Preservation of Reproductive Function in the Female

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By OBGYN.net Staff [1]

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Introduction
The female reproductive function depends on the healthy ovaries (primary reproductive organ) and genital tract organs. Infertility is one of the main gynecological problems which affect between 10-15% of females in the reproductive age. Preservation of the reproductive function in the female begins by prophylactic prevention and efficient treatment of which lead to impaired reproductive function as: sexually transmitted diseases, pelvic inflammatory disease, and puerperal sepsis. And through screening and early detection of pre-malignant conditions of the genital organs which allow conservative management for these conditions and preservation of the reproductive function. Gonadal failure as a result of germ cell depletion can occur at any age, and from the effects of chemical cytotoxicity, disease, and infections, as well as genetic predisposition.(1) Advances in cancer therapy have improved the long-term survival of young patients suffering from malignancies. However the adverse effects of the treatment leads to sterility and loss of gonadal function due to premature ovarian failure.(2)

Cryopreservation of human gametes and embryos has become an integral part of assisted reproduction. The major developments in cryopreservation technology have mirrored the rapid expansion of reproductive technology over the past 2 decades. It is now possible to cryopreserve sperms, oocytes, embryos at their various stages of development and more recently the ovarian tissue. The ovarian tissue cryopreservation offers the hope of fertility preservation for women who are exposed to potentially sterilizing medical, surgical or radiological treatments. Parallelizing the
introduction of cryobiology to assisted reproduction; has been the realization of a number of moral and ethical issues related to gamete and/or embryo storage.\(^{(3)}\)

**Causes of Reproductive Function Impairment in the female**

The female reproductive function can be affected by different pathological factors which can be summarized as follow:

1. Congenital causes: as aplasia, atresia, and deformities
2. Traumatic causes: whether obstetric or general surgical trauma
3. Inflammatory causes: as sexually transmitted diseases, pelvic inflammatory disease, and chronic specific or non specific infections
4. Neoplastic causes: either benign or malignant tumors of the genital organs
5. Iatrogenic: either medical, surgical or irradiation
6. Miscellaneous causes: as obesity, cachexia, chronic medical diseases, chromosomal abnormalities, endometriosis, immunological, endocrinopathy and environmental toxicants

**Environmental Endocrine Disrupters and Ovarian Function**

Within the last decade it has been proved that environmental toxicants can mimic endogenous hormones and act as endocrine toxicants. Organochlorine chemicals as: pentachlorophenol, polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and hexachlorobenzene (HCB), were found in ovarian follicular fluid in infertile women.\(^{(4)}\) These disrupting chemicals can bind to steroids receptors with effect as estrogens, anti-estrogens, anti-androgens. These endocrine disrupters can hinder fertility through its effect on hypothalamus, pituitary gland, and reproductive tract of both sexes, as well as direct toxic effects on the conceptus. The ovary is of particular interest; because it is a dynamic organ (undergoing profound hormone regulated changes with each menstrual cycle. The recruitment of cohort of follicles from the pool of primordial follicles, folliculogenesis, steroidogenesis, and ovulation of the dominant follicle represent targets for dysregulation by endocrine toxicants. Selective destruction of primordial follicles is a serious consequence of exposure to endocrine disrupters, which can lead to premature ovarian failure in exposed women. Endocrine disrupters can have a serious consequences on ovulation, oocyte maturation, blastocyst development and cleavage, and luteal phase competency.\(^{(5)}\)

**Indications for reproduction preservation in women**

1. Chemotherapy and pelvic irradiation for malignant diseases as: , non-Hodgkin lymphomas, Breast carcinoma
2. Surgical extirpation of gonadal tissue
3. Pelvic disease as severe endometriosis
4. Incipient ovarian failure as premature menopause
5. Natural aging of the ovaries
6. Autoimmune diseases as systemic lupus erythematosus, treated by chemotherapy

The biggest factor is natural aging, as increased numbers of women are postponing pregnancy until ages when fertility has declined. This trend is most advanced in northern Europe and the peak age for child-bearing is now 30-36 years.\(^{(6)}\)

The oocytes fertility is often poor at that age and the IVF success rates are so low after 40 years age even though most women at this age still ovulate regularly.\(^{(7)}\)

The number of primordial follicles declines steadily from birth (approximately 1x10^6) until about 35 years of age (approximately 2x10^4). After 35 to 40 years of age, the rate of primordial follicle loss increases with the result that most women enter menopause (with no remaining follicles) between 45 to 55 years.\(^{(6)}\)

Premature menopause can be the result of:

1. Small starting pool of primordial follicles due to genetic predisposition as in Turner's syndrome (25% of cases)
2. Rapid depletion of the follicles due to mumps, oophoritis, auto-immune disorders, chemotherapy and radiotherapy
3. Surgical oophorectomy
Breast cancer is the most common malignancy in women of reproductive age, and of 180,000 new cases in USA each year, 25% occur before menopause and 15% are diagnosed in the reproductive age group many of them will be treated by chemotherapy as cyclophosphamide with its adverse effects on reproduction have been well established with the likelihood of immediate ovarian failure between 38-78% of cases.\(^8\) Even those who do not immediately become menopausal following chemotherapy, are likely to experience infertility and early menopause. As pregnancy is not recommended for at least 2-5 years recurrence-free interval after breast cancer treatment, by that time passed many women become infertile due to aging and diminished ovarian reserve.\(^9\) Hodgkin's disease is the most common malignancy in the population aged 15-24 years. Prolonged survival of >90% is now expected due to introduction of effective chemotherapy protocols. Premature ovarian failure (POF) is a common long term consequence of chemotherapy and radiotherapy due to progressive and irreversible damage to the germ cells, with its fixed number and inability to regenerate.\(^10\)

**Choice of the method for fertility preservation**

There is a range of alternative options to preserve fertility. Based on the type and timing of chemotherapy, the type of cancer, the patient's age and the partner status, a different strategy of fertility preservation may be needed.

If the patient has a partner or accepts donor sperm, embryo cryopreservation should be considered first, since this is a clinically well established procedure. When the patient is single, oocyte cryopreservation may be preferred to ovarian tissue banking. In breast cancer patients, tamoxifen or aromatase inhibitors can be used for ovarian stimulation prior to oocyte or embryo cryopreservation. In patients, aromatase inhibitors may be the only choice for ovarian stimulation.\(^11\)

When the pelvic radiotherapy is used, ovarian transposition can be preformed.

Fertility preservation should be an integral part of improving the quality of life in cancer survivors, however, it is neither possible nor ethical to recommend the same recipe for every cancer patient.\(^9\)

**Cryopreservation of oocytes**

**Introduction**

The cryopreservation of gametes and embryos involves an initial exposure to cryoprotectants, cooling to subzero temperatures, storage, thawing, and finally, dilution and removal of the cryoprotectants with return to a physiological environment which allows further development. The cells must maintain their structural integrity throughout the cryopreservation procedure. The single most important principle of cryopreservation is to reduce damage caused by intracellular ice formation. This is usually achieved by dehydrating the cells before or during the cooling procedure. If dehydration is inadequate the growth of large intracellular crystals of ice may occur which can damage the cells. Factors known to affect survival of cryopreserved cells gametes and embryos include: the species, their developmental stage, the type of cryoprotectant, and method of cryopreservation.\(^12\)

Oocytes and embryos of some, but not all, mammalian species can be cryopreserved. In the mouse it is possible to freeze immature, mature, and pronuclear oocytes to hatched blastocyst stage embryos without a significant reduction in viability. This degree of success has yet to be achieved for other species. Current research is therefore aimed at improving freezing procedures for gametes and zygotes of the human and other mammals, vertebrates, and invertebrates.

In vitro fertilization (IVF) programs for the human usually cryopreserve embryos. Storing unfertilized human oocytes has the potential advantage of preserving germ cells for individual women and avoids complications which may arise if partners separate (social, moral, and legal) oocyte freezing is, however, rarely performed because of the very poor prospects of achieving a pregnancy with frozen oocytes. This may change as ongoing research is aimed at developing simpler and more effective cryopreservation procedures for oocytes and embryos of a range of species including man. In 1957, Lin et al.\(^13\) demonstrated that the mouse oocyte can survive cooling to -5°C in a medium containing 5% glycerol. In 1958, Sherman and Lin reported the birth of live young following in vitro fertilization of mouse oocytes that had been "frozen" at -10°C in a medium containing 5% glycerol.\(^14\)

It was not the human oocyte, however, but the human embryo that was first to be successfully cryopreserved.\(^15\)

The first report of the birth of live young after storage of mouse embryos at -196°C in liquid nitrogen.\(^16\) The protocols that had been shown to work well with mouse embryos were largely
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It was in 1983 that the first paper on a pregnancy from a cryopreserved human embryo was published by Trounson and Mohr (1983). Unfortunately, a live birth did not ensue and it was the Dutch group of Zeilmaker and colleagues (1984) who reported the first recorded live birth after cryopreservation of human embryos. It was again David Whittingham (1977) who reported on the first successful cryoconservation of the mouse oocyte at -196°C in liquid nitrogen followed by the birth of live young. Based on these protocols, and encouraged by the successful start of human embryo cryopreservation, several teams started to investigate the cryoconservation of the human oocyte. It was Chen who reported the first birth ensuing from a pregnancy obtained from a frozen oocyte in 1986. A limited number of live births were announced in the following years. Despite the early successes, oocyte cryopreservation did not find widespread clinical application. The reasons for this were that the efficiency of oocyte cryopreservation was low due to low survival rates and that alarming reports questioning the genetic safety appeared in the literature. It was found that the degree of polyplody increased significantly after cryopreservation of the mouse oocyte (Glenister et al. 1987). Oocyte cryopreservation was stopped in almost all clinical settings, while a few research teams continued to investigate the effects of cryopreservation on the oocyte. Recently, oocyte cryopreservation has been moved back to the clinic following reports by Gook et al. (1995) that proposed a workable protocol for cryopreservation of human oocytes. Using the proposed protocols, some successful pregnancies following oocyte cryopreservation have been obtained.

The problems encountered in the freezing of mature oocyte stimulated research on the cryopreservation of immature oocytes in the germinal vesicle stage. In this stage, the chromosomes are in the prophase of the first meiotic division and are protected within a nuclear membrane, rather than being exposed in a condensed state on an assembled spindle. It was estimated therefore, that cryopreservation of the oocyte in the germinal vesicle stage would be less problematic in terms of spindle damage and genetic risk. It has been shown that the freezing of immature mouse and bovine oocytes can be followed by in vitro maturation, in vitro fertilization, embryo development, and even the production of live offspring. The cryopreservation of human immature oocytes has been shown to be more problematic in terms of survival and maturation to the MII stage. A limited number of pregnancies have been achieved form in vitro matured human oocytes derived from fresh ovarian biopsies, collected during IVF treatment cycles or from unstimulated ovaries.

The need for oocyte cryopreservation:

1) Auto-conservation of Oocytes: In the routine of assisted procreation, oocyte cryopreservation can be of importance in the case of cycles where transfer of embryos to the uterus is contraindicated due to an increased risk for server ovarian hyperstimulation syndrome, or where no sperm can be produced at the moment of pick-up. At present, the greatest interest in oocyte cryopreservation comes from the filed of oncological medicine. With the progress in screening and improved treatments for several types of cancers, an increased survival prognosis and eventually total cure have become possibilities. However, cancer treatment by radiotherapy, often in combination with chemotherapy, puts a serious burden on the function of reproductive organs. It is often the case that systemic cancer treatment leads to ovary burnout, so that the frequently young patient loses any hope of ever becoming pregnant, at least with her genetically own child. Chemotherapy for breast cancer may induce amenorrhea in up to 62% of patients. As long-term survival rates for young women with cancer tend to improve, more attention is being focused on prevention of detrimental effects of radio-or chemotherapy on the patient's germinal cells.

Oocyte cryopreservation prior to anticancer treatment is therefore the only resort for young patients without a partner. In analogy to cryopreservation of human sperm, freezing of mature oocytes was considered an elegant option for preservation of fertility. Although interest in mature oocyte cryopreservation recently seemed to be renewed, clinical practice has proven it to be rather disappointing, resulting in only a handful of successful pregnancies. Furthermore, the medical and psychological burden of ovarian stimulation and the delay it causes in starting cancer treatment prevents this attitude from being widely applied.

2) Donor Oocyte Cryopreservation: Oocyte cryopreservation would also be a great tool in cases of oocyte donation. In the current situation oocyte donation is often cumbersome, not only as a result of the psychological aspects involved, but also because of the strict endocrinological requirements for synchronization of the ovarian cycles of the donor and the acceptor. The preservation of the oocyte until the appropriate moment of fertilization and embryo transfer would
circumvent elegantly this now-mandatory synchronization system.\(^\text{(27)}\)

3) **Oocyte Cryopreservation for Legal, Ethical, or Religious Indications:** In countries, or groups the cryopreservation of embryos is prohibited by law or is strongly disapproved of by religious and ethical guidelines, the cryopreservation of oocytes is the only way out. Thus it is clear that the challenge of and necessity for establishing oocyte cryopreservation are still very prevalent.\(^\text{(27)}\)

**Cryobiological factors influencing cryoefficiency:**

1) **Cell Survival:** Cryopreservation of a cell involves the cooling of this cell to a subzero temperature at which all metabolic processes are arrested, followed by the return from this frozen condition to a physiologically active state. In other words, cryopreservation comes down to stopping biological time. In practice, cells are stored in liquid nitrogen at -196°C. The real challenge associated with cryopreservation is not to survive storage at -196°C but to avoid intracellular ice formation in the transition across a temperature zone between -15°C and -60°C, where intracellular ice formation is possible. Cells have to pass this zone twice, once during cooling and once during thawing. As will become clear from the following, it is absolutely necessary to control the cooling and thawing rate in this zone. Another prerequisite for cell survival is the presence of cryoprotective agents in the freezing medium. Evidence is accumulating for the occurrence of cryoprotective agent as a part of a natural defense system. The discovery of the cryoprotectant glycoprotein in the circulation fluid of arctic fish is a living example of this.\(^\text{(28)}\)

2) **The Addition of Cryoprotectants:** As stated above, glycerol was discovered to act as a cryoprotective agent in 1949. Since then several other chemical substances have been found to have a cryoprotective action. The most commonly used cryoprotectants besides glycerol are dimethylsulfoxide (DMSO) and 1,2-propanediol (PROH). Cryoprotectants are highly miscible with water and have a low molecular weight, so that they can readily permeate the cell. Cryoprotectants are commonly used in concentrations of 1-2 M and can exert a prefreezing dehydrating effect due to the hypertonicity they bring to the freezing medium. Often, non-permeating cryoprotectants such as sucrose are used to promote prefreeze dehydration. Furthermore, cryoprotectants cause a freezing-point depression so that the temperature at which the cellular contents will freeze is lowered. In addition, cryoprotectants exhibit a high glass-forming tendency upon freezing. Also, at any time during cooling, cryoprotectants reduce the absolute concentration of salts that remain in the unfrozen solution. Thus, we can say that cryoprotectants confer protection by reducing the risks of intracellular ice formation and of solution effects. However, the use of cryoprotectants must be considered a knife that cuts two ways: as well as affording cryoprotection, cryoprotectants can also become toxic when exposure conditions are inappropriate.\(^\text{(27)}\)

3) **Control of Cooling and Thawing Rate:** Consider a cell that is suspended in an isotonic medium. When the temperature drops below 0°C, the medium and the cell will initially remain unfrozen but super cooled, i.e., in a liquid state below the freezing point. Since the cell contains no efficient ice nucleators, ice will form first in the extra cellular medium. Due to this extracellular ice formation, the extracellular concentration of solutes will increase. The cell will respond osmotically, and water will start to leave the cell to restore the chemical water potential. In other words, the cell will start to dehydrate, and this is the cell's first step in the prevention of intracellular ice formation. All further physical events can be described in terms of the cooling rate. If the cycle of dehydration can be repeated often enough, i.e., if the cooling rate is slow enough, the cell will have lost all freezable water by the time the cell freezing point is reached. If cooling is done rapidly or ultra rapidly, water cannot leave the cell fast enough to restore the osmotic equilibrium and the equilibrium will be restored by a sudden and fast intracellular freezing, so that intra and extracellular solute concentrations will match again. It therefore seems imperative to cool a cell slowly. If this is done, the question then is how to thaw this cell. What happens if a slowly frozen cell that is almost fully dehydrated is thawed rapidly? The extracellular water will melt massively and due to the large solute gradient with the hypertonic cell, water will rush into the cell and cause lyses of the cell through osmotic burst. If thawing is done slowly, the rehydration of the cell can take place gradually. Slow cooling therefore requires slow thawing. Cooling and thawing may not be too slow, however, since then the cellular components are exposed for a long time to high concentrations of slats, which may be harmful in terms of lipid and protein destabilization. This type of injury is referred to as solution effects.\(^\text{(27)}\)

If cooling is done very rapidly or slowly but to a high subzero temperature such a -30°C, small ice
crystals will be present when the cell is plunged into liquid nitrogen. It is imperative then to thaw rapidly so that the typical ice crystal aggregation tendency is overcome by quick melting of the ice crystals.

In conclusion, the challenge is to find a cooling rate that is slow enough to prevent intracellular ice formation and fast enough to prevent solution effects according to the Two-factor hypothesis proposed by Mazur (1984). Every cell type will have an optimal cooling rate dictated by conditions of cell permeability and surface-to-volume ratio. For the oocyte, which is a large cell, the optimal cooling rate is below 1°C/min. In practice, cooling and thawing are done with computer-controlled biological freezers. To make sure that the cycle of dehydration is started, the necessary triggering of extracellular ice formation is induced manually by touching the solution with a cooled tool. This is called "seeding".

4) Equilibrium and Nonequilibrium Freezing: Equilibrium freezing refers to the above-described procedures. It means that during the cooling and thawing phases the aim is to maintain chemical equilibrium between intra- and extracellular water potential. The cryoprotectants are used in 1-2 M concentrations and prefreeze exposure times are on the order of 10-20 min. Cryodamage is done either through intracellular ice formation or through solution effects.

Nonequilibrium freezing is a total departure from classic freezing theories. It is characterized by high concentrations of permeating cryoprotectants in combination with nonpermeating cryoprotectants, by short prefreeze exposure times of less than 5 min, and by immediate high cooling rates. Plunging into liquid nitrogen is done directly after the short exposure time. A distinction can be made between ultra rapid freezing and vitrification, which are both types of nonequilibrium freezing. In ultra rapid freezing, one permeating cryoprotectant in combination with a nonpermeating agent such as sucrose is often used. In vitrification, a mixture of different cryoprotective agents in combination with a nonpermeating agent is used. The concept of vitrification (literally: glass formation) was introduced as early as late 1930s and 1940s and was rediscovered by Rall and Gahy in 1985. Vitrification is a process of solidification whereby an aqueous, viscous solution does not crystallize upon cooling but immediately forms a glass on ultra rapid freezing.

A) Equilibrium Freezing of Oocytes: In the first successful experiment on mouse oocyte cryopreservation, reported by Whittingham (1977), a slow-cooling equilibrium-freezing method was used with 1.5M DMSO as the cryoprotectant. Oocytes were equilibrated in 1.5M DMSO at 0°C and cooled slowly at a rate of less than 1°C/min to -80°C; this was followed by storage in liquid nitrogen and slow thawing at a rate of 8°C/min. A survival rate of approximately 70% was obtained and in vitro fertilizability was lower than for unfrozen control oocytes, but live young were obtained after transfer of embryos at the two-cell or blastocyst stage to pseudo pregnant foster mice.

In the first successful attempt at human oocyte cryopreservation by Chen (1986) a modification of the technique by Whittingham (1977) was used. The prefreeze equilibration was done similarly in 1.5 M DMSO at 0°C followed by slow cooling, but at -32°C the oocytes were plunged into liquid nitrogen, followed by rapid thawing. A new wave of research reports by Gook et al. (1995) in the 1990s proposed a workable protocol for cryopreservation of human oocytes. The freezing of human oocytes with PROH was advocated, linked to the use of intracytoplasmic sperm injection (ICSI), making it possible to obtain high levels of fertilization and embryo cleavage rates. The risk of chromosomal abnormalities did not seem to be increased. A few pregnancies following oocyte cryopreservation have recently been obtained.

B) Non-equilibrium Freezing of Oocytes: Ultrarapid freezing, technique was revolutionary, easy to perform, and much cheaper in terms of equipment than the slow-freezing techniques which require a programmable biological freezer.

Results on ultra rapid freezing of the human oocyte have been very scarce. Trounson (1986) reported survival rates of around 50% upon thawing, but after culture the oocytes degenerated. Feichtinger (1987) reported a 12% survival rate, while Al-Hasani et al. (1987) found only one of 25 oocytes surviving.

Vitrification of the human oocyte was investigated by Trounson (1986) Feichtinger (1987) and Hunter et al. (1995). Good survival rates have been reported, but embryonic development seems limited, vitrification is done as the ultrarapid freezing technique with the use of a high concentration cryoprotective media (more than 40%).

In conclusion, different cryobiological systems have been tried out on the oocyte, be it slow equilibrium freezing or rapid nonequilibrium freezing. Results in terms of survival and fertilization rates tend to be quite variable.

Generally, fertilization rates seem lower and disturbances to the genetic integrity of the resulting embryos have been found.
Oocyte structures affected by cryopreservation

Whatever type of cryopreservation was used for the oocyte, be it equilibrium or nonequilibrium freezing, the freezing efficiency and genetic safety were under review. The oocyte is a highly unique structure containing the information for the body plan of the organism. Several research teams have been investigating which type of structure(s) in the oocyte is sensitive to the stresses imposed by cryopreservation. Several targets have been studied: microtubules, the zona pellucida, the fertilization machinery (parthenogenetic activation), and the chromosomes.

1) Microtubules: Microtubules are structures essential to intracellular. The oocyte contains a highly structured microtubular system in the form of the meiotic spindle, which carries the chromosomes at the metaphase plate. The microtubules of the spindle are in steady state equilibrium with the pool of free tubulin in the cytoplasm. Cryopreservation involves cooling and exposure to cryoprotectants. Microtubules are sensitive to cooling (depolymerization) and to chemical agents that interfere greatly with the water hydrogen bonds which, in particular, are also involved in building microtubules. Since microtubules are essential to the architecture of the oocyte as well as to normal fertilization and development, it is important to consider that damage to the microtubules during freezing may have an effect on further development. (27)

Cooling and cryoprotectants have an influence on the organization of the microtubular system of the oocyte, but restoration of the normal condition can be obtained. (32)

2) Zona Pellucida: The zona pellucida is a very characteristic integument of the oocyte and is of primordial importance in the sperm’s interaction with the oocyte and in the prevention of polyspermy by means of the zona reaction. The zona reaction leads, after the penetration of one sperm in the oocyte, to release of the content of the oocyte’s submembranous cortical granules in order to alter the glycoprotein of the zona coat, so causing zona hardening.

It has been demonstrated that both cooling and exposure to DMSO can cause premature zona hardening and reduced fertilization in mouse and human oocytes. (33) The best conditions with the least effect on the zona involved exposure to DMSO at 4°C for the mouse oocyte as well as for the human oocyte. The use of fetal bovine serum in the freezing medium also seems to be beneficial in preventing premature zona reaction. (34)

3) Chromosomes: Since the cytoskeletal integrity of the oocyte can be altered by cryopreservation, and since the chromosomes in particular are carried by the spindle consisting of microtubules, the induction of genetic abnormalities by cryopreservation is a major concern. It was demonstrated that slow freezing of mouse oocytes with DMSO induced increased polyploidy in the first-cleavage embryos. (35) The increased polyploidy seemed to be triploidy type caused by retention of the second polar body. For ultrarapid freezing of mouse oocytes as for slow freezing, increased polyploidy occurred in the first-cleavage embryos. The most important finding from these studies, however, is that there was no increase in the frequency of aneuploidy. (36)

The frequency of aneuploidy in vitrified mouse oocytes was not increased, provided that the time of exposure to the cryoprotectant was carefully controlled.

For the human oocyte the data are scarce. The birth of healthy children, however few, indicates that normal fertilization and normal human development without chromosomal abnormalities are possible after freezing of the human oocyte. (37) There was no increase in the frequency of aneuploidy in cryopreserved mature human oocytes. Moreover, after cryopreservation of the human oocyte with PROH there were no abnormal karyotypes in pronuclear embryos. (38)

4) Parthenogenetic Activation: Since the cytoskeleton is involved in the steps of fertilization, it was useful to consider the effect of cryopreservation on the possible precocious activation of the fertilization machinery. (39) It was shown that PROH can cause activation of the mouse oocyte but that the degree of activation can be controlled by lowering the temperature and time of exposure for concentrations of up to 1.5M. For DMSO no pathenogenetic activating effect has been demonstrated. (40)

For the human oocyte, no increased frequency of activation has been demonstrated after freezing with DMSO, (41) after vitrification or after freezing with PROH. (38)

Future prospects

The search for clinical application of oocyte cryopreservation is still going on. The investigation of possible damage to the oocyte is being given this much attention in current reproductive research projects. A few teams have taken up the clinical practice of mature oocyte cryopreservation again
but the general attitude is one of caution, since in particular the efficiency of the procedure is still questionable.\textsuperscript{(27)}

A major task for the future will be the establishment of proven, safe cryopreservation protocols designed specifically for human oocytes or ovarian tissue. Furthermore, techniques to prevent transmission of infectious organisms during cryostorage should be given great attention. Finally, the long-term follow up of children originating from cryopreserved gametes or tissues has to be well organized in order to monitor the long-term effect of freezing on the offspring. Keeping in mind the enormous impact assisted reproduction has had in the field of human procreation during the final decades of the last century of the second millennium.\textsuperscript{(27)}

**Cryopreservation of Fertilized Ova or Embryos**

A much more clinical option is the cryopreservation of fertilized ova or embryos after (IVF) before chemotherapy. However, this alternative is relevant to married women, and almost inapplicable to the very young, or single women. Moreover the ovarian stimulation with hMG/hCG before IVF, egg retrieval, requires the initiation of chemotherapy to be postponed, which is often contraindicated by hematologists and oncologists.\textsuperscript{(8)}

Furthermore, the increase in estradiol concentration, following hMG/hCG ovarian stimulation may aggravate the clinical situation of patients with breast carcinoma or other estrogen-sensitive tumors, or that of systemic lupus erythematosus patients by inducing a flare-up of the autoimmune disease.\textsuperscript{(12)}

The preliminary clinical results suggest that it may be possible to retrieve immature ova from developing antral follicles, mature them in vitro for few days, fertilize them by intracytoplasmic sperm injection (ICSI) and subsequently cryopreserve the embryos generated. This option may enable a resolution for preservation of future fertility without postponing the initiation of chemotherapy and without exposure of these patients to ovarian stimulation and hyperestrogenism.\textsuperscript{(8)}

To date all available evidence suggests that human babies resulting from frozen embryos are normal. Too few babies have been born from frozen human oocytes for any trend to emerge. Research aimed at establishing the efficacy and safety of ovarian tissue cryopreservation is needed.

**Preservation of ovarian tissue**

1. Ovarian transposition
2. Transplantation of ovarian tissue
   a. Autografting (orthotopic-heterotopic)
   b. Xenografting
3. In Vitro maturation
4. Cryopreservation
5. Chemotherapy-GnRh analog Co-treatment

**Introduction**

In 1960 Parrott was able to obtain live young mouse from transplanted ovarian wedge that had been frozen with glycerol to -79°C.\textsuperscript{(42)} Such therapy could provide a source of ovarian tissue that, when autotransplanted, would maintain an adequate estrogenic milieu that protects against and osteoporosis. It has been suggested that it may be best to restrict ovarian transplantation to women whose ovaries are disease free, because cancer can be transmitted with ovarian tissue grafts in animals.

A second role for this therapy could be in the form of oocyte banking as a strategy to preserve the reproductive potential of younger women or girls before cancer therapy. Cryopreservation of ovarian tissue before initiation of oncological treatment, followed by autologous transplantation after remission, could provide a means of protecting fertility. Obviously, an option for these cases involves oocyte retrieval (today probably better accomplished with gonadotropin stimulation, although the natural cycle will be preferable when improved oocyte in vitro maturation protocols become available), followed by IVF and embryo cryopreservation.

The potential therapeutic use of ovarian autotransplantation mandates continued efforts to achieve better results with ovarian tissue cryopreservation (i.e. freezing of the isolated germ cell at different stages of maturity, cumulus-encased oocytes, follicles and sliced tissue) and with in vitro oocyte maturation procedures. Ovarian tissue can be recovered at the time of laparotomy, or ovarian biopsy specimens can be taken by laparoscopy. When autografting to an orthotopic site succeeds in
restoring ovulatory menstrual cycles, hormonal replacement therapy and medical intervention in the process of conception may not be needed. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196°C has been reported. Alternatively, oocytes could be recovered from an ectopically located graft and matured in vitro or after gonadotropin stimulation, mature oocytes could be retrieved and used in ART. (43)

Cryopreservation of ovarian tissue has several potential advantages over both oocyte and embryo freezing. Hundreds of immature oocytes may thus be cryopreserved without the necessity of ovarian stimulation and delay in initiating cancer treatment. Cryopreservation of ovarian tissue is of great benefit, since immature oocytes are relatively quiescent, smaller, and lack zona pellucida and cortical granules. These properties make them far more tolerant to freezing and thawing injuries than mature oocytes. Furthermore, it has been hypothesized that primordial follicles have a greater potential to repair sublethal damage to organelles and other intracellular structures during their prolonged growth phase. However, a significant follicular loss occurs with freezing, thawing and grafting. It is unknown as yet how well and how long a given frozen-thawed ovarian segment will function after auto-transplantation in human. (44)

There are three optional methods for utilization of cryopreserved ovarian tissues: autotransplantation, xenotransplantation and in vitro maturation.

The principal advantages of ovarian tissue banking over the storage of oocytes and embryos are:

1. Large numbers of primordial follicles can be collected and stored
2. The primordial follicles appear less vulnerable to cryoinjury than growing or mature oocytes
3. A single ovarian tissue graft has the potential to restore normal ovarian cyclicity and may remain functional for an extended period of time (years)
4. The follicles in the ovarian tissue graft are naturally selected prior to ovulation
5. No hormonal treatments are required as the graft itself supports the processes leading up to ovulation, conception, and pregnancy
6. That the ovarian tissue can be collected at any time from patients of any age by laparoscopy or laparotomy
7. A male partner is not required at the time of cryopreservation (45)

1) Ovarian transportation: The whole ovaries are mobilized away it’s normal position to preserve it’s function in young patients who are in need of pelvic radiotherapy for malignant disease as cervical carcinoma, malignant lymphomas or GIT malignancy. It can be done through:

   1. Laparotomy: (done for hysterectomy) the ovaries are dissociated from it’s connections and put in a subcutaneous tunnel in the abdominal wall. (47)
   2. Laparoscopic ovariopexy: by suturing the ovary to the round ligament (high and laterally) using polypropylene suture. Repositioning of the ovaries after the end of radiotherapy can be done laparoscopically by cutting the suture with possible return of natural fertility. (48)
   3. Robotically assisted endoscopic ovarian transposition was described by Malpus et al. (2003). They mobilize the ovaries on their infundibulopelvic ligaments and sutured it to the ipsilateral pericolic gutters. (49)

Ovarian transposition proved its value in prevention of premature menopause and maintaining ovarian function. Zinger et al. (2004) reported successful pregnancy using ART, transcutaneous oocytes retrieval from a transpositioned ovary and surrogate mother, 11 years later after hysterectomy in a case of carcinoma of the cervix (stage IB). (50)

A case of twins pregnancy following bilateral ovarian transposition (and hysterectomy) was reported by Azem et al. (2003). Morice et al. (2001) found 2 cases of ovarian metastasis out of 107 transpositioned ovaries in patients of stage IB carcinoma of the cervix. They suggested that ovarian transplantation to be avoided if the cervical lesion is > 3cm or the lymph vessels spaces are involved. (52)

2) Transplantation of ovarian tissue: The concept of using a graft to restore reproductive function is not new. Last century a variety of gonadial tissues were transplanted for both males and females, and the first reported pregnancy resulted from a relocated ovary over one hundred years ago when Morris autografted a fragment of ovarian tissue in a woman suffering from Pelvic Inflammatory Disease, to a stump of the fallopian tube. However, the overall success was low as it
was not until this century that the problem of tissue rejection was fully appreciated. Ovarian tissue is not immunologically privileged. It expresses markers which allow it to be recognized as self and nonself. Ovarian tissue grafts placed in recipients which are not histocompatible will therefore be recognized as foreign and be rejected. Transplants in mice are therefore largely restricted to inbred lines. In humans, it is restricted to autografting. The importance of the immune system in the rejection procedure is illustrated by the observation that animals with severely compromised immune system accept ovarian tissue, not only of other strains of mice but also cats, sheep, human.

Primary follicles in both fresh and cryopreserved marmoset and human ovarian tissue appear to develop normally following grafting into immunologically compromised mice.[46] Ovarian transplantation has been used as a tool to aid scientific research; it has also been used to propagate animal lines. Whole ovaries have been grafted into more accessible sites (e.g., the neck) to facilitate endocrine and other studies of ovarian function. Many of these studies have been performed on large animals and the blood supply to the ovaries has usually been reanastomosed with the recipients blood supply immediately. Under these circumstances the ovaries very rapidly resume their normal function. However, it is not necessary to transplant the whole ovary or even to reanastomose it with a blood supply in order for the ovary to resume normal function. In species such as the human and sheep the primordial follicles are concentrated in the cortex very close to the surface of the ovary and it has been demonstrated in sheep that a slice of ovarian cortical tissue sutured onto the ovarian pedicle after ovariectomy can recover fully and will support normal cycling, conception, and term pregnancy. In mice, grafts of primordial follicles alone, without any ovarian stromal tissue can restore fertility. Ovaries from small animals and pieces of ovarian cortex or isolated primordial follicles contained within an artificial matrix are not anastomosed with the recipient’s blood supply. This exposes the tissue to a period of hypoxia and may cause a reduction in the number of viable follicles in the ovary but most transplants do survive and recover normal function. Early studies in mice established that on average around 42% of all oocytes survived transplantation. Studies in rats have shown that most ovarian grafts are revascularized in 24 to 72 hours. There is currently little comparable information on reestablishment of vascularization of ovarian grafts in the human. It is not known how much tissue needs to be replaced to restore regular menses and fertility in a woman. However, it has been estimated that ovulation can occur with as few as 100 (women, mice) to 400 (cattle) oocytes remaining in the ovary. Ovarian tissue transplantation is a feasible and practical procedure. [53]

**A) Autotransplantation:** Either fresh or cryopreserved ovarian tissue can be autotransplanted (autografted). Live offspring have been produced by autografted ovarian tissue in mice, sheep and recently in monkey.[53] The ovarian tissue can be autografted either: orthotopically (fixed to the ovarian fossa) or heterotopically (far transplanted to a far subcutaneous area in the arm or the abdominal wall). As yet, it is not known which will turn to be more practical and effective.

**B) Xenotransplantation:** It is the transplantation of ovarian graft to subcutaneous area in immunodeficient mouse. The advantage of this technique is the possibility of its application to patients for whom hormonal treatment is contraindicated (as in breast cancer). It was reported that after xenotransplantation, exogenous gonadotropin stimulation of human ovarian tissue, generated follicular growth in 51% of grafts, with subsequent formation of corpus luteum. However, there is a serious concern of possible transmission of xenoses and animal pathogens to human.[54]

**3) In Vitro maturation:** The in vitro growth and maturation of human primordial follicles followed by IVF, is very attractive option, but it is technically challenging due to the prolonged growing phase of primordial follicles and lack of knowledge of the optimal conditions for growth and maturation of human oocytes. Although preliminary encouraging experiments have been generated in animal models.[54] The ability to completely grow and mature human primordial follicles in vitro, will not be available until the development of an optimal culture system.[55]

**4) Cryopreservation:** Parkes and Smith were the first to show that follicles in cryopreserved ovarian slices cooled to -79°C at >1°C/min grew and produced ovarian steroid hormones after grafting. The cooling rate that they used is unlikely to have permitted full dehydration of the tissue, and 95 to 99% of the follicles were destroyed. Slower cooling rates (0.3°C/min to -40°C) have recently been tested and this rate allowed 80% of isolated mouse primordial follicles and 72% of bovine fetal germ cell suspensions to survive cryopreservation. The cryopreservation of isolated human ovarian follicles may be more difficult as the enzymatic procedures required to liberate primordial follicles form the cortex can destroy the integrity of the follicle.[53]
Preservation of Reproductive Function in the Female
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Ovarian tissue cryopreservation may help overcome many of these problems. A wedge of ovary or an ovarian biopsy can contain large numbers of germ cells (each mm³ of ovarian cortex may contain several hundreds of primordial follicles) and unlike oocytes, can be collected from patients before or after puberty and at any stage of the menstrual cycle. It has yet to be established how many follicles would need to be grafted in order to restore fertility in the human.

Ovarian tissue cryopreservation may be a particularly valuable approach for species which have oocytes that are difficult to cryopreserve because primordial follicle possess many characteristics which should make them less vulnerable to cryoinjury than a mature oocyte.

The main advantages are:

1. Their small size (30 to 60µm)
2. Low metabolic rate
3. Cell cycle stage (arrested at propose of meiosis I)
4. Absence of zona and cortical granules
5. Smaller amount of cyrosensitive intracytoplasmic lipid

Their smaller size results in a larger surface to volume ratio which reduces the problems associated with water and cryoprotectants diffusing into and out of the cell. The resting cell cycle stage avoids problems associated with chromosomes arranged on a temperature-sensitive metaphase spindle. The absence of a zona and cortical granules avoids the problem of zona hardening. The lipid content may be important as pig and bovine embryos which contain large amounts of intracellular lipid become less sensitive to cooling and cryopreservation if their lipids are removed by centrifugation and micromanipulation. The mouse has eggs and embryos which are not sensitive to chilling or cryopreservation. Unlike mature oocytes with an intact cumulus, isolated primordial follicles have a relatively consistent size with no more than a single layer of follicular cells surrounding the oocytes. Clearly the volume of ovarian or cortical tissue which is to be frozen and the position of the follicles within that tissue will influence the amount of cryoprotectant which reaches the follicles. There have to date been no abnormal young born from cryopreserved ovarian transplants. While this may reflect the limited number of studies reported it is likely that any follicles which are injured by the freeze-thaw procedure will not survive the natural processes of recruitment, development, ovulation, and fertilization. (53)

Varying composition of cryoprotectant solutions have been used to prevent tissue damage during cryopreservation and thawing. Hreinsson et al. (2003)(56) compared the use of human serum (20%) VS. human serum albumin (HSA) 25 mg/ml in cryoprotectant solutions. They used slices of ovarian cortical tissue 1-1.5 mm³ each to be cryopreserved in cryoprotectant solutions. After thawing they studied 1,318 follicles using light microscope, and transmission electron microscope, they found that viability of follicles was 99.3% in freshly dissected tissue. After thawing 65% of follicles and 75% of oocytes were viable with serum VS. 69 and 74% respectively with HAS with no significant difference between both groups. (56)

**Risks of storing biological materials at low temperatures**

Cryopreservation will in most instances reduce the viability of a tissue to some extent, but once at -196°C, the loss of viability is largely independent of the time in storage. Storage in liquid nitrogen is therefore in theory a suitable way of storing eggs, embryos, or ovarian tissue for a patient. Storage in the liquid phase of the liquid nitrogen does, however, carry potential risks. Infectious agents such a viruses can be spread though liquid nitrogen. Viruses are highly resilient and if straws, vials, or ampoules break while in liquid nitrogen storage, viruses contained within it may contaminate the liquid nitrogen itself. It has been established that viruses in the liquid nitrogen can contact and contaminate other containers within that storage tank. This is known to have caused the transmission of hepatitis. (58) The risk of transmission must therefore be considered for patients with stored ovarian tissue as this tissue is replaced into the body surgically. This risk may be reduced or eliminated by using alternative storage methods including storage in the vapor phase of liquid nitrogen, or storage in -140°C to -150°C biological freezers. (52) Unfortunately there is little information about the effects of long-term storage of biological tissues at these slightly higher subzero temperatures. This may prove crucial for ovarian tissue, as this is likely to be stored for prolonged periods of time. Because in liquid nitrogen (-196°C) it is so cold that there is virtually no movement of atoms or molecules, therefore, there is practically no deterioration of the stored tissues. At higher temperatures (e.g., above -130°C) atoms and molecules can move and gradual
changes are likely to occur. At temperatures of -90°C and above changes occur more rapidly. Short periods of exposure to such “high” temperatures can cause lethal damage to embryos. Even if tissue are to be stored in liquid nitrogen vapor (approximately -196°C) or in ultra low temperature biological freezers (-140°C to -150°C) it may be necessary to develop cryopreservation solutions which are designed for use at these higher subzero temperatures.\(^{(57)}\) The stability of the cryoprotectant solutions may be enhanced by establishing which cryoprotectant agent forms the most stable glass (vitrification), and by adding macromolecules or polymers such as Ficoll, Poly Vinyl Pyrrolidone (PVP), Dextran, Poly Vinyl Alcohol (PVA), and Sucrose. The use of polymers has the further advantage that it can replace serum, which would remove a potential source of infectious agents, and reduce the variability associated with patient serum. It has already been demonstrated that some (but no all) polymers are as effective as or more effective than serum in protecting oocytes and embryos from freeze-thaw injury. This may be attributed to the polymers ability to reduce the brittleness of frozen solutions and reduce ice crystal growth during warming. Further research in this area is needed.\(^{(53)}\)

**Surgical Procedures**

Collection of ovarian tissue is done laparoscopically. The amount of ovary removed depends upon the extent of normal ovarian tissue and the patient’s desire to retain normal ovarian tissue. In women with severe endometriosis, both ovaries may require removal to increase the chance of cure, but in younger women, one or part of one ovary may be retained to avoid hormone replacement therapy. Women having chemotherapy or radiation therapy, which is known to cause ovarian failure, can have both ovaries removed prior to therapy. In young women who wish to store ovarian tissue as an insurance against future infertility and increased risk of chromosomal abnormalities with aging, a small portion may be removed.

Removal of a part or the whole ovary is achieved by laparoscopy in 90% of women. It is a simple technique that can be done as a day or overnight procedure in a hospital dealing with operative laparoscopy techniques.

Operative technique for ovarian removal: Patients are placed in the lithotomy position and an intrauterine manipulator is inserted. Four small incisions are used, 1 of 10 mm and 3 of 5 mm. On the left side the sigmoid colon occasionally requires mobilization. The course of the ureter is identified at or below the pelvic brim.

The operative procedure is commenced at the most difficult place of dissection. The ovary is retracted medially away from the lateral pelvic wall. Failure to achieve safe laparoscopic dissection is determined quickly and laparotomy performed immediately if necessary. The ovarian pedicles are dealt with by bipolar forceps, sutures, staples, or endoloops.

The specimen is removed in one of two ways. The ovary is removed in a bag through the posterior fornix of the vagina or through the 1 cm umbilical incision. After removal of the specimen all pedicles are checked for complete hemostasis. Continuous irrigation ensures complete peritoneal toilet and when the solution in the pelvis is clear, 1 liter of Hartmann solution is infused and left in situ. If the bowel has been adhered to excised tissue, Interceed (Johnson & Johnson Pty Ltd), a soluble adhesion barrier, is used to cover the peritoneal surfaces. The patients are discharged home 12 h after surgery if clinical observations are normal; if surgery has been difficult or involved bowel resection, they remain in hospital for 24 to 48 h.

Wedge resection or biopsy of the ovary is performed using scissor excision with minimal use of electro-coagulation to avoid damage to the ovary. Interceed is placed over the ovarian incision to reduce the risk of adhesions.

Replacement of ovarian tissue: This can be done by placing small 1 to 3 mm pieces of the ovary in the ovarian fossa below the fallopian tube. The pieces can be kept in place by an Interceed, over, scarification of the peritoneal surface, or retroperitoneal placement. The most suitable technique has not been determined.

It is possible that the tubal fimbria would be a more suitable site of transplantation as it has adhesive properties for oocytes pick-up which may facilitate ovarian implantation.\(^{(53)}\)

**Freezing-Thawing protocols**

The cooling-thawing procedures used for cryopreservation of mouse, sheep, and human ovarian tissues are similar to that used for cryopreservation of oocytes and embryos as mentioned before.
5) Chemotherapy and Gonadotrophin-releasing hormone (GnRh) Analog co-treatment: The possibility of administering an adjuvant treatment that might limit the gonadal damage caused by an otherwise successful treatment of malignant disease is an attractive idea. Several investigators have demonstrated that GnRh-a inhibits chemotherapy-induced ovarian follicular depletion in rats, and monkeys by decreasing the total amount of follicle loss during the chemotherapeutic insult, and by decreasing the daily rate of follicular decline. (59) Blumenfeld et al. (2002)(60) had comparative controlled study of chemotherapy and GnRh-a co-treatment VS. Chemotherapy alone. They used depot i.m. monthly injection of 3.75 mg decapeptyl for 6 months parallel to chemotherapy (and combined radiotherapy in many of the cases). They found that 95% of cases in co-treatment group (GnRh-a) resumed spontaneous ovulation and menses within 12 months VS. 45% in chemotherapy alone group. And premature ovarian failure was diagnosed in 5% VS. 55% in both groups respectively.

Preservation of the fallopian tubes

The tubal factor of infertility represents up to 15% of the causes in infertile women. The infertile couple due to tubal disease has two therapeutic options to achieve pregnancy; reconstructive tubal surgery and in vitro fertilization (IVF). Tubal surgery has indisputable benefits for the patient if infertility is cured by intervention. It gives the patient the possibility of conceiving more than once without further treatment. It also gives the couple the psychological advantage of being able to conceive spontaneously. The reproductive surgery and IVF must be considered as complementary options directed towards increasing the overall probability of achieving pregnancy. (61)

Not all tubal damages are suitable for surgery. It is contraindicated in the presence of bilateral multisites tubal obstruction, dense pelvic adhesions, and bilateral salpingectomy. For these cases, IVF is clearly the only therapeutic option. In other situations, the decision-making process requires detailed discussion on the effectiveness, adverse effects and cost of the procedure. Tubal surgery should be done only by gynecologist who has appropriate training in microsurgery and laparoscopy, and maintaining high level of skill by regularly operating steady volume of patients.

1) Hydrosalpinx: It is the distal tubal obstruction with variable degrees of tubal distension and mucosal damage. Two different treatment choices can be used: (1) Distal tubal surgical repair. (2) IVF. The preoperative investigations are crucial to determine patients suitable for surgical repair through laparoscopy and salpingoscopy for testing the tubal mucosa, before management as follows:

- Salpingostomy: to create a new ostium with well everted fimbrial or ampullary mucosa. It can be done through laparotomy and microsurgical techniques or laparoscopy. The pregnancy rates after this operation is directly correlated to the tubal mucosal condition(62), pregnancy rates varies between 27%-43% after laparotomy and between 20-40% after laparoscopy. The introduction of CO₂ laser for salpingostomy and the use of prosthesis to maintain tubal potency after salpingostomy were tried with no significant differences in pregnancy rates. (63)

- Salpingectomy: hydrosalpinges are associated with low implantation rates and low clinical pregnancy rates after IVF due to negative effects of secretions in the hydrosalpinx on both the endometrium and the embryo. Surgical treatment of hydrosalpinx before IVF, improved the pregnancy rates in comparison to non surgical management. (63) The efficacy of salpingectomy to treat cases of severe and irreversible tubal pathology before IVF remains a subject of debate. The use of endoscopy (salpingoscopy) for evaluation of tubal mucosa is mandatory for selection of cases for salpingostomy( in presence of healthy mucosa) or salpingectomy specially in cases of severe and irreversible tubal pathology. (63) Other surgical procedures were described for treating hydrosalpinx before IVF: (1) transvaginal needle aspiration before ovarian stimulation or at the time of oocytes retrieval, (2) proximal tubal occlusion to prevent uterine spill of hydrosalpinx contents,(3) salpingostomy in selected cases of hydrosalpinges, or (4) the use of antibiotics. All these procedures have to be evaluated in a positive well designed trials. (64)

2) Proximal tubal occlusion: Salpingitis isthmica nodosa occurs around the intramural and proximal isthmic endosalpinx. Lesion grows over time and obliterates the lumen. Endometriosis is a known cause of proximal tubal blockage involving the intramural oviduct.(65) treatment of proximal tubal occlusion can be performed through : (1) Surgical, using microsurgical techniques, through laparotomy or laparoscopy, (2) Transcervical catheterization or balloon tuboplasty, under fluoroscopic guidance (radiographic) or (3) Hysteroscopic tubal catheterization. (66) The microsurgical tubo-cornual anastomosis is the standard treatment of proximal blockage, with 47.4% pregnancy rate. The hysteroscopic transcervical tubal catheterization was followed by
pregnancy rate of 48.9%, while the radiographic approach had lower pregnancy rates between 8.7-28.8%.\(^{(67)}\)

3) **Tubal phimosis:** This is the partial obstruction of the distal end of the fallopian tube due to presence of peritoneal adhesive bands surrounding the terminal end. The treatment of choice is fimbrioplasty. The pregnancy rates up to 61% after laparotomy and microsurgical fimbrioplasty VS. 74.3% after laparoscopic fimbrioplasty had been reported.\(^{(68)}\)

4) **Tubal sterilization:** More than 1% of sterilized women seek restoration of fertility. Microsurgical tubal anastomosis was followed by pregnancy rates between 55% and 78%.\(^{(69)}\) While laparoscopic anastomosis was followed by pregnancy rates as high as 82.8%.\(^{(67)}\)

5) **Peritubal adhesions:** Pelvic adhesions are implicated in the etiology of up to 20% of infertility cases and are frequently associated with tubal damage. The subsequent fertility depends upon the severity and extent of adhesions, with best results of adhesiolsysis for mild and filmy adhesions. The rates of intrauterine pregnancy followed adhesiolsysis salpingo-ovariolysis) were 46.5% after laparoscopy and 54.8% after microsurgical laparotomy procedures. The postoperative steroids reduce the incidence and severity of adhesions reformation. While other liquids as dextran appeared neither to reduce the incidence or severity of adhesions.\(^{(70)}\) The use of second-look laparoscopy and adhesiolsysis versus no second look laparoscopy, proved no difference in the pregnancy rates. Also no difference in the pregnancy rates following postoperative hydrotubation VS. no hydrotubation.

6) **Ectopic tubal pregnancy:** The fallopian tube is the site of ectopic pregnancy in 95% of cases. The incidence of ectopic pregnancy has doubled in western countries during the last 2 decades, with incidence rate of 11.5/1000 pregnancies. The conventional management of tubal ectopic pregnancy by laparotomy and salpingectomy is followed by decrease of the subsequent intrauterine pregnancy rates to 40-60%, which necessitates the conservative management to maintain fertility. Different conservative management modalities are practiced with preference of laparoscopic approach as diagnostic and therapeutic management, specially in early, undisturbed ectopic pregnancy.

1. Laparoscopic salpingostomy with suction of gestational sac and bipolar diathermy hemostasis.\(^{(71)}\)
2. CO\(_2\) laser salpingostomy with vaporization of gestational sac, with high safety and success rates as regard to tubal patency and subsequent intrauterine pregnancy.\(^{(72)}\)
3. Methotrexate injection (of 12.5-25mg) into small undisturbed gestation sac followed by 90% tubal patency with avoidance of toxicity of systemic methotrexate.\(^{(73)}\)
4. Prostaglandin (PGF\(_2\alpha\)) injection was tried with comparable success but due to postoperative abdominal pain (PG effect), this approach is less commonly used.\(^{(74)}\)
5. Laparoscopic salpingectomy : in cases of severely damaged tube, excision and haemostasis can be done through laparoscopic approach, with comparable success as laparotomy.\(^{(75)}\)
6. Laparotomy : When laparoscopic approach is not available, conservative management through laparotomy for tubal milking, linear salpingostomy can be done, or radical management by salpingectomy in severely compromised patients.

### Preservation of the uterine body

Uterine fibroids (leiomyomas) are the most common tumour in women, with reported incidence of 20-25%.\(^{(76)}\) The incidence increases with age until the time of menopause and incidence as high as 50% was reported.\(^{(77)}\) Fibroids are associated with a number of reproductive problems as infertility, pregnancy loss, and menorrhagia. The relationship between fibroids and infertility remains a subject of debate.\(^{(78)}\) Although the prevalence of fibroids in infertile women is high, no causal relationship between fibroids and infertility has been demonstrated. Impaired gamete transport, distorted uterine cavity, and abnormal blood supply to the endometrium may be a reason for poor implantation in cases of fibroids.\(^{(79)}\) More than 40 reports of myomectomy and subsequent pregnancy rates are found in the literature. Pregnancy rates vary from 10% to 77%.\(^{(78, 79)}\) The large variations in pregnancy rates following myomectomy is likely to reflect differences in patients characteristics, the size and position of the fibroids. The duration of infertility and the age of the women. It is also likely to reflect differences in the skill of the surgeons. In one study that compared the pregnancy rates between women who had undergone myomectomy with those who had not, there was an increased pregnancy rate in the surgery groups.\(^{(79)}\) Another aspect of its effect on reproductive function is the impact of fibroids on assisted reproductive technology (ART) cycles. A number of studies have consistently shown a decrease in
pregnancy rates in women with fibroids, particularly in women who have a distorted uterine cavity when compared with women without distortion of the cavity and women with no fibroids. Most of these studies concluded that pregnancy and implantation rates were significantly lower in the groups of women with intramural and submucous fibroids. In two studies a subgroup of women with submucosal fibroids was considered and the results suggested that hysteroscopic myomectomy was beneficial. However, even when there was no deformation of the uterine cavity, the pregnancy rates were lower. Other study failed to demonstrate an effect of intramural or subserous fibroids, (women with submucous fibroids were excluded), on results of intracytoplasmic sperm injection (ICSI) cycles, compared with women without fibroids.

Preservation treatment of uterine fibroids

Fibroids are the primary indication for hysterectomy, they represent over 30% of the total number of hysterectomies. For women in reproductive age, other treatment options of fibroids for preservation of the uterus included expectant management, medical treatment, conservative surgical treatment and uterine fibroid embolization.

1. Abdominal Myomectomy: It is indicated in women with deep intramural fibroids, with one large fibroid > 15 cm in diameter, or with 3 or more fibroids of > 5cm in diameter. Abdominal myomectomy is associated with more blood transfusion due to primary haemorrhage, higher rates of fibril morbidity, and postoperative adhesions.

2. Laparoscopic Myomectomy: Compared with laparotomy, laparoscopic procedures are associated with less postoperative pain, short hospital stay, rapid recovery and reduced adhesions formation. The surgeon should be familiar with laparoscopic suturing and multiple layered suturing is recommended. No significant difference was found between laparotomy and laparoscopic myomectomy as regard to the incidence of abortion, preterm labour, and C.S. rate.

3. Hysteroscopic myomectomy: It is the treatment of choice for submucous fibroids of < 5 cm in diameter. It is followed by pregnancy rates of up to 73% in infertile women. Gervaise and Fernandez (2003) recommended the preoperative reduction of submucous myomas of > 3cm in diameter using 3 courses of GnRH analog. The long term complications included intrauterine adhesions in 10% of cases.

4. Uterine Fibroid Embolization (UFE): It is one of the newest treatment options of fibroids. It is an alternative treatment to hysterectomy, with the aim of reduction of the uterine size and cessation of excessive bleeding. Up to 70% of cases had immediate cessation of menorrhagia and improvement of pain and pressure symptoms after this procedure. At 6 months follow-up, the total uterine volume decreased by 56% and the average diameter of the largest myoma decreased by 36%. UFE was followed by premature menopause in up to 1% of cases due to embollization of utero-ovarian collateral circulation compromising the ovarian blood supply. But the premenopausal women with declining ovarian function tend to be more affected than younger women. There are several reports of pregnancy following (UFE).

5. Laparoscopic Bipolar Uterine Artery Coagulation: It was followed by 76% reduction in the mean fibroid volume and 46% reduction of uterine volume. Cases of pregnancy following this procedure was reported. The utero-ovarian collaterals are not interrupted by this procedure, so premature ovarian failure is unlikely to occur. It can be considered as an alternative to uterine fibroid embolization specially in young women.

6. Laparoscopic Myolysis: It is done by coagulating the central part of the myoma either by a long bipolar needle or with laser. Coagulation necrosis of the fibroid results in vascular damage and shrinkage of the fibroid, with 41% mean decrease of fibroid volume. The main disadvantage of this procedure is adhesion formation.

Preservation of the uterine cervix

1) Preservative management of CIN: Many women seen with cervical intraepithelial neoplasia (CIN) are young and wanting children in the future. The cone biopsy of the cervix is the standard diagnostic and therapeutic management in these cases. The conization of the cervix may be complicated by primary haemorrhage in up to 10% of cases. Less commonly perforation of the uterus which may precedes local inflection or peritonitis. Scarring of the cervix may lead to cervical...
stenosis and subsequent cryptomenorrhoea. Subsequent fertility can be impaired by virtue of cervical scanning or the loss of cervical mucus. Pregnancy, should it occur, may be complicated by an increased incidence of abortion, preterm labour, or cervical dystocia in labour.

In an effort to avoid complications which sometimes follow cone biopsy, pre-malignant disease of the cervix has been treated by local destruction using one of a variety of methods. Before the use of such methods the patient must be examined by expert colposcopist who is satisfied that all the lesion can be seen and colposcopically directed biopsy for histopathological examination excluded the presence of invasive carcinoma. It is also important that the patient is available for regular cytological and if necessary colposcopic surveillance after treatment. The local destruction of CIN lesion can be done by: cryocautery, electrocautery, electrodialthermy, cold coagulation (cryosurgery), carbon dioxide laser ablation, or excision by loop diathermy. (92)

2) Preservative management of carcinoma of the cervix:
Many of the patients with cervical carcinoma are young and need to reserve their fertility. The treatment of choice in stage I carcinoma is radical hysterectomy with pelvic lymph nodes dissection. Recently, it has been published the alternative treatment modality for preservation of fertility of these patients without having a considerable adverse effects on the cure rates. This treatment is done by: (1) radical trachelectomy through vaginal - abdominal or laparoscopic approach, (2) laparoscopic pelvic lymphadenectomy. (3) Laparoscopic ovarian transposition. Followed by (4) Radiotherapy.

The reported recurrence rates were between 0% and 8% which are comparable to that after radical hysterectomy. 35 live births were reported out of 210 women treated by this preservative management. However the rates of 2nd trimester pregnancy losses and preterm deliveries were high, due to cervical weakness.

Large randomized controlled study comparing the safety and survival rates between conservative and radical treatment of these cases are needed. (93)

Ethical aspects of reproductive preservation

Oocytes and ovarian tissue harvesting and implantation promise in restoring fertility in adolescent cancer survivors. Additional problems that can raise their own ethical issues, depending on circumstances of treatment. For example, preservation of ovarian tissue and ability to generate viable oocytes for IVF, create another set of issues if the women’s uterus has received full radiation or has been removed. Ability to carry a pregnancy following full radiation is clearly questionable, and after hysterectomy the need of surrogacy for gestation with it’s own ethical dilemmas, must be considered. (94)

More researches are required in the near future and before patients can ethically be enrolled in the clinical trials, Dudjinski (2004) (95) suggested the following ethical points to be regarded by clinician investigators:

1. Ensuring that the intervention doesn’t harm the patient by delaying cancer treatment
2. Ensuring that no remnant cancer cells well be reintroduced during transplantation or fertilization
3. Preventing damaged cryopreserved oocytes from being fertilized and implanted
4. Seeking informed assent from adolescent patient and informed consent form their parents or guardians
5. Developing polices to protect the patients’ future rights to her gametes
6. Developing polices addressing the disposition of gametes if the patient dies
7. Respecting the patient by protecting her from harm while honoring her right to self determination

References:

References

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