at 20 weeks and was achieved 24 weeks after transplantation and followed by the first live birth.

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 post-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 made to improve follicle survival rates in order 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 place 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 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 case of severe pelvic adhesions that preclude orthotopic transplantation; and 5) straightforward removal and/or 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 (Figure 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 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).

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% (Figure 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.

Combined technique: ovarian tissue cryopreservation followed by immediate oocyte vitrification (Figure 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 (Figure 1). The combined technique increases the efficacy of the procedure, thereby giving young cancer patients greater chances of success.

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, in order 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 case of leukemia, neuroblastoma and Burkitt lymphoma, and moderate (0.2-11%) in 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 (83) 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 chimerism, and ovarian allografting was performed, with the tissue donor being the sister who had already donated bone marrow. The technique is shown in Figure 9, as described by Donnez and Dolmans (1). No immunosuppressive therapy was administered and no signs of rejection were observed. 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). Since 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 (Figure 1)

One alternative to obtaining mature oocytes would be 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

379 times, have proved successful in purging malignant cells (87). Growing antral follicles were
380 observed after autografting human primordial follicles inside a fibrin scaffold in a mouse
381 model (88).

382 In vitro development of primordial follicles

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

388 Ovarian stem cells

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

394

395

396

397 **CONCLUSIONS**

398 Ensuring safe ovarian tissue transit to allow and extend access to fertility preservation in
399 large countries and low-resource areas is another formidable challenge in this field. While
400 improvements in freezing techniques and strategies to minimize the risks of fertility
401 preservation are still at the research stage in cancer patients, they will in all likehood be
402 implemented in women with benign diseases (like recurrent endometriosis) in the near

403 future. For non-oncological indications, vitrification of oocytes has emerged as the technique
404 of choice. There is no doubt that the combined technique (ovarian tissue cryopreservation
405 immediately followed by oocyte vitrification) boosts the chances of pregnancy and should at
406 least be contemplated in women with a low ovarian reserve. It was clearly time to move on
407 from experimental studies to more widespread clinical application and this has now been
408 approved; the American Society for Reproductive Medicine no longer considers ovarian
409 tissue cryopreservation an experimental technique (93).
410

ACKNOWLEDGEMENTS

The authors thank Mira Hryniuk, BA, for reviewing the English language of the manuscript and Deborah Godefroidt for her administrative help.

AUTHORS' ROLES

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

FUNDING

This study was supported by grants from the Fonds National de la Recherche Scientifique de Belgique (FNRS-PDR Convention T.0077.14, FNRS grant no 5/4/150/5 awarded to Marie-Madeleine Dolmans, EOS grant 30443682) the Fonds Speciaux de Recherche, the Foundation Against Cancer, the family Ferrero and viscount de Spoelberch.

CONFLICTS OF 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 premature ovarian insufficiency 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 preseravtion 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
   Artificial ovary and in vitro culture of primordial follicles

References

1. Donnez J, Dolmans MM. Fertility preservation in women. N Engl J Med. 2017;377(17):1657-1665.
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;33:2222-2231.
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, 1469–1475.
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;33:1201-1204.
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;154:175–184.
11. Meirow D & Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum. Reprod. Update, 2001;7:535–543.
12. Wallace WH, Thomson AB & Kelsey TW. The radio sensitivity of the human oocyte. Hum. Reprod. 2003;18:117–121.
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;70:385-386.
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;110:932-940
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: 606-14. 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;23:139-155.
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;36:543-551.
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.e1-589.e6.
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;113:836-844.
37. Donnez J. Women with endometrioma-related infertility face a dilemma when choosing the appropriate therapy: surgery or in vitro fertilization. Fertil Steril. 2018;110:1216-1217.
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;110:1218-1226.
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. 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;33:1615-1620.
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;34:325-336.
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;34:323-334.
60. Donnez J, Manavella DD, Dolmans MM. Techniques for ovarian tissue transplantation and results. Minerva Ginecol. 2018;70:424-431.

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;37:101-108.
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;39:196-198.
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;33:270-279.
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;33:1107-1116.
69. Silber SJ, DeRosaM, 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;33:1595-1603.
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;35:516-570.
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 (in press)
76. Shapira M, Dolmans MM, Silber S, Meirow D. Evaluation of ovarian tissue transplantation- results from three clinical centers. Fertil Steril. 2020 (in press).
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-F25.

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, Squifflet J. 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 a. 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;178:231-239.
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;112:1022-1033.

Table

Table 1: Indications for fertility preservation

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

B) **Benign conditions** for which fertility preservation is indicated:
1. Non-oncological systemic diseases requiring chemotherapy/radiotherapy and/or bone marrow transplantation
2. Non-malignant 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

Table 2:

Risk of ovarian metastasis according to cancer type

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

LEGEND TO FIGURES

Figure 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 thawing

- 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 vitrification of oocytes. This combined technique theoretically yields a 50-60% chance of obtaining a live birth.

From Donnez J, Dolmans M. N Engl J Med 2017;377:1657-1665

Figure 2:

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

Figure 3:

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

Figure 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.

Figure 5:

Orthotopic ovarian tissue transplantation on the ovarian medulla, 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 (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).

Figure 6:

Orthotopic ovarian tissue transplantation in a peritoneal window, when no ovaries 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).

Figure 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).

Figure 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).

Figure 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.

Figure 1

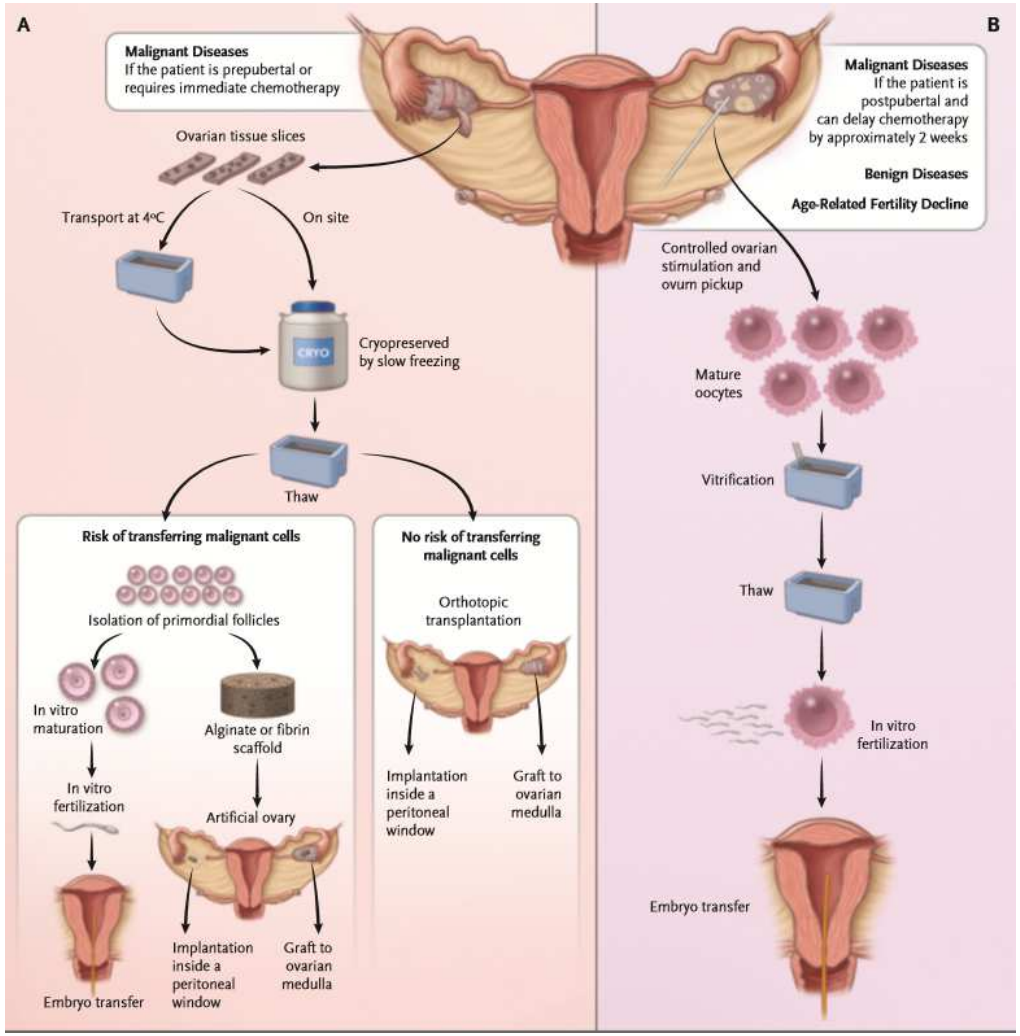
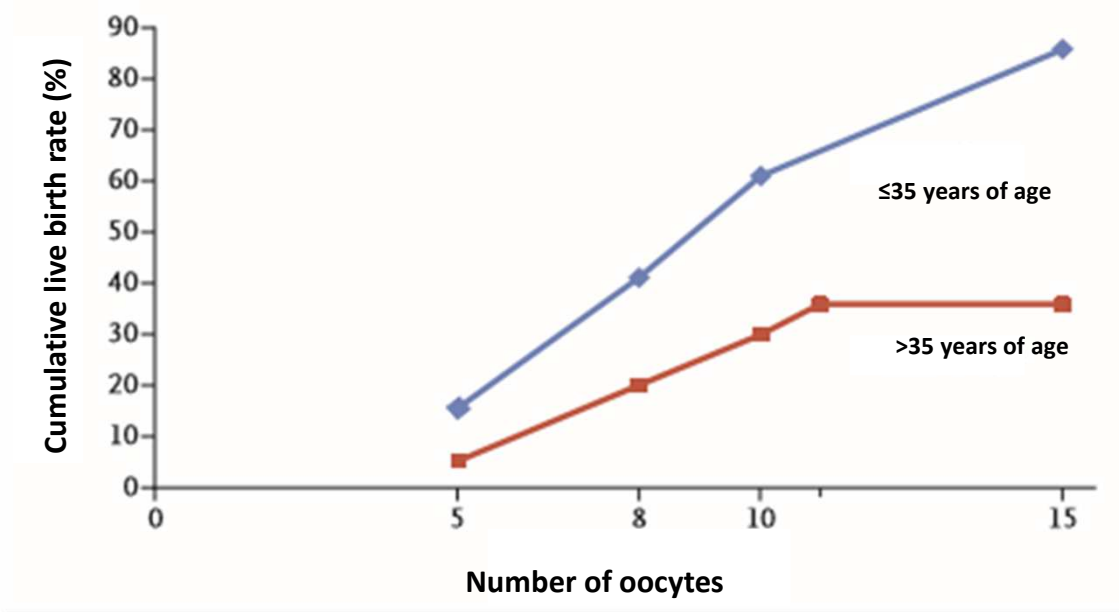

A
Malignant Diseases
If the patient is prepubertal or requires immediate chemotherapy
Ovarian tissue slices
Transport at 4°C
On site
Cryopreserved by slow freezing
Thaw
Risk of transferring malignant cells
Isolation of primordial follicles
In vitro maturation
In vitro fertilization
Embryo transfer
Alginate or fibrin scaffold
Artificial ovary
Implantation inside a peritoneal window
Graft to ovarian medulla
No risk of transferring malignant cells
Orthotopic transplantation
Implantation inside a peritoneal window
Graft to ovarian medulla
B
Malignant Diseases
If the patient is postpubertal and can delay chemotherapy by approximately 2 weeks
Benign Diseases
Age-Related Fertility Decline
Controlled ovarian stimulation and ovum pickup
Mature oocytes
Vitrification
Thaw
In vitro fertilization
Embryo transfer

Figure 2



Adapted from Cobo A et al. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril. 2016;105:755-64.

Figure 3

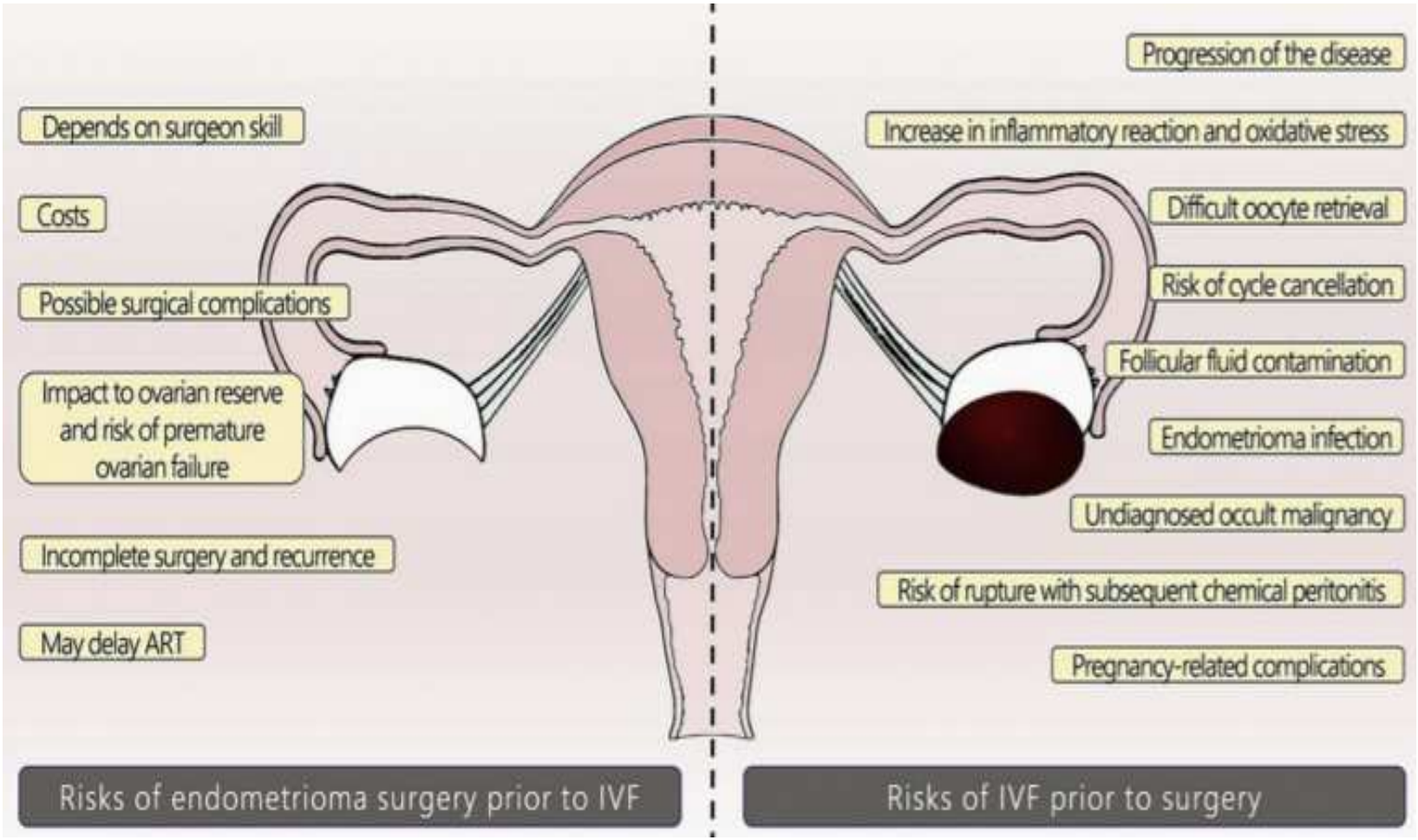

Depends on surgeon skill
Costs
Possible surgical complications
Impact to ovarian reserve and risk of premature ovarian failure
Incomplete surgery and recurrence
May delay ART
Progression of the disease
Increase in inflammatory reaction and oxidative stress
Difficult oocyte retrieval
Risk of cycle cancellation
Follicular fluid contamination
Endometrioma infection
Undiagnosed occult malignancy
Risk of rupture with subsequent chemical peritonitis
Pregnancy-related complications
Risks of endometrioma surgery prior to IVF
Risks of IVF prior to surgery

Figure 4

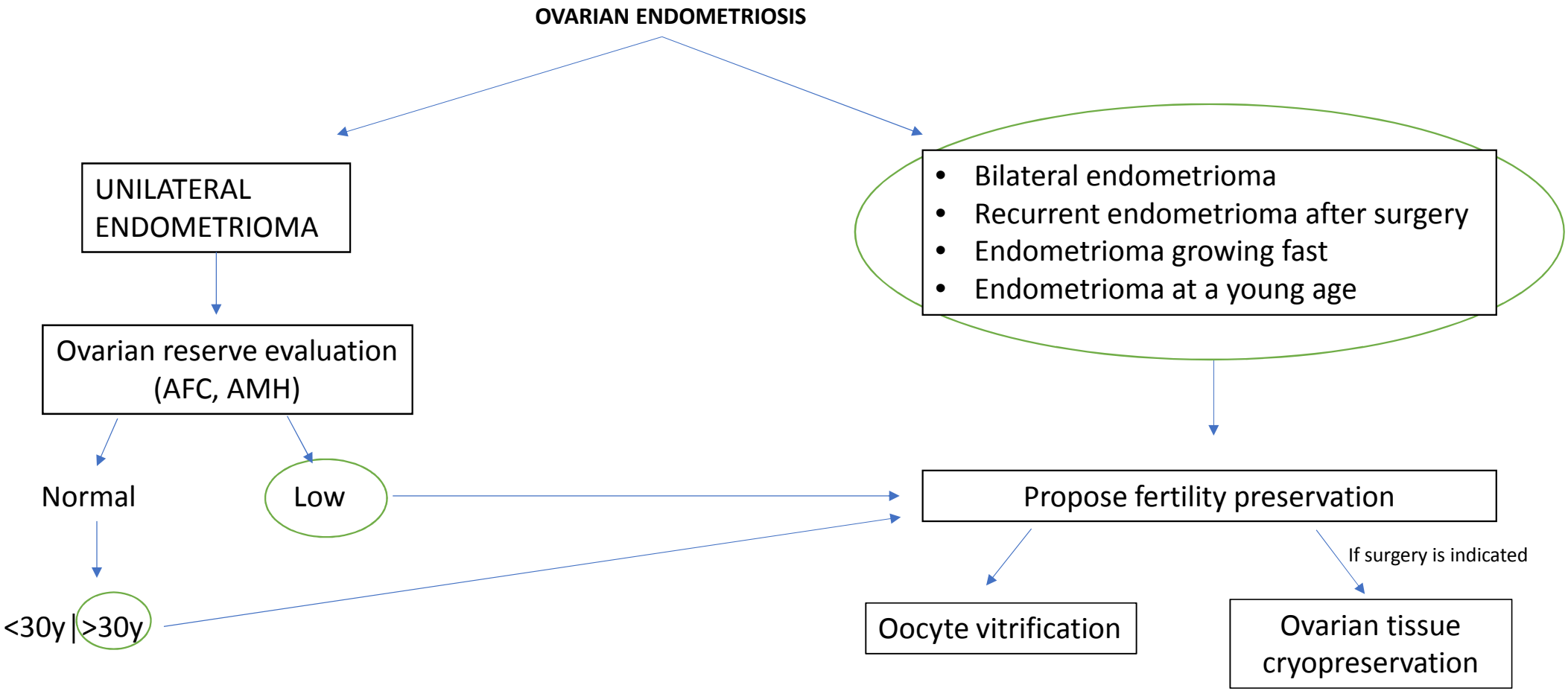

OVARIAN ENDOMETRIOSIS
UNILATERAL ENDOMETRIOMA
Ovarian reserve evaluation (AFC, AMH)
Normal
Low
<30y
>30y
• Bilateral endometrioma
• Recurrent endometrioma after surgery
• Endometrioma growing fast
• Endometrioma at a young age
Propose fertility preservation
If surgery is indicated
Oocyte vitrification
Ovarian tissue cryopreservation

Figure 5

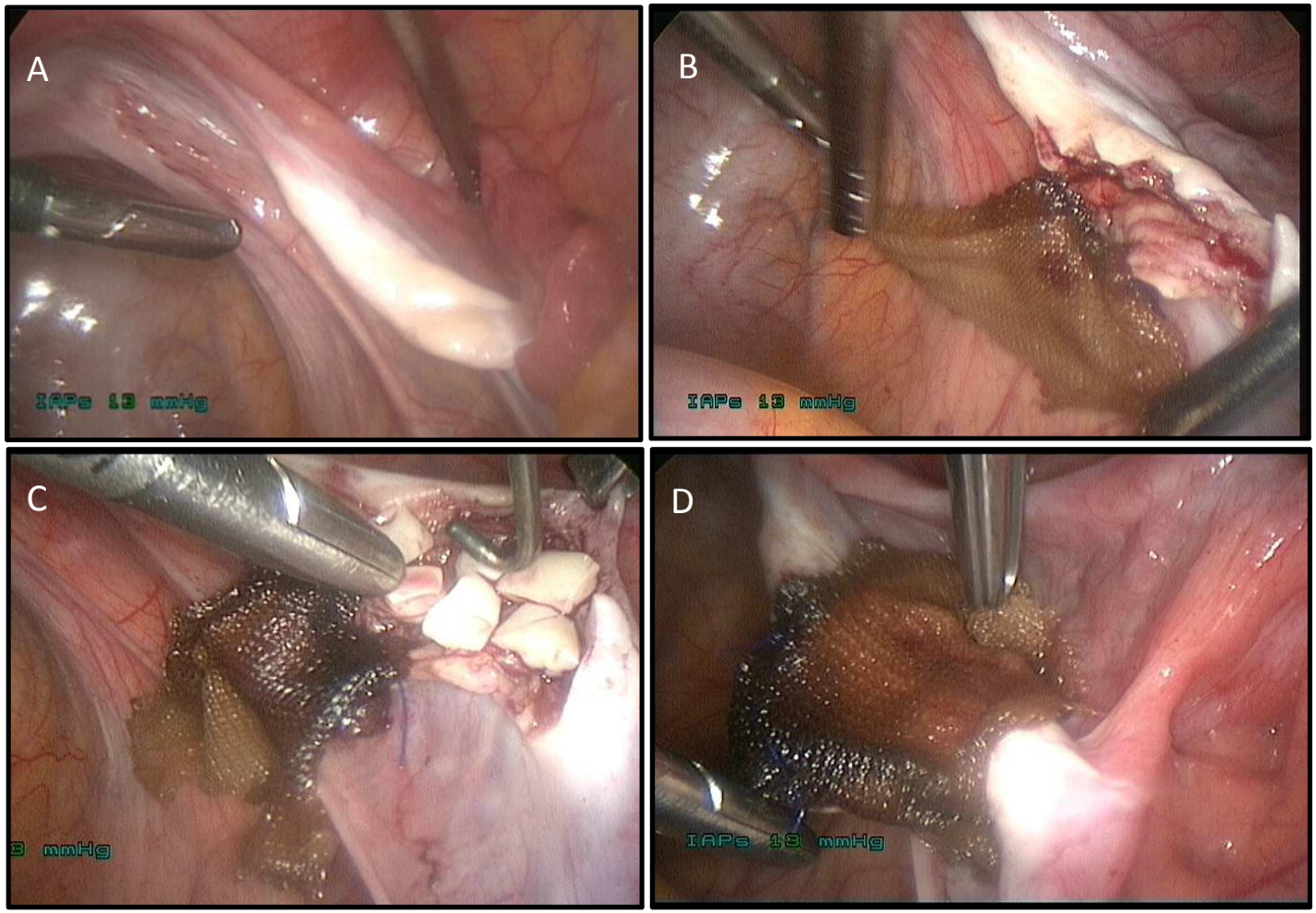


Figure 6

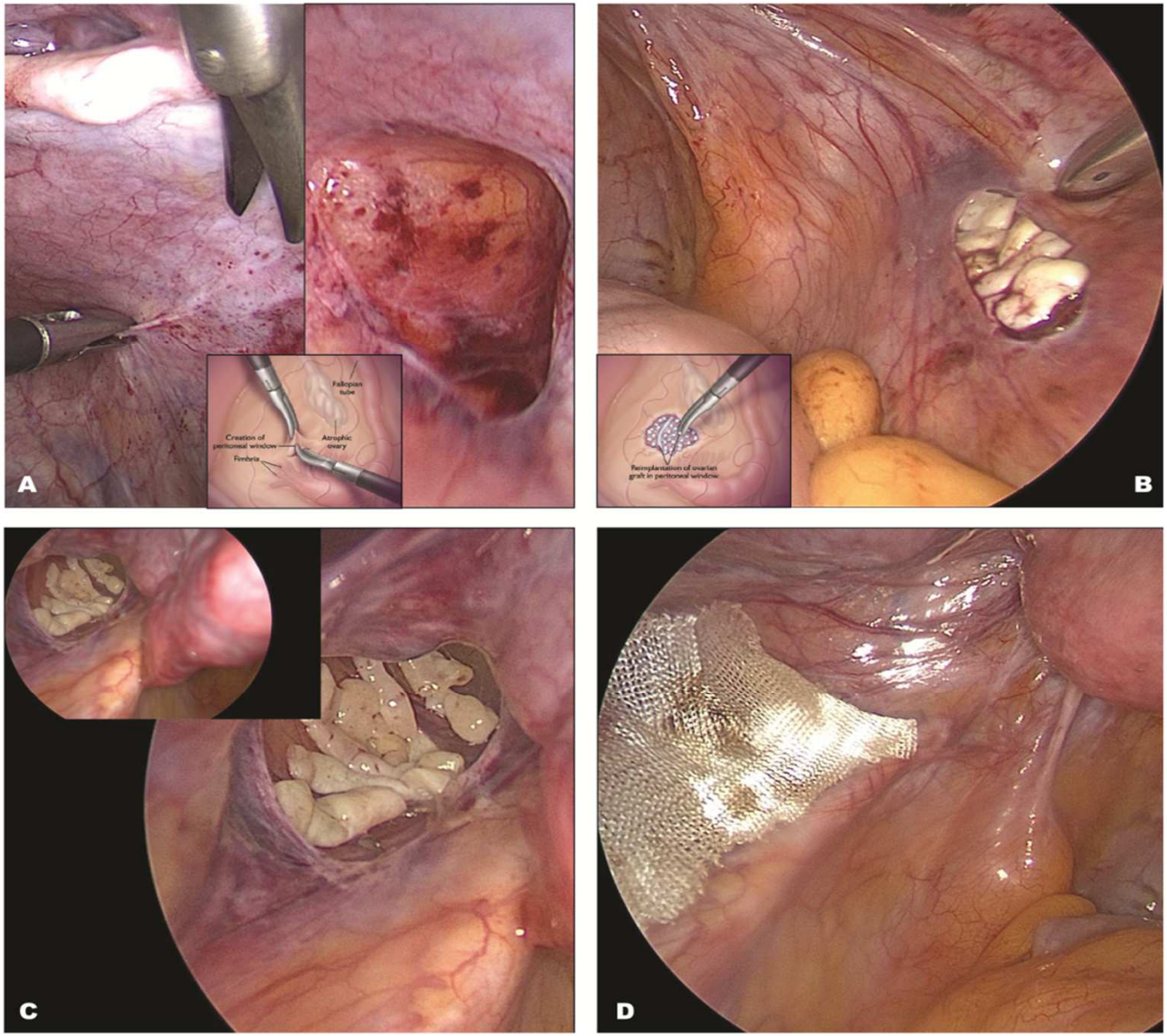

Fallopian tube
Creation of peritoneal window
Atrophic ovary
Fimbria
A
Reimplantation of ovarian graft in peritoneal window
B
C
D

Figure 7

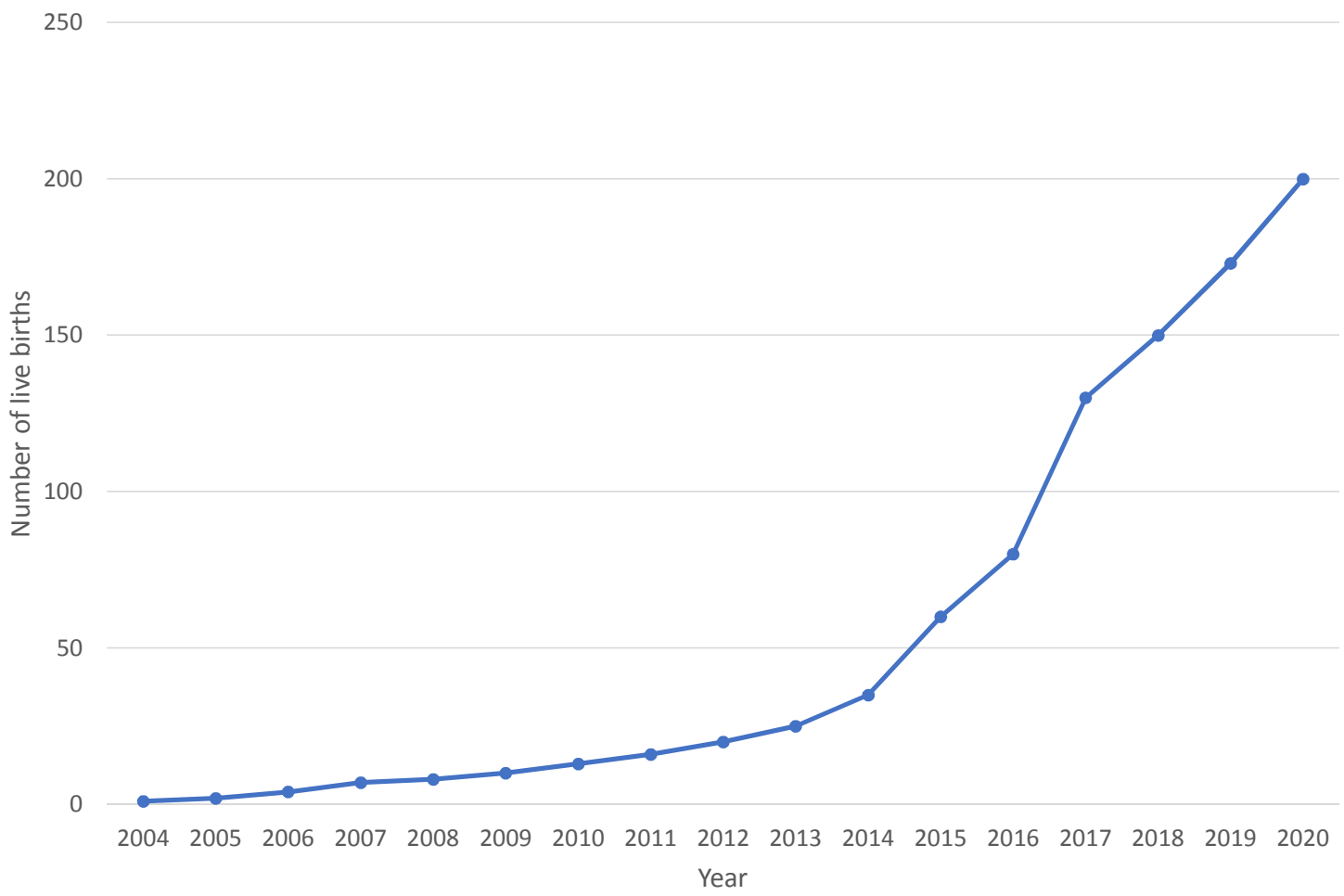


Figure 8

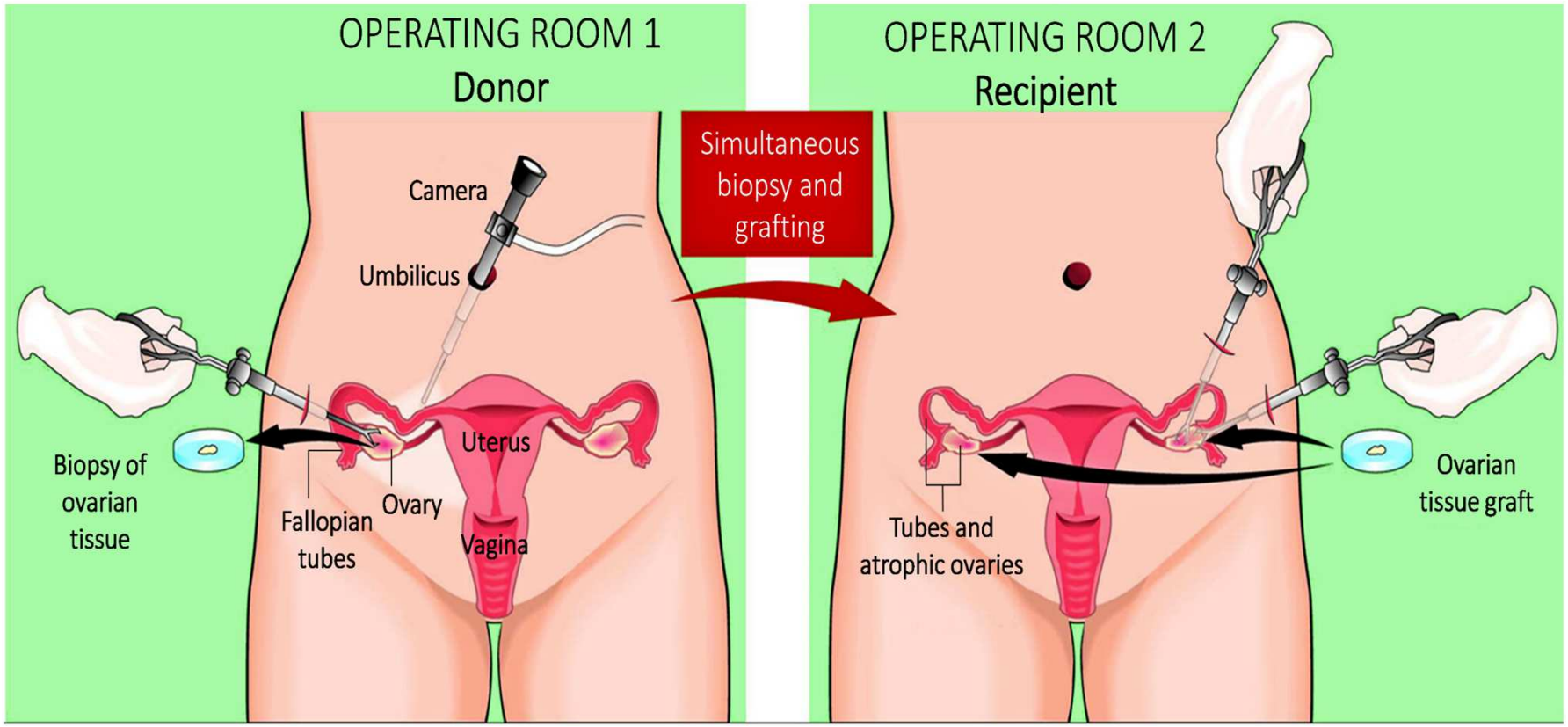
	Sheba Medical Center Tel Aviv	Cliniques Saint Luc Brussels	Infertility Center St Louis
Number of auto-transplantations	32	23	5

Total : 60 patients

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

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

Figure 9


OPERATING ROOM 1
Donor
OPERATING ROOM 2
Recipient
Simultaneous biopsy and grafting
Camera
Umbilicus
Biopsy of ovarian tissue
Fallopian tubes
Ovary
Uterus
Vagina
Tubes and atrophic ovaries
Ovarian tissue graft

HIGHLIGHTS

- Fertility preservation should be considered prior to each gonadotoxic treatment
- Embryo and oocyte cryopreservation are efficacious
- Both techniques need time for ovarian stimulation
- If no time for ovarian stimulation, ovarian tissue cryopreservation should be proposed
- Ovarian tissue cryopreservation should be proposed for prepubertal girls
- Orthotopic transplantation of frozen thawed tissue allows natural conception