
بسم الله الرحمن الرحیم

Female Fertility Preservation

Introduction

- Reproductive capacity may be seriously affected by age, different conditions, including genetic syndromes, and also by treatments, especially those with gonadal toxicity.

- Fertility preservation (FP) is a fundamental issue for individuals of reproductive age, both male and female, or prepubescent boys and girls whose future fertility may be compromised.

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

Indications for FP

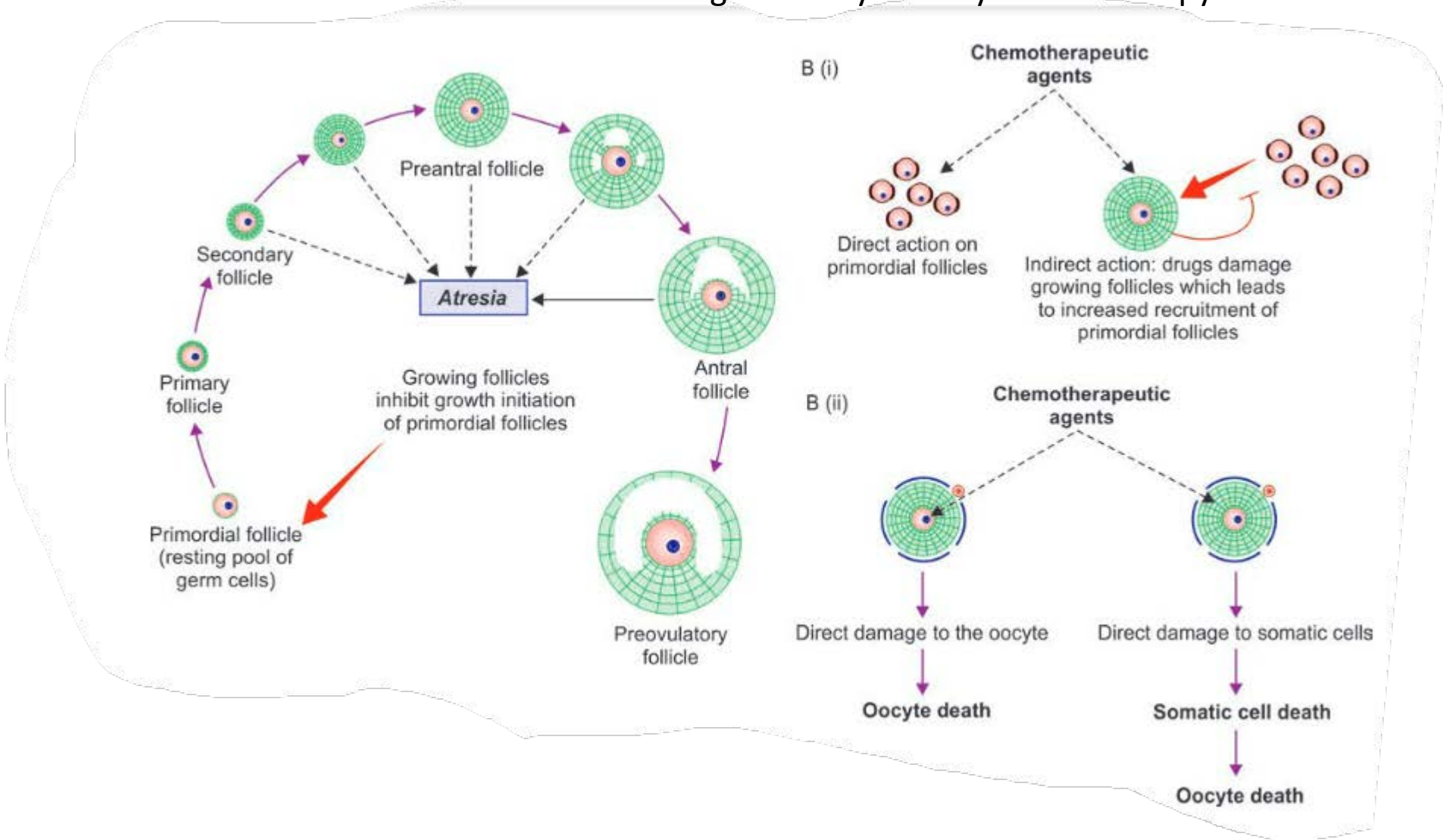
1. Cancer

- Many forms of cancer are associated with impaired ovarian function at the time of cancer diagnosis. However, the main effect on fertility arises from commonly used treatments such as chemotherapy with alkylating agents and pelvic radiation that present well-known gonadotoxic side effects.

- Gonadal failure resulting from these treatments may affect different aspects of reproductive health, including:
  pubertal development, hormone production, and sexual function

- The fact that more than 80% of children and adolescents with cancer become long-term survivors has raised an increased interest in the long-term effects of cancer treatment on fertility.

Mechanism of cellular damage in ovary after cytotoxic therapy



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Chemotherapy and radiotherapy may induce premature ovarian insufficiency (POI) in women. Ovarian damage is drug- and dose-dependent and increases as the patient ages.

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Radiotherapy not only damages the ovaries but also reduces uterine vascularity and causes myometrial fibrosis, endometrial damage, and reduction in uterine volume.

- It had been seen that an irradiation dose of as less as 2 Gy can destroy 50% of the primordial follicles in the ovary.
- Abdominal irradiation dose of 20–30 Gy in childhood resulted in 97% of women to suffer from ovarian failure and 67% of women who received irradiation in prepuberty to suffer from ovarian failure.

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2. Non-oncological conditions requiring fertility preservation

Autoimmune diseases

Systemic lupus erythematosus (SLE)
Behcet's disease
Churg-Strauss syndrome (eosinophilic granulomatosis)
Steroid resistant glomerulonephritis
Granulomatosis with polyangiitis (formerly Wegener's granulomatosis)
Inflammatory bowel diseases
Rheumatoid arthritis
Pemphigus vulgaris

Hematopoietic stem cell transplantation

Autoimmune diseases unresponsive to immunosuppressive therapy
Hematological diseases (sickle cell anemia, thalassemia major, plastic anemia)

Medical conditions causing POI

Ovarian oophoritis
Benign ovarian tumors
Mosaic Turner's syndrome
Fragile X Mental Retardation 1
Galactosemia
Beta-thalassemia, Endometriosis

- Hematological and autoimmune conditions, usually require chemotherapy or radiotherapy, especially for those in need of a bone marrow or hematopoietic stem cell transplantation

- POI in women with autoimmune disease is also affected by disease duration and presence of anti-Ro and anti-U1RNP (ribonucleoprotein) antibodies

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Endometriosis:

Endometriosis is associated with declining fertility, not only due to the disease process but also because of the corrective surgeries

The local inflammation caused by the endometrioma increases the follicular burnout and the cystectomy surgery for removal of the endometrioma has a negative impact on the ovarian reserve due to loss of germinal vesicles (GV) attached to the cyst.

Fertility preservation should be offered in endometriosis if:

- There is quantitative alteration in ovarian reserve due to repetitive surgeries
- Recurrent endometriosis
- Bilateral endometrioma
- A large (>5 cm) unilateral endometrioma

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Delayed childbearing

Female fertility starts declining after the age of 32 years and this decline accelerates after the age of 37 years. Women who delay childbearing should therefore consider oocyte freezing in order to preserve their fertility.

Gender reassignment procedures

Sex affirmation procedures in transgender men who have been assigned female at birth involve surgical removal of ovaries and cross sex hormone therapy. This has a deleterious effect on the fertility of these individuals.
Therefore, fertility preservation may be offered to them before they undergo any of the sex changing treatments.

Techniques of Fertility Preservation in Females

The choice of the optimal method for fertility preservation depends on the time available to start cancer treatment,cancer type and its treatment, age of the patient, and presence or absence of a partner.

Ovarian stimulation

Both, embryo and oocyte cryopreservation (slow freezing or vitrification) are first-line FP methods.

Unlike oocytes, the embryos are sturdy and can safely withstand the process of vitrification and thawing. They have over 90% survival rate post thawing and 40% of them implant in the first attempt. Cumulative pregnancy rate with frozen embryos is above 65% in infertility patients.

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- It should be noted that fertilized oocytes can only be transferred to the woman after the consent of both partners, which is why preserving some oocytes in an unfertilized state should be considered, even in the case of a stable partnership.

- The decisive factor in ovarian stimulation is maximization of the oocyte yield and minimization of the complication rate, so that oncological treatment can be started immediately after follicular aspiration.

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- It requires 2 weeks for ovarian stimulation, followed by egg retrieval and cryopreservation This can be a limitation for women with highly malignant tumors requiring immediate initiation of treatment and in prepubertal girls.

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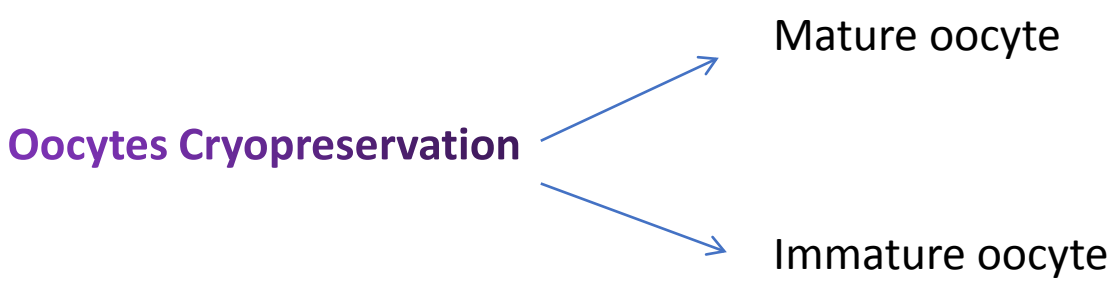

Mature oocyte cryopreservation:

It is the preferred method in postpubertal girls and in women without partners, provided enough time is available for the whole procedure of oocytes collection prior to starting cancer treatment, which takes about 2 weeks.

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Immature oocyte cryopreservation and IVM:

IVM is a technique where immature oocytes are retrieved transvaginally from either unstimulated or minimally stimulated ovary.

It takes 24–48 hours for the immature oocytes to mature in vitro once kept in a specially formulated medium. Results are better if priming with HCG is done prior To retrieval. Mature oocytes are then cryopreserved and if partner sperms are available, then embryos are first formed and then cryopreserved.

This technique is important for a woman who needs to start anticancer treatment immediately or who has a hormone-sensitive tumor.

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- For IVM, the chances of success with a fresh transfer are relatively high in some centres. However, after additional oocyte cryopreservation, which is necessary for use as a fertility preservation measure, the success rates are low.

- Cao et al. reported that only 13% of in vitro-matured, cryopreserved, and fertilized oocytes developed into embryos compared to 33% without prior cryopreservation.

- Alternatively, IVM can be used to aspirate the oocytes from the follicles in ovarian tissue prior to its cryopreservation.

- Because of low blastocyst development rates and birth rates are considered, it is evident that this method is not very efective.

- The standard protocol for stimulation is the antagonist protocol with ovulation induction using GnRH agonists (triptore_x0002_lin 0.2 mg s.c.) to minimize the risk of ovarian hyperstimulation syndrome (OHSS).

- Ovarian stimulation can now be initiated at any time during the menstrual cycle.

- In addition, double stimulation and stimulation directly after ovarian tissue removal are also possible.

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- According to a study by Kuang et al. pregnancy rates after stimulation start in the luteal phase are similarly high to those after a stimulation start in the early follicular phase and the malformation rate is unaffected.

- According to studies to date, stimulation with stimulation starting in the luteal phase takes 1–2 days longer, and a slightly higher gonadotropin dose is required per day than for a stimulation start in the early follicular phase.

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Depending on the cycle phase, stimulation can be carried out as follows:

- Stimulation start in the early to mid follicular phase: the conventional antagonist protocol with HMG or FSH,addition of the antagonist when control follicle ≥ 14 mm and GnRH-agonist triggering with triptorelin 0.2 mg s.c. when 3 follicles ≥ 17 mm. Stimulation dose is approx.50 IU higher than for an intended fresh transfer.

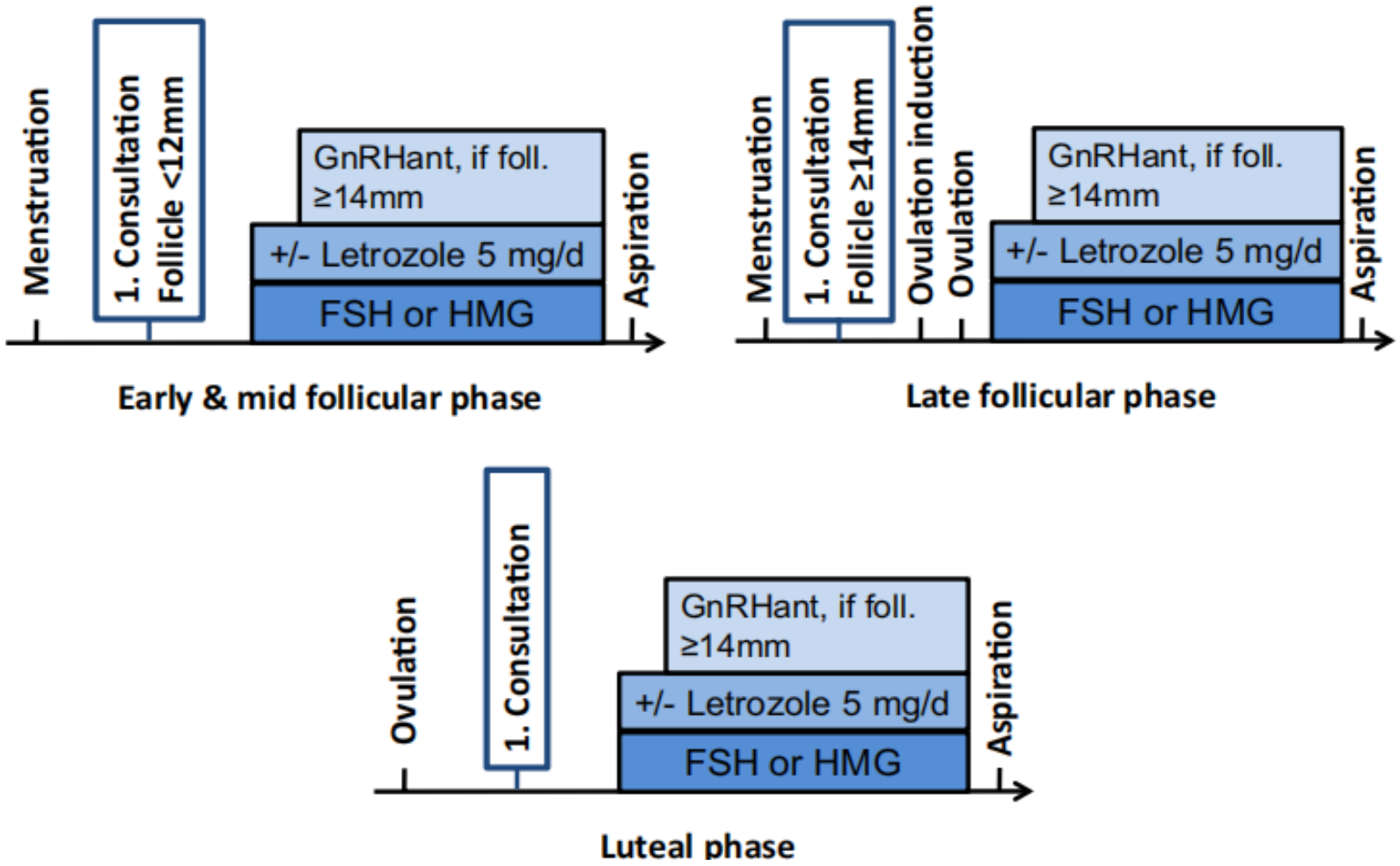
- Stimulation starts in the late follicular phase with a leading follicle ≥ 14 mm: ovulation induction with follicular size of 14 mm with triptorelin 0.2 mg s.c. followed by luteal phase stimulation immediately after ovulation.

• Stimulation start in the luteal phase: the conventional antagonist protocol with hMG or FSH and GnRH-agonist triggering with triptorelin 0.2 mg s.c. Stimulation dose is slightly higher than after stimulation start in the early follicular phase. Start of antagonists as soon as the newly developed leading follicle is ≥ around 14 mm.

Double stimulation

Stimulation with a classical antagonist protocol as well as ovulation induction with a GnRH-agonist is initially performed. It can be assumed that the first stimulation can also be started in any cycle phase (random start stimulation). Small follicles are not aspirated.

A second stimulation is started around 5 days later. To exclude premature ovulation, GnRH antagonists are administered as soon as the leading follicle exceeds 14 mm. The time required for double stimulation is ca. 30 days.



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Reducing the estradiol concentration in estrogen-sensitive tumours

To reduce the increasing estrogen concentrations during ovarian stimulation, the addition of aromatase inhibitors, e.g., letrozole 5 mg (2.5 mg each morning and evening from the first day of stimulation) is recommended.

The number of mature oocytes obtained and their fertilization capacity is not reduced by the addition of letrozole.

The previous studies have not shown increased malformation rates in children after low dose stimulation with letrozole.

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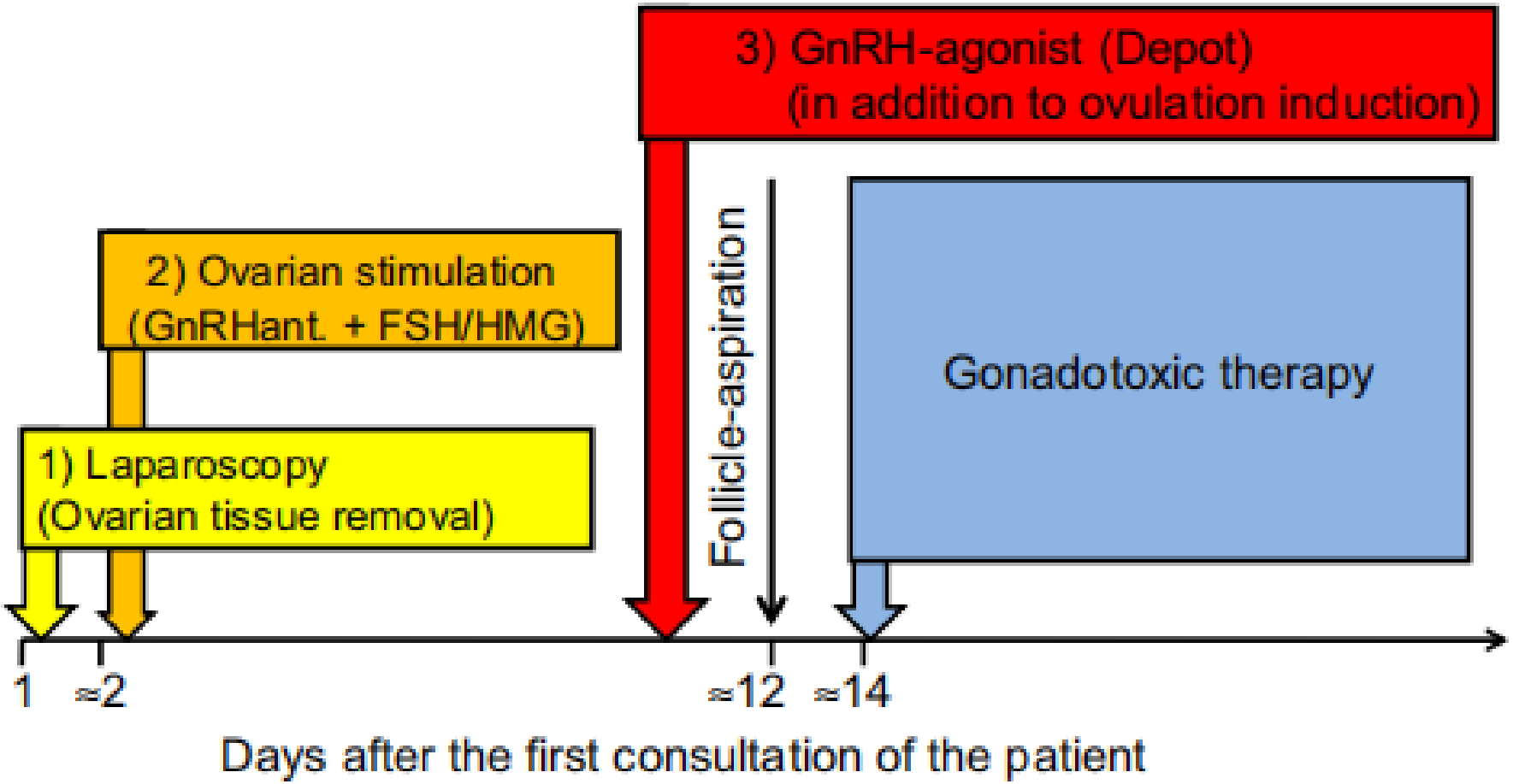
Combination of ovarian stimulation with the removal of ovarian tissue

Ovarian stimulation can be combined with cryopreservation of ovarian tissue to increase the success rate after highly gonadotoxic treatment.

50% of an ovary is removed laparoscopically and ovarian stimulation is started 1–2 days later. According to the studies carried out so far, there is no increased complication risk and the number of oocytes obtained is not significantly reduced after removal of ovarian tissue.

The time required for the combination of both treatments is about 2.5 weeks.



Combination of the three main techniques to preserve fertility

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Cryopreservation and transplantation of ovarian tissue

If there is a suffciently high ovarian reserve, part of the ovarian tissue can be cryopreserved for later transplantation. Since the cryopreserved tissue volume is not very large and some of the follicles degenerate during cryopreservation and transplantation, the transplants are only active for a few years.

Therefore, the transplant does not serve to restore long-term ovarian function, which would replace hormone replacement therapy, but only to achieve a pregnancy.

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As per the ESHRE Guidelines on Female Fertility Preservation (2020) OTC can be offered under the following conditions:

■ In patients undergoing gonadotoxic treatment where oocyte/embryo cryopreservation is not feasible or if the patient so prefers.

■ In patients with POI-associated genetic and chromosomal disorders Ovarian tissue cryopreservation may also be considered in prepubertal girls.

However, OTC should not be offered to patients with low ovarian reserve (AMH <0.5 ng/mL and AFC <5) or advanced age (>36 years).

- Transplantation for the induction of puberty as well as postponement of the menopause have been reported; however, since transplantations for these indications are of limited use endocrinologically , the cryopreservation of ovarian tissue should only be performed to establish a fertility reserve.

- In principle, the higher the ovarian reserve (i.e., the follicle density in cryopreserved and transplanted ovarian tissue), the greater the chances of pregnancy; therefore, cryopreservation of ovarian tissue is ideal for younger women or even children.

- The upper age limit is often stated as 35 years,although this limit can be around 38 years for women with a high ovarian reserve.

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Efficacy

The data for assessing efectiveness are still limited. However, since data from previously published case series show similar success rates, the probability of birth per transplant can already be roughly estimated.

In a case series from Denmark with 41 women, 53 transplantations were performed. A total of 42 transplants were performed in 32 women because of a wish to conceive.31% of transplanted women gave birth to at least one child.

In a case series from the FertiPROTEKT network, 95 transplants were performed in 74 women . A sub-analysis of the 40 women who received their frst transplant for POI included 11 pregnancies and 9 births. This corresponds with birth rate of 23% per transplant.

A case report has been published that reported a birth after the cryopreservation of ovarian tissue at the age of 13 years (premenarchal) and a transplant at the age of 27.

This case report shows that transplantation can lead to pregnancies even after prepubertal cryopreservation of ovarian tissue. This is supported by two case reports, which h ave shown an induction of puberty by the transplantation of prepubertal cryopreserved ovarian tissue.

Risks

The removal and transplantation of ovarian tissue requires a laparoscopy, the transplant another laparoscopy, possibly a laparotomy. When removing ovarian tissue, there is an increased risk of infection and bleeding depending on the oncological disease (e.g., in leukemia patients).

According to the FertiPROTEKT register, one complication per 500 laparoscopies which necessitates surgical revision is expected for the removal of ovarian tissue.

The surgical risks for the transplantation of ovarian tissue are not higher than for any other laparoscopy.

Based on knowledge of the ovarian metastasis potential of oncological diseases and based on systematic studies of cryopreserved ovarian tissue, a preliminary, still incomplete risk category for ovarian metastases has been developed for individual oncological diseases.

In high-risk cases:

cryopreservation of ovarian tissue should be considered as experimental and the patient should be informed that the tissue might not be used or can only be used after further establishment of the techniques mentioned below.

Risks of ovarian metastasis

Low risk	Medium risk	High risk
Breast cancer stage I–II and infiltrating ductal subtype	Breast cancer stage IV and infiltrating lobular subtype	Leukaemia
Squamous cell carcinoma of the cervix	Colon cancer	Neuroblastoma
Hodgkin's lymphoma	Adeno carcinoma of the cervix	Burkitt lymphoma
Osteogenic carcinoma	Non-Hodgkin's lymphoma	Ovarian carcinoma
Wilms tumour	Ewing sarcoma	
Non-genital rhabdomyosarcoma		

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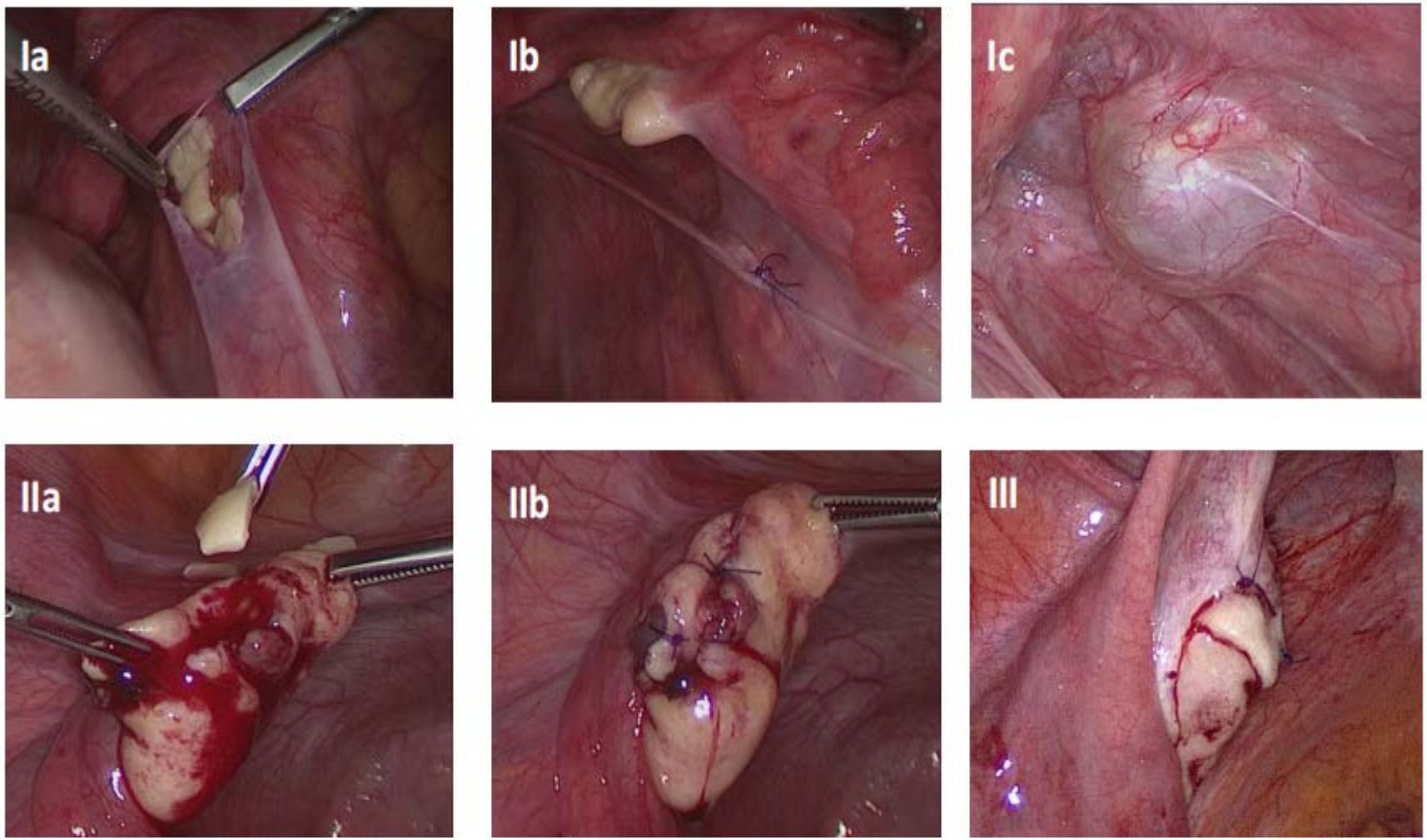
Transplantation of ovarian tissue

The number of tissue fragments to be transplanted is determined using the ovarian reserve, and if possible the follicular density after histological determination, and the age of the patient at the time of removal of the tissue.

The amount of ovarian tissue to be transplanted usually corresponds to approximately 15–25% of an entire ovary.

The ovarian tissue is mainly transplanted orthotopically, i.e., into the pelvic wall lateral to the ovaries, into or onto the ovary . A transplant onto the ovary best imitates the physiological anatomy, but requires the greatest laparo_x0002_scopic-microsurgical expertise and the duration of surgery (1–2 h) is the longest. A transplant into the pelvic wall is the least physiological localization, but surgically the easiest to perform and the operation only takes about ½–1 h.



Transplantation of ovarian tissue I: subperitoneally in the ovarian fossa (Ic: 12 months later), II: into the ovary, and III: onto the ovary (University women's hospital, Bern, Switzerland)

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Which localization leads to the highest chances of pregnancy is still unclear.

How much ovarian tissue should be transplanted is also still open to discussion. In the transplantations that led to a pregnancy in the FertiPROTEKT network, approximately 15–20% of the amount of an entire ovary was transplanted.

During the transplantation, the patency of the tubes should be checked and, if necessary, a hysteroscopy should also be considered.

Follow-up after transplantation

The first signs of ovarian activity are seen after about 3months. If the tubes are open and no other relevant sterility factor is present, a spontaneous pregnancy may be attempted.

If a pregnancy does not occur or a different relevant sterility factor is present, then IVF, possibly combined with ICSI, can be carried out.

Since it is currently unclear which transplant location is ideal and how much tissue should be transplanted, the transplantation of ovarian tissue should only be carried out within the scope of clinical trials if possible.

Fertoprotective agents

- GnRH analogues/agonists (GnRH-a) may protect follicles from destruction during chemotherapy, probably by suppression of gonadotropin levels and reduction of utero-ovarian perfusion (Meirow et al., 2007). These agents have long been used for the prevention of ovarian damage, despite their efficacy being a subject of debate owing to inconsistent results from randomized trials using GnRH-a.

Update on fertility preservation from the Barcelona International Society forFertility Preservation–ESHRE–ASRM 2017.

- The effect of GnRH-a is based on the hypothesis that the resulting pituitary down regulation and “inactivation” of the ovarian activity would lead to a reduced sensitivity to cytotoxic effects. However, activation of the primordial to secondary follicles is gonadotropin-independent, so a protective influence cannot be plausibly explained this way. Thus, the mode of action of GnRH-a is currently unclear.

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- ❑ After the initial GnRHa application, a gonadotropin fare up is noted. However, whether this fare up is relevant is currently unclear, as the primordial follicles are not gonadotropin sensitive.

- ❑ GnRHa are usually administered s.c. or i.m. as monthly or 3-monthly depots.

A depot-GnRHa should be repeatedly injected, so that the downregulation lasts about 1–2 weeks beyond the end of the last chemotherapy cycle.

Ovarian transposition

- The aims of ovarian transposition are the preservation of hormonal ovarian function and the possibility of pregnancy, even after oncological treatment.

- The effects of radiotherapy on ovarian function are considerable. A dose of 2 Gy to the ovaries (LD50) reduces the follicular density by half.

➢ The dose for induction of a premature ovarian insufficiency in a 30-year-old woman is specified as 16 Gy.

- Ovarian transposition should be considered when targeted radiotherapy is performed in the pelvic area. In addition to the benefits and risks, alternatives such as cryopreservation should be discussed.

➢ A combination of different fertility preservation techniques is also possible.

- Ovarian transposition is not appropriate in patients who undergo total body irradiation. Pelvic radiotherapy is often performed in Hodgkin's and non-Hodgkin's lymphoma, rectal cancer, Ewing's sarcoma of the pelvis, and cervical cancer.

- The importance of the new position of the ovaries has been proven. The distance from the radiation field Is decisive, since 10% of the radiation dose is still active at a distance of 10 cm from the radiation field.

- Radiotherapy to the entire pelvis, despite transposition, is significantly more likely to lead to ovarian failure than localized after loading (35 versus 6%).

- In this respect, close collaboration between the surgeon and radiologist is necessary before the planned transposition.

- The positioning height of the ovary is also a relevant prognostic factor. In a multivariate analysis, the positioning height with an odds ratio of 11.7 was the most relevant prognostic factor for the ovarian function.

- The ovary should lie at least 2 cm above the iliac crest. In addition, a safety margin of approximately 2 cm must be included, because the position of the ovaries can change postoperatively.

- The surgical risks of ovarian transposition are low. In most cases, the procedure is possible via laparoscopy. If laparotomy is performed because of another indication, ovarian transposition can be carried out simultaneously without a substantial increase in the complication rate.

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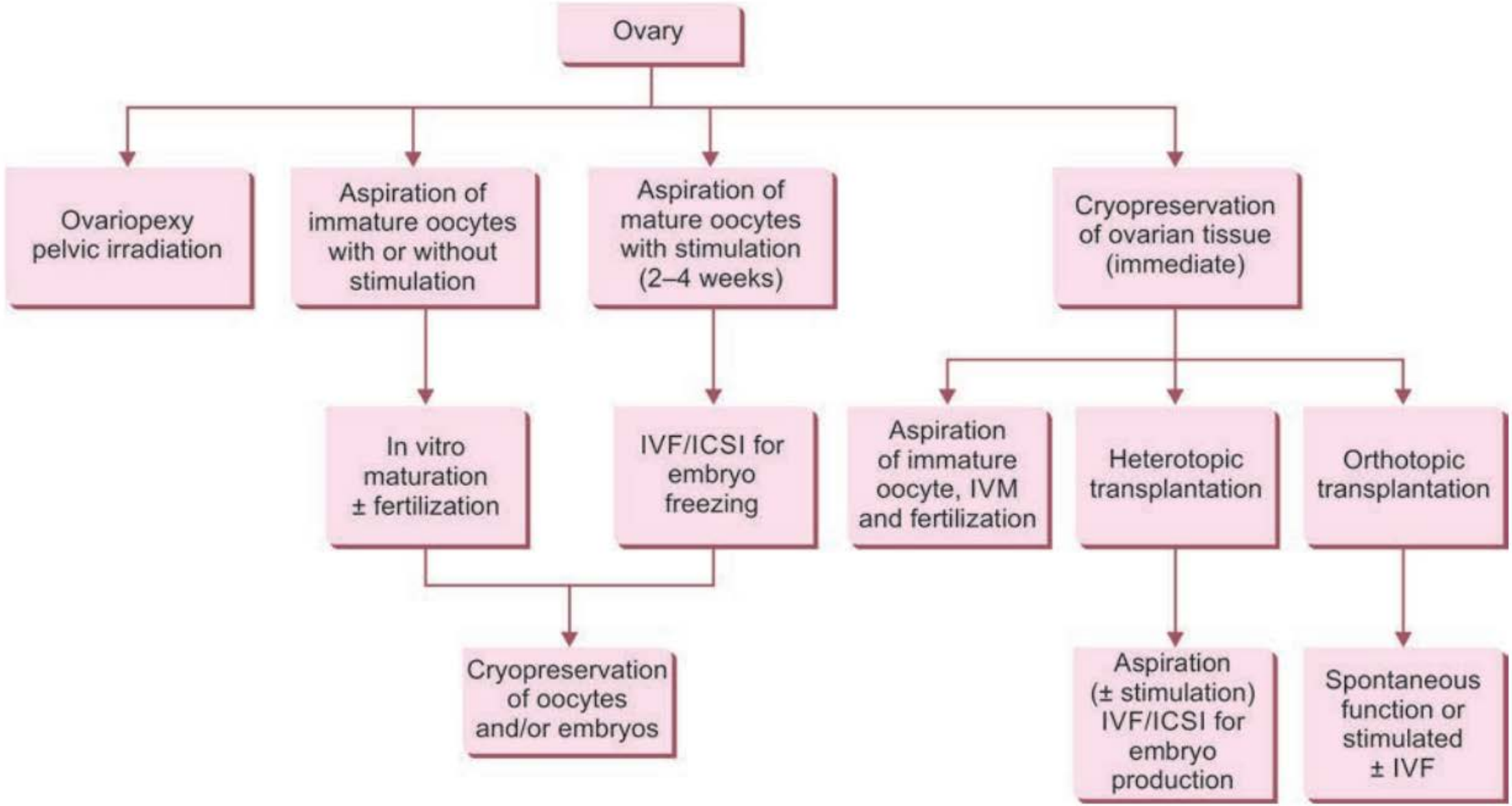
Options for fertility preservation in women



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Various techniques are being developed to enable the use of ovarian tissue in patients with haematological cancers with the purpose of avoiding the transmission of tumour cells.

However, these techniques, which are shown in Table, are still purely experimental.

Technique	Detail	Effectiveness in animal model	Effectiveness in human system
Xenotransplantation [31]	Transplantation of ovarian tissue into immunodeficient animals for to generate oocytes, especially in diseases with a high risk of tumour cell contamination of ovarian tissue	Development of offspring in mouse/rat model	Oocytes were obtained after transplantation into SCID mice. So far, no generation of embryos
In vitro growth (IvG) [57]	Cultivation of ovary tissue or isolated follicles to generate oocytes, especially in diseases with a high risk of ovarian tumour cell contamination	Development of offspring in mouse model	Development of embryos from primates in the mouse. Development of human germinal vesicles in the mouse
"Artificial ovary" [58, 59]	Isolation of preantral follicles from ovarian tissue, fixation in a matrix and orthotopic transplantation of the matrix Especially in diseases with a high risk of ovarian tumour cell contamination	Development of follicles in the mouse	No application in the human system as yet

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متشکرم!