

Seminar Article

Assisted Reproductive Technology for Women Seeking Fertility Preservation

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Introduction

Procreation and parenthood has always been central to human culture and society. There are many who experience situations that affect their future pregnancy adversely. These range from voluntary choice to delay conception till a later date or diseases and treatments that affect future fertility. Earlier there were not too many options for fertility preserving other than planning pregnancy early or cryopreserving sperm. With the advent of assisted reproductive technologies (ART) new tools for fertility conservation have emerged; ovarian cortex, oocyte or embryo cryopreservation. Surgical and pharmacotherapeutic advancements have helped in improving the scope of fertility preservation in oncology.

Indications for Fertility Preservation

- Cancer
- Diseases that can cause premature ovarian insufficiency or testicular failure
- Auto immune diseases
- Prior to myeloablative techniques for hematopoietic stem cell therapy
- Hematologic diseases like thalassemia, sickle cell anemia
- Severe Gonadal damage- iatrogenic/traumatic
- Gender reassignment procedures

Available Fertility Preservation (FP) Strategies

- Cryopreservation Technologies
 - Semen /testicular tissue Cryopreservation
 - Embryo Cryopreservation
 - Mature Oocyte Cryopreservation

- In-vitro maturation
- Ovarian Tissue Cryo preservation
- Surgical strategies
 - Fertility sparing surgery
 - Uterine transplant
- Medical adjuvants to chemotherapy
 - Use of gonadotropin-releasing hormone agonists

Cryopreservation Technologies

1) Semen/Sperm cryopreservation

Semen is cryopreserved most often prior to chemotherapy or radiotherapy for cancer as these are known to affect the quality and quantity of gametes significantly. This is the established fertility preservation technique for men who can produce a semen sample. Some men cryopreserve sperm as an 'insurance policy' or 'back up' before a fertility treatment cycle as they may not be able to provide a sample on demand or when the quality of the semen parameters have been highly variable earlier.

Spermatozoa obtained for cryopreservation are obtained by masturbations or after stimulation when with ejaculatory problems, or sperm retrieved by various techniques like Percutaneous epididymal sperm aspiration (PESA), Testicular aspiration (TESA), testicular biopsy and sperm extraction etc.

When an ejaculate is obtained; the semen liquefies, the parameters are analyzed, and motile viable sperms are isolated by swim-up or density gradient methods and then cryopreserved. The sperm pellet obtained after processing is mixed with cryoprotectants and are frozen. Two most commonly used methods are slow freezing and rapid freezing. The slow freezing technique was introduced by Behrman and Sawada¹ which involves cooling sperm over a period of 2–4 hours in two or three steps, manually or automatically

using a semi programmable freezer. Rapid freezing proposed by Sherman² involves exposure of sperm containing straws to nitrogen vapors for 8–10 min and immersion into liquid nitrogen at -196°C .

Smaller number of spermatozoa are obtained in sperm retrieval techniques. They must be cryopreserved by novel approaches involving biological or non-biological sperm carriers. ICSI pipettes, mini straws, cryo loop, agarose microspheres, empty zona pellucida are used as a carrier for freezing small number of spermatozoa.³⁻⁷

Cryopreservation affects the DNA integrity of some of the stored spermatozoa.⁸ Never the less, the cryopreserved sperm have been used over decades to achieve pregnancies safely either by insemination or by IVF/ICSI.

Testicular tissue/sperm preservation in pre-pubertal boys

Testicular biopsy and tissue of immature testis are cryopreserved for pre-pubertal boys in whom semen cryopreservation is not possible. Testicular tissue cryopreservation should be recommended in pre-pubertal boys even though fertility restoration strategies by auto-transplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans.⁹

2) Embryo cryo preservation

Embryo cryopreservation for fertility preservation are opted by the couple where the female partner undergoing ovarian depleting or gonadotoxic treatment of oncologic origin. This is one of the first line strategies for post pubertal fertility preservation and the other being metaphase II (mature) oocyte vitrification which has high success rates.⁹ Women who wants to delay their childbearing but does not want to risk of not having adequate ovarian reserve undergo oocyte cryopreservation. Embryo cryopreservation requires the oocytes to be retrieved from the woman and spermatozoa to fertilize the mature oocytes. Hence, the woman needs to go through IVF and should have a source for spermatozoa- husband/partner or donor spermatozoa. The woman may need controlled ovarian stimulation which may delay the cancer treatment. Embryo cryopreservation is not suitable for pre-pubertal patients in whom ovaries are still pre-pubertal.¹⁰

Embryo cryopreservation provides a good success rate depending on the number and quality of embryos stored. A retrospective analysis done by Cardoza et al in 2015¹¹ noted a 37% pregnancy rate and 30% cumulative live birth rate in cancer patients who underwent Frozen Embryo Transfer (FET) which was comparable to 43% pregnancy rate and 32% cumulative pregnancy rates ($p=0.49$ and $p=0.85$ respectively) in women undergoing IVF for tubal infertility. The cancer patients were found to have significantly higher rates of twinning ($p=0.035$) which may have been because there were no underlying factors causing infertility.

3) Mature Oocyte Cryopreservation

Oocyte preservation is a good option for women to maintain reproductive autonomy. Metaphase II oocyte vitrification is considered the best technique.⁹ Here the woman undergoes controlled ovarian (COS) stimulation, trigger and oocyte retrieval and the mature oocytes are cryopreserved. Hence it goes without saying that mature oocyte cryopreservation is preferred only in women in whom COS is safe and is expected to respond well.

The advancements in the freezing and thawing techniques, especially the introduction of vitrification techniques, have considerably increased the pregnancy rates from cryopreserved, warmed and fertilized oocytes. Cobo et al¹² and Rienzi et al¹³ noted that the implantation rates and pregnancy rates of embryos derived from fresh versus vitrified oocytes were comparable.

Both embryo and oocyte cryopreservation cannot be performed on prepubertal girls as they are not suited for ovarian stimulation or for transvaginal oocyte retrieval.

Ovarian stimulation for embryo or mature oocyte cryopreservation

Ovarian stimulation for fertility preservation is advocated only if it is safe for the patient and ovarian response is expected to be good. Gonadotropin-releasing hormone (GnRH) antagonist protocols are referred as they are shorter, cost effective and yield outcomes comparable to the longer GnRH agonist protocol. Dose of gonadotropins can be decided based on age, ovarian reserve and body mass index to get an appropriate response.

When fertility preservation is to be done especially when the patient has cancer, time constraints may pose limitations in waiting till a conventional day 2 start of COS. In such scenarios, luteal phase stimulation and random start protocols may be used so that the wait till menstruation is avoided. In the luteal phase protocol, GnRH antagonist is given for 3–4 days to achieve a quick down-regulation and controlled ovarian stimulation (COS) is started subsequently with or without the onset of a menstrual bleed. The random start protocol was introduced by Cakmak et al¹⁴ where COS is started as soon as the patient is ready irrespective of the menstrual cycle phase she is in at that point of time. The follicles secondary to the lead follicles are followed and when they reach a size of 12 mm GnRH antagonist control is started till oocyte trigger and retrieval is done. Normal follicular growth and development is observed despite the increased progesterone levels seen in the luteal phase or a spontaneous luteinizing hormone surge, which may occur when the initial lead follicle reaches maturity. The random and conventional start cycles were comparable in the number of eggs retrieved, mature oocyte yield, and fertilization rates. The duration of stimulation is needed till oocyte retrieval are longer compared to the conventional start COS.

Anti-estrogens; letrozole and tamoxifen-based COS or addition to routine COS are associated substantially to reduced peak estradiol levels, making them a safer and effective protocol in women with estrogen sensitive cancers.¹⁵⁻¹⁶

4) In vitro maturation (IVM) of immature oocytes

Here immature oocytes are retrieved either invitro after minimal or no stimulation or ex vivo from ovarian tissue (ovarian cortex biopsy or oophorectomy specimen) followed by IVM and fertilization and embryo cryopreservation or mature oocyte freezing. Ex vivo immature oocyte retrieval and IVM and preservation is a good strategy for prepubertal girls requiring fertility preservation. Immature oocyte aspiration and invitro maturation for IVF is most commonly used in PCOS patients to retrieve more oocytes without ovarian stimulation, thereby completely avoiding the risk of ovarian hyperstimulation syndrome. This is a promising technique to preserve fertility in cancer patients who do not want to risk ovarian stimulation and in prepubertal girls. It also offers hope to preserve fertility in prepubertal girls who have had hematological malignancies like leukemia where ovarian tissue cannot be transplanted due to risk of reintroduction of cancer cells.

First live birth from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient was achieved in Singapore and the case report was published in 2014.¹⁷ The first ongoing pregnancy from fertilization of IVM oocyte was reported in by Segersl et al in 2015.¹⁸ They had retrieved immature oocytes from oophorectomy specimen of 34 patients and had a mean immature oocyte yield of 14 per patient. The IVM rate was 36%, fertilization rate 64% and at least one good day 3 embryo could be frozen in seven of eight couples who underwent embryo cryopreservation. One of these patients conceived and had an ongoing pregnancy in 2015.

Giovanna Fasano et al¹⁹ assessed the efficiency of oocyte in-vitro maturation (IVM) and vitrification procedures. They studied 130 adults and six prepubertal girls. A higher mean oocyte yield was obtained from the girls compared with adults (11.5 ± 8.0 versus 3.8 ± 4.2 , respectively, $P < 0.001$) but degenerated oocytes were significantly higher in girls (35.5% versus 17.1%, respectively, $P < 0.001$). IVM rates were significantly more in the post pubertal compared to pre-pubertal girls (28.1% versus 10.3%, $P = 0.002$).

5) Ovarian tissue cryopreservation

Ovarian tissue cryopreservation (OTC) is procuring and the procedure involves freezing of ovarian cortical tissue. This technique has many advantages over oocyte and embryo cryopreservation. It does not delay the start of cancer therapy and avoids the risk of ovarian stimulation. There is no need for partner or donor sperm.

The disadvantage is the chance of reintroduction of cancer cells when tissues are auto transplanted. Hence auto transplantation is not advised in women who have

been treated for leukemia. The ovarian cortex is dissected, free cut in to small fragments and are either vitrified or frozen by a slow cooling technique. This ovarian cortex may be harvested leaving the ovaries behind or be done ex vivo after performing oophorectomy. OTC can be done both in prepubertal children and adults. The tissue is later warmed and transplanted into the pelvis- either onto ovarian remnant or pelvic peritoneum (orthotopic) or subcutaneously in the fore arm or abdominal wall (heterotopic), when the patient is fit and chooses to regain fertility. Spontaneous pregnancies can occur following orthotopic ovarian tissue transplantation while IVF and oocyte retrieval, fertilization and then embryo transfer is required to achieve pregnancy from the heterotopic cortex transplant.

Complete restoration of ovarian activity has been seen in all women whose transplanted ovarian cortex had primordial follicles. The restored ovarian cortical function has been documented to last up to 7 years following transplant, with the women resuming cyclical menstruation. Jadoul P et al in 2017 reports that more than 86 live births from auto transplanted ovarian tissue have already been documented.²⁰ They analyzed 545 cases and found that ovarian tissue transplant resulted in a 30% pregnancy rate. Donnez et al in 2004 reported the first live birth following laparoscopic orthotopic transplant of cryopreserved ovarian tissue in a Hodgkin's lymphoma survivor who had gone into premature ovarian insufficiency due to her cancer treatment. The first live birth from orthotopic transplantation of cryopreserved ovarian tissue frozen prior to achieving menarche was reported in 2015.²¹ The ovarian tissue was cryopreserved at the age of 14 as the girl needed myeloablative treatment followed by hematopoietic stem cell transplantation to cure severe sickle cell anemia. The first ongoing pregnancy following IVF and ET from a heterotopic auto transplant of cryopreserved ovarian tissue was reported by Stern et al²² in 2013.

Surgical Strategies

Medical and surgical fertility sparing approaches are applied in cancer patients so that they have a reasonable hope to conceive naturally or with ART

Fertility sparing Surgery (FSS) in cancer patients

Ovarian transposition in women who need radiotherapy

In women who require pelvic or irradiation laparoscopy/laparotomy and ovarian transposition to above pelvic brim by mobilizing the ovaries after cutting the utero ovarian ligaments. When craniospinal irradiation must be given, the ovaries are fixed as laterally as away from the spine as possible. Titanium clips are placed on the borders of the ovaries for radiological identification. These women require IVF and transabdominal oocyte retrieval when they plan to conceive.

Cervical cancer

Radical trachelectomy (RT), and in selected cases conization and simple trachelectomy have been pioneered to treat early-stage cervical carcinoma as the fertility sparing option. While subsequent pregnancies have been reported, after RT. Fertility may be impaired by anatomical and physiological changes, such as adhesions, cervical stenosis and/or loss of cervical function. The surgical procedure can be followed by subfertility and a need for assisted reproduction.²³

Ovarian tumors

Fertility-sparing surgery by retaining one ovary or resorting to only partial cystectomy with extensive staging may be offered to young women with stage I ovarian epithelial cancer, after counselling about the risk of recurrence and need for complete surgery after family is completed.

Borderline ovarian tumors (BOT) account for 10–15% of all epithelial tumors, and these women are typically younger than 40 years of age when diagnosed. Although conservative surgery is done, most of these women require ART techniques in view of altered ovarian reserve or post-operative adhesions. In vitro data have suggested that gonadotrophins and/or high-dose estrogens do not induce proliferation in BOT cell cultures. Hence, IVF may be considered for patients who select conservative fertility-sparing management for borderline tumors as there is no evidence for any adverse effects of pregnancy on the course of BOT.²³

Endometrial carcinoma

In patients who desire fertility preservation with detailed counselling, conservative management with high-dose progestin treatment may be considered to allow a disease-free window in which to attempt pregnancy.

For younger patients, with a shorter duration of infertility and reassuring ovarian reserve without anovulation or severe male factor, spontaneous conception may be attempted for a limited time but may take several months, which can lead to anxiety about the risk of recurrent disease. Thus, assisted reproductive treatments may be performed for attaining early pregnancy. Efficient ART therapies have helped successful pregnancies to be increasingly reported.²³ The data that are derived from these cases do not seem to show worse prognoses as ART probably increases the chances of gestation and cuts the interval to conception.

Uterine Transplant

Uterine transplant is the surgical procedure where a healthy uterus is transplanted when the uterus is absent congenitally/ iatrogenically. Absolute uterine factor infertility (AUI) affects approximately one in 500 women of childbearing age. Till the advent of uterine transplantation, adoption or surrogacy were the only options for these women. In 2000, the first human uterine transplant was done in Saudi Arabia although

the uterus had to be removed within three hours.²⁴ The second uterine transplant was successfully done in Turkey in 2011, but although the graft survived under immunosuppressive therapy and the patient resumed menstrual cycles, no live birth occurred although IVF was tried multiple times.²⁵ The first series of livebirths following uterine transplants from close relatives were reported by the Swedish team headed by Matt Brannstrom in 2014.²⁶

Medical Strategy-Gonadotropin releasing Hormone (GnRH) Agonist

“Fertoprotective adjuvant therapy” is a term used for administration of adjuvant therapy during or prior to chemotherapy with an agent that can prevent loss of ovarian reserve. So far, the only drug used in clinical practice is the gonadotropin-releasing hormone (GnRH) agonist.²⁷⁻²⁸

Gonadotropin-releasing hormone agonist causes suppression of the gonadotropin levels to prepubertal levels and decreases utero-ovarian perfusion. It is frequently used in conjunction with chemotherapy hoping to minimize the gameto toxic effects. The GnRH agonist should be started at least 10 days prior to chemotherapy so that the suppressive effect is well established, and should be continued till the chemotherapy is over.¹⁰

The 2011 Cochrane database suggests considering the ‘off label’ use of GnRH agonists in women of reproductive age receiving chemotherapy.²⁹

Future Perspectives

Activation of follicles

A live birth has been achieved by IVF and ET in a woman with POI using a double approach which resulted in rapid folliculogenesis and mature oocyte production following auto transplantation of ovarian cryopreserved tissues (OTC)

- activating in vivo primordial follicles in by interrupting the Hippo signaling pathway mechanically using ovarian fragmentation/laser/ drilling
- In vitro primordial follicular activation of OCT prior to auto transplantation by acting on the PI3K-PTEN-AKT-FOXO3 pathway

In vitro follicle culture

Cryopreserved tissue transplantation carries the risk of re-seeding cancer cells into the patient. This risk can be minimized by using complete In Vitro Gametogenesis IVG and maturation of oocytes as a means of fertility restoration.

In Vitro Gametogenesis (IVG) by in situ culture of primordial follicles from cryopreserved tissue using three dimensional (3D) multi step culture methods have yielded meiotically competent metaphase II non-human primate and human oocytes. However, considerable researches are needed before the proven safety of using these oocytes.

Artificial ovaries

Instead of above (in vitro culture) primordial follicles may be engineered into an 'artificial ovary', consisting of preantral follicles and other ovarian cells assembled in a 3D matrix, or scaffold. Once transplanted to the patient, this 'artificial ovary' is expected to restore ovarian function and fertility.

New fertoprotective agents

Current researches focuses on agents with anti-apoptotic properties (imatinib, sphingosine-1-phosphate, and tamoxifen) and on agents that also prevent follicle activation such as, an immune modulator (AS101) that acts on the PI3K/PTEN/AKT follicle activation pathway and the anti-Mullerian hormone.⁹

In Vitro spermatogenesis

Spermatogonial stem cells (SSC) are cultured in 3D systems that resemble the in vivo situation. This is still in the early experimental stage.

Artificial gametes

The use of primordial germ cells (PGC) and Pluripotent stem cells (PSC) are potential sources of gametes. It is reported that the generation of haploid mouse spermatid-like cells are able to produce viable and fertile offspring.

Barcelona International Society for Fertility Preservation-eshre-asrm 2015 Expert Working Group Recommendations

- Patients suffering from cancer and non-cancer diseases that may affect their fertility, should be counselled regarding the potential fertility loss and should be referred to fertility specialists to discuss options for FP and current results
- Semen cryopreservation is the only established FP technique in men
- Embryo and oocyte cryopreservation are first-line FP methods in post pubertal women. Metaphase II oocyte cryopreservation (vitrification) is the preferred option.
- Cumulative evidence supports future use of orthotopic transplantation of cryopreserved ovarian tissue to regain ovarian function as well the fertility.
- Testicular tissue cryopreservation should be recommended in pre- pubertal boys even though fertility restoration strategies by auto transplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans.
- The international registries on the short- and long-term outcomes of FP should be established.

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