

Fertility Preservation for Women

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The use of cryopreservation has become a relevant addition to reproductive medicine.

The options of preserving fertility by cryopreservation are summarised in Table 1

TABLE 1.
OPTIONS OF PRESERVING FERTILITY IN WOMEN BY CRYOPRESERVATION
• Embryo cryopreservation
• Oocyte freezing <ul style="list-style-type: none"> • Mature oocytes • Immature oocytes-followed by In vitro maturation (IVM)
• Ovarian tissue preservation <ul style="list-style-type: none"> • Thawing and transplantation <ul style="list-style-type: none"> • Orthotopic • Heterotopic • Primordial follicle dissection and IVM

Embryo cryopreservation

Since the first successful birth of a child after the transfer of a human frozen embryo in 1984, (Mohr L R, Trounson A, Freeman L. Deep freezing and transfer of human embryos. *Journal of In Vitro Fertilization and Embryo Transfer* 1985; 2:1-10) embryo cryopreservation has been used for over 20 years with live birth rates per frozen embryo transfer about 14 per cent. (NPSU report 2002. Bryant J, Sullivan E & Dean J 2004. Assisted reproductive technology in Australia and New Zealand 2002. AIHW Cat. No. PER 26. Sydney. Australian Institute of Health and Welfare National Perinatal Statistics Unit (Assisted Reproductive Technology Series No.8)).

However, in order to have embryos to freeze, the woman must have a life partner, and she needs to undergo stimulated cycles of IVF. Let us calculate the number of oocytes needed to be collected in order to build a supply of frozen embryos that may enable a woman to complete her family in the future. With a fertilisation rate of 70 per cent and about 70 per cent the embryos obtained being of sufficient quality to freeze, with an average survival rate of 70% after thawing, with an average live birth rate of 13.7 per cent where embryos were thawed (NPSU report 2002), an average of 21 oocytes will have to be collected

to expect to produce one live offspring. With an average oocyte collection rate of 11 per cycle (Monash IVF average oocyte harvest), (Table 2) a woman would need four stimulated cycles to have a probability of 1 (4x11x0.7x0.7x0.7x0.137) of achieving a family of two children after successful treatment. Even with back to back cycles, using down-regulation protocol, this would take (four x eight weeks) in excess of six months. This length of delay and often the use of controlled ovarian hyperstimulation with raised levels of oestrogen are contra-indicated for some cancer sufferers. Natural cycle IVF avoids the risk posed by increased oestrogen levels, but only produces one embryo. The addition of Tamoxifen, a Selective Oestrogen Receptor Modulator (SERM), or letrozole (an aromatase inhibitor) can increase the yield of oocytes and embryos while blocking the effect of oestrogen on the breast and uterus (Oktay K, Buyuk E, Libertella N *et al*). Fertility preservation in breast cancer patients: a prospective comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. *J Clin Oncol*) However, the cryopreservation of fewer embryos may have some psychological benefit, but not a realistic chance of completing a family.

In the unfortunate situation where the woman does not survive, or is not well enough to embark on a pregnancy, a large number of 'unwanted' embryos will remain in cryostorage.

TABLE 2.
NUMBER OF OCCYTES NEEDED TO COMPLETE FAMILY USING (hypothetical calculation)
44 oocytes collected
30 embryos resulting
21 of sufficient quality to freeze
15 embryos suitable for transfer after thawing
2 live births

Oocyte cryostorage

The ability to cryopreserve oocytes would be the most desirable method of preserving fertility. The freezing of mature oocytes would still require controlled ovarian hyper stimulation with its limitations of time and hormone levels. As the efficiency of oocyte freezing and subsequent thawing is currently so low, even more oocytes than if utilising IVF and embryo freezing, would need to be collected.

Despite the first description of successfully freezing oocytes being in 1987, fewer than 150 pregnancies have been reported since. (Coticchio G, Bonu MA, Bianchi V, Flamigni C, Borini A. Criteria to assess human oocyte quality after cryopreservation. *Reprod Biomed Online*. 2005;11:421-7). The barriers are poor post-thaw survival and low fertilisation rates despite Intra Cytoplasmic Sperm Injection (ICSI), and development failure even at post-implantation stages. This could be due to critical perturbations of cell components, effecting the cytoskeleton, chromosomes, or intracellular calcium signalling system (Coticchio G, Bonu MA, Borini A, Flamigni C. Oocyte cryopreservation : a biological perspective. *Eur J Obstet Gynecol Reprod Biol* 2004;1115:Suppl 1:S 2-7).

Recently, attempts have been made to measure the osmotic stress experienced by the oocyte during exposure to cryoprotectant, revealing that there can be dramatic changes in cell volume. (Paynter S J. A rational approach to oocyte cryopreservation. *Reprod Biomed Online*. 2005; 10:578-86). The accurate assessment of the clinical efficiency of oocyte cryopreservation is difficult, due to a lack of controlled studies.

New developments that may increase the efficiency of oocyte freezing are the use of vitrification and ultra-rapid freezing, and the freezing of immature oocytes with subsequent in vitro maturation may offer theoretical and practical advantages. (Gosden R G. Prospects for oocyte banking and in vitro maturation. *J Natl Cancer Inst Monogr*. 2005; 60-3).

Whilst this is attractive, the technology of In Vitro Maturation (IVM) is in its infancy. This area has recently been reviewed in a comprehensive publication (Tan S L, Chian R, and Buckett W M. (eds) In-vitro maturation of Human Oocytes: basic research to clinical applications. Taylor and Francis, UK publishers, 2006). Due to the lack of efficacy, alternate approaches to preserving fertility need to be researched.

Ovarian tissue freezing

The stimulus for developing human ovarian cryopreservation is the limitations for oocyte and embryo freezing described above, and the promising outcome of experimentation with animal ovarian tissue cryopreservation and transplantation.

Over a decade ago in Edinburgh, successful experiments using sheep were reported whereby freeze thawed ovarian cortex was successful grafted back to the orthotopic site with evidence of prolonged function and subsequent live births. (Gosden R G, Baird DT, Wade J C and Webb R . Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196C. *Human Reproduction* 1994; 9 : 597 - 603.) (Baird D T, Webb R, Campbell B K, Harkness L M, and Gosden R G. Long-term ovarian function in sheep after ovariectomy and transplantation of autografts stored at -196 C. *Endocrinology* 1999; 140: 462 - 471).

Attempts have been made to freeze entire ovaries, (Martinez-Madrid B, Dolmans MM, Van Langendonck A, Defrere S, Donnez J Freeze-thawing intact human ovary with its vascular pedicle with a passive cooling device. *Fertil Steril*. 2004;82:1390-4) whilst reporting high survival rates for follicles, the technique should still be considered experimental. Although a successful autotransplantation of an ovary with its intact vascular pedicle to the upper arm has been used in a patient with cervical cancer (Hilders, C G, Baranski A G, Peters L, Ramkhelawan A, Trimpos J B. Successful human ovarian autotransplantation to the upper arm, *Cancer* 101, (12), 2771 - 2778), others working on sheep have shown poor graft survival (3/11) after grafting frozen thawed intact ovaries (Bedaiwy MA, Jeremias E, Gurunluoglu R, Hussein MR, Siemianow M, Biscotti CV, Falcone T. Restoration of ovarian function after autotransplantation of intact frozen thawed sheep ovaries with microvascular anastomosis. *Fertility and Sterility* 2005, 79 (3), 594 - 601).

The first reported birth of one naturally conceived child after orthotopic transplantation of thawed -frozen ovarian tissue (Donnez J, Dolmans M M, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B, vanLangendonck A. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 2004; 364:1405-10) has given encouragement to research in this area. Another pregnancy has been reported where routine IVF with controlled ovarian hyperstimulation and transvaginal oocyte collection was carried out after orthotopic transplantation of ovarian tissue. (Mierow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, Schiff E, Dor J. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *N Engl J Med* 2005; 353:318-21). These case reports illustrate that ovarian tissue grafting can prove successful in selected individuals, but to date we have no idea of the true efficacy of the process.

The site of the subsequent transplant after cryopreservation can be heterotopic (transplanted into a site other than the ovarian fossa) with subsequent oocyte collection and IVF. If the site is superficial, oocytes can easily be harvested and undergo IVF. The most advanced development in this area is the report of Oktay and colleagues (K Oktay, E Buyuk, L Veeck, N Zaninovic, K Xu, T Takeuchi, M Opsahl, Z Rosenwaks Development after heterotopic transplantation of cryopreserved ovarian tissue *Lancet* 2004;363:837-4) who reported the case of a 30-year old woman with stage IIB breast cancer who had ovarian cortex stored before she underwent high dose chemotherapy and bone marrow transplant. The thawed ovarian tissue was subsequently transplanted beneath the skin of the lower abdominal wall, IVF with ICSI was carried out with two embryos resulting. Unfortunately a pregnancy did not result.

Nawroth and colleagues have reviewed the use of vitrification as a possible alternative to slow freezing, applied to both oocytes and embryos. They suggest that it may also have a place in freezing ovarian tissue, but that the technique is still only in its infancy. (Nawroth F, Rahimi G, Isachenko E, Liebermann M, Tucker M J, Liebermann J. Cryopreservation in assisted reproductive technology: new trends. *Semin Reprod Med*. 2005 23:325-35).

Questions remain as to how to optimise tissue preparation, which cryoprotectant to use, and how much ovarian tissue is required.

Safety

One of the problems with transplanted frozen-thawed ovarian tissue is the risk of transmitting a malignancy. Shaw and colleagues (Shaw J M, Bowles J, Koopman P, Wood E C, Trounson A O Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. *Hum Reprod*. 1996;11:1668-73) studied ovarian tissue from AKR strain mice with lymphoma transplanted fresh or cryopreserved tissue into young healthy mice. The cancer transmitted in 7/7 with fresh tissue and 6/7 with cryopreserved tissue. However, research using human ovarian tissue from donors with Hodgkin's and non-Hodgkin's lymphoma grafted into immune deficient mice was reassuring with no cases of disease transmission in grafted animals, although animals grafted with material from a human lymph node taken from a patient with lymphoma demonstrated transmission of disease, illustrating that SKID mice were good models to study safety (Kim S S, Radford J, Harris M, Varley J, Rutherford AJ, Lieberman B, Shalet S, Gosden R. Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation. *Human Reproduction* 2001; 16: 2056 - 2060.)

TABLE 3.

Risk of ovarian involvement by various cancers (after Oktay 2001)

Low risk < 0.2%	Moderate risk (0.2-11%)	High risk > 11% ³
Wilm's tumour	Breast cancer Adenocarcinoma of uterine cervix	Leukaemia Neuroblastoma
Non-Hodgkin lymphoma		
Hodgkin lymphoma		
Nongenital- rhabdomyosarcoma Osteogenic sarcoma Squamous cell carcinoma of uterine cervix Ewing's sarcoma		

The risk of ovarian involvement in different cancers the risk of transmission was classified by Oktay in 2001 (Ovarian tissue cryopreservation and transplantation: preliminary findings and implications for cancer patients. *Human Reprod Update*, 2001; 7: 526).

Conclusions

Freezing of eggs or ovarian tissue should still be regarded as 'experimental' and should only be offered as a research protocol approved by ethics committees. (Robertson J A. Cancer and fertility: ethical and legal challenges. *J Natl Cancer Inst Monogr*. 2005;104-6). No doubt oocyte freezing and banking will become an option for patients seeking fertility preservation (Gosden 2005), but patients undergoing cancer treatment should be appropriately and honestly counselled, keeping in mind the small chance of success.

The use of cryopreservation for social reasons can not yet be justified.



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