Published in final edited form as:

Best Pract Res Clin Obstet Gynaecol. 2012 June ; 26(3): 391–405. doi:10.1016/j.bpobgyn.2012.01.001.

Recent advances in oocyte and ovarian tissue cryopreservation and transplantation

Kenny A. Rodriguez-Wallberg, MD, PhD* and

Karolinska Institute, Department of Clinical Science, Intervention and Technology, Division of Obstetrics and Gynecology K57, Karolinska University Hospital Huddinge SE-141 86 Stockholm, Sweden; and ²Karolinska University Hospital Huddinge, Fertility Unit, Stockholm, Sweden

Kutluk Oktay, MD, FACOG

Institute for Fertility Preservation, Department of Obstetrics and Gynecology, New York Medical College, Valhalla, NY, USA

Abstract

Options for preserving fertility in women include well-established methods such as fertility-sparing surgery, shielding to reduce radiation damage to reproductive organs, and emergency in-vitro fertilisation after controlled ovarian stimulation, with the aim of freezing embryos. The practice of transferring frozen or thawed embryos has been in place for over 25 years, and today is a routine clinical treatment in fertility clinics. Oocytes may also be frozen unfertilised for later thawing and fertilisation by intracytoplasmic sperm injection *in vitro*. In recent years, oocyte cryopreservation methods have further developed, reaching promising standards. More than 1000 children are born worldwide after fertilisation of frozen and thawed oocytes. Nevertheless, this technique is still considered experimental. In this chapter, we focus on options for fertility preservation still in development that can be offered to women. These include freezing of oocytes and ovarian cortex and the transplantation of ovarian tissue.

Keywords

fertility preservation; female; children; oocyte cryopreservation; egg freezing; ovarian tissue cryopreservation; ovarian tissue transplantation; reproductive health; cancer treatment; laparoscopy; gonadotoxicity; radiotherapy; chemotherapy; adolescents; prepubertal girls

Introduction

Established fertility-preservation methods for women recognised at this time include fertility-sparing surgery and surgical ovarian transposition, shielding to reduce radiation damage to reproductive organs, and embryo cryopreservation after ovarian stimulation with gonadotropins and in-vitro fertilization (IVF). Currently, all remaining options are still considered experimental.

© 2012 Elsevier Ltd. All rights reserved.

*Corresponding author. kenny.rodriguez-wallberg@karolinska.se (K. Rodriguez-Wallberg), Tel +46 8 585 800 00; Fax +46 8 585 872 50.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Cryopreservation techniques play a central role in assisted reproduction. Since initial reports that sperm could survive after being cooled at low temperatures,¹ methods of preserving spermatozoa developed rapidly after accidental discovery of the cryoprotective properties of glycerol on sperm cells.² Research on cryopreservation of sperm could advance relatively fast owing to the small size of sperm cells and their high number available for experiments. Since the first reported live birth after insemination with spermatozoa that had been frozen and thawed,³ clinical treatments have developed fast. Further development of sperm cryopreservation techniques has made the introduction of fertility treatments possible with banked frozen and thawed sperm from donors, and the establishment of sperm banking as a method for preserving male fertility.

With the development of IVF,⁴⁻⁷ methods for cryopreservation of embryos were required, as IVF treatment often resulted in surplus embryos.⁸ The human embryo has a relatively high permeability to cryoprotectants, and it is notably more resistant to cryodamage than the oocyte. After the first reported human pregnancy after cryopreservation, and thaw of an eight-cell embryo in 1983,⁹ methods for cryopreservation of embryos developed successfully. It is estimated that more than half a million babies have been born worldwide after intrauterine replacement of frozen and thawed embryos.¹⁰

Cryopreservation of oocytes

Freezing of oocytes for fertility preservation is an option for women who do not have a partner and for women who do not wish to preserve embryos because of religious or ethical concerns. Oocyte cryopreservation for fertility preservation may also be an option for adolescent girls in selected cases, as shown in a recent report of a 14-year old girl interested in undergoing hormonal stimulation and oocyte freezing instead of ovarian tissue cryopreservation, which is usually the preferred option for children.¹¹

A valid concern in carrying out ovarian stimulation for oocyte freezing in women with breast cancer and other hormone-sensitive tumours is the exposure to supraphysiological oestradiol levels during gonadotropin stimulation, which has been a main obstacle in offering fertility preservation by oocyte or embryo cryopreservation to those women. Alternative and potentially safer stimulation protocols with tamoxifen,¹² and aromatase inhibitors have been developed.¹³ Those stimulation treatments have been shown to be effective,^{14,15} with no detrimental effects on relapse or survival rates in these women.¹⁶

Freezing unfertilised oocytes, with the aim of later thawing and fertilising them by IVF or intracytoplasmic sperm injection (ICSI) is today a promising option for preserving fertility.¹⁷⁻¹⁹ The road to its current success, however, was long and difficult. The human oocyte is the largest cell in the body, and its large size and large volume with a high content of water makes it extremely fragile to intracellular ice formation during the freezing and thawing processes. Oocytes, in contrast to embryos and zygotes, also present with lower permeability to cryoprotectants. Research on permeability coefficients of human oocytes collected from different women has also revealed significant differences among individuals regarding permeability to water and cryoprotectants.²⁰ Similar findings have been reported in several species, including rodents and cattle.^{21,22} For that reason, finding appropriate cooling and warming rates to ensure oocyte survival after cryopreservation has not been an easy task, and has been a great challenge in assisted reproductive technologies.²³

Chilling injury and disruption of the meiotic spindle

Concern has also been raised about the disruption of the meiotic spindle observed when oocytes are being cooled to temperatures near 0°C, which has been described as a sign of chilling injury. At low temperatures, tubulin depolymerises, and the support for the structure

of the spindle, which maintains the chromosomes aligned on the metaphase plate, disassembles.^{24,25} The meiotic spindle is needed for the correct chromosome segregation during the oocyte maturational processes.

Variations in oocytes' sensitivity to chilling injury have also been reported among individuals in human oocytes²⁴ as well as in several animal species, including non-human primates.²⁶

Nevertheless, it has been shown that the spindle reappears in the cytoplasm after warming up the oocytes at 37°C,^{27–29} and chromosomal aberrations do not seem to be increased in embryos obtained from frozen thawed oocytes compared with fresh embryos.³⁰

Increasing fertilisation rates by intra-cytoplasmic sperm injection

Over many years, the techniques for cryopreservation of oocytes were based on slow freezing protocols originally developed for embryo freezing,³¹ and success rates of oocyte survival and fertilisation were low. Since the first pregnancy reported in 1986,³² only a few live births have been achieved.³³ Cryopreservation of oocytes induces hardening of the zona pellucida, which could only be overcome after the development of ICSI.³⁴ In 1997, microinjection of a sperm *in vitro* was first used on oocytes that had been frozen and thawed, which resulted in a live birth,³⁵ and over 100 deliveries were reported in the following decade by using ICSI.^{36,37}

As a result of legal restrictions on freezing embryos, research on cryopreservation of oocytes has been particularly successful in Italy, where reports of improvement of freezing protocols have abounded.^{36,38,39} Methods of evaluating oocyte quality have been further developed,⁴⁰ and increasing survival rates of up to 75.9%, fertilisation rates of 76.2% after ICSI, and pregnancy rates of 21.3% per embryo transfer have been reported.⁴¹ The miscarriage rates reported in pregnancies obtained after fertilisation of frozen and thawed oocytes have also been similar to those found in control participants when embryos were obtained from fresh oocytes.⁴¹

Oocyte freezing by vitrification

The process of vitrification involves the use of high concentrations of cryoprotectants and rapid cooling to achieve a glass-like highly viscous solution without formation of ice crystals. Given the high inter-individual variation in oocyte sensitivity to chilling, it has been suggested that rapid cooling might prevent such injury.²³ In animal studies, cattle oocytes presented with increased survival and fertilisation rates when cooling them at high rates.⁴²

The first pregnancy after fertilisation of thawed vitrified oocytes was achieved in 1999,⁴³ and few groups reported using this technique in the following years.⁴⁴ Significant improvements in vitrification investigational protocols were reported in 2003, when Japanese researchers cryopreserved oocytes by vitrification in specially constructed devices aimed at cooling at the fastest rates, with the objective of avoiding problems with chilling and cytoskeleton damages. With those methods, a high survival rate after thawing and clinical pregnancies was obtained.^{45–47} The method of vitrification in those studies reached an oocyte survival rate of 91% after thawing and a 50% blastocyst stage development.⁴⁶

The efficiency of vitrification of oocytes has made possible the current establishment of oocyte banking in donor programmes.^{48,49} Survival rates of oocytes thawed after vitrification have been reported to be as high as 96.9%, and the fertilisation rates are currently non-significantly different from those of fresh oocytes (76.3% v 82.2%, respectively).⁵⁰ Pregnancy rates have been reported to be as high as 65.2%, and implantation

rates 40.8% with vitrified and thawed oocytes.⁵⁰ In the largest, prospective, randomised-controlled study, including 600 recipients of donated eggs conducted in Spain, no superiority of using fresh oocytes over vitrified egg-banked and thawed oocytes could be demonstrated.⁴⁹

Commercially available vitrification kits use either open system containers with a direct contact with liquid nitrogen or closed containers with no direct contact. Open systems offer the possibility of more rapid cooling; however, those systems might present with a risk of contamination and transmission of diseases.⁵¹ New approaches for preventing the potential risk of cross-contamination are being investigated, such as avoiding direct contact with liquid nitrogen by long-term vapour-phase nitrogen storage,⁵² and ultraviolet radiation applied to the liquid nitrogen, with the aim of sterilising it when using open vitrification systems.^{53,54}

Cryopreservation of immature oocytes

Because immature germinal vesicle stage oocytes have not yet formed spindle, and because they have a higher membrane permeability, it has been suggested that germinal vesicle stage oocytes might be more resistant to chilling injury than mature metaphase II oocytes.⁵⁵ Reported survival rates of immature oocytes cryopreserved with slow freezing protocols after thawing vary from 37%⁵⁶ to 63%.⁵⁷ Spindle abnormalities, however, have also been reported after in-vitro maturation,^{58,59} suggesting impaired developmental competence when oocytes are frozen at germinal vesicle stage.

Few reports have been published of live births after immature oocyte cryopreservation with subsequent in-vitro maturation and IVF.^{60–62}

Rates of fertilisation and cleavage in cryopreserved and in vitro-matured oocytes are still significantly lower compared with non-frozen control participants. Additionally, in-vitro maturation programmes have only developed in a few centres worldwide. For those reasons, current clinical treatments aim to obtain mature metaphase II oocytes for cryopreservation.

In a recent study,⁶³ in-vitro maturation of immature collected oocytes after controlled ovarian hyperstimulation significantly increased the number of mature oocytes yielded and embryos obtained for cryopreservation in women with breast cancer (45% increase in mature oocyte yield with 86% fertilisation rate of in-vitro matured oocytes). This suggests that it might be a complementary strategy for the preservation of fertility.

Perinatal outcome after oocyte cryopreservation

Over 1000 pregnancies and infants born after fertilisation of frozen-thawed oocytes have been reported worldwide, and these figures are constantly increasing.¹⁰ Studies indicate that pregnancies and infants conceived after oocyte cryopreservation do not present with increased risk of adverse obstetric outcomes or congenital anomalies.^{64–67} Nevertheless, most of the oocytes have been frozen by slow freezing methods, and the use of vitrification is relatively recent in clinical programmes. Vitrification techniques, however, had previously been introduced for the cryopreservation of blastocysts in clinical IVF programmes. A Swedish report of neonatal outcomes of children born after transfer of vitrified blastocysts⁶⁸ has presented with reassuring results, as no apparent negative effects in neonatal outcomes have been found when comparing children born after transfer of vitrified blastocysts to those born after slow-freezing or after the transfer of fresh blastocysts.

Although overall pregnancy rates are improving, freezing of oocytes is still considered experimental, and it is recommended that the method should be carried out as part of research projects under institutional review boards.^{17,37,69,70}

Cryopreservation and transplantation of ovarian tissue

Cryopreservation of ovarian tissue combined with orthotopic transplantation into an irradiated ovary restored fertility in rodents 50 years ago.⁷¹ It took more than 30 years, however, to improve the freezing protocols and transplantation procedures in larger animals and, in particular, in species that could be considered applicable to humans. In fact, the only available cryoprotectant during since the 1930s was glycerol. Although it was successful for freezing sperm, it was highly ineffective for cryopreserving oocytes and ovarian tissue.⁷² In the 1970s, more effective cryoprotectants emerged, such as propanediol, ethylene glycol and dimethylsulphoxide (DMSO).

The exhaustive studies of ovarian cryopreservation and transplantation conducted in animals, and particularly those in the lamb model, finally led to significant improvements in cryopreservation and transplantation techniques, and the establishment of a model for applying these investigational procedures to humans.⁷³ In this model, the ovarian tissue was cryopreserved after improving current slow freezing protocols using DMSO as a cryoprotectant. The ovarian tissue was frozen in slices and the thawed slices were later transplanted orthotopically. In the lamb, recovery of cyclic ovarian activity was demonstrated, and pregnancies and live born lambs achieved, as well as long-term ovarian function.⁷⁴ Since then, ovarian tissue has been cryopreserved from several species, with the aim of improving the methods, optimising existing freezing protocols, and developing new techniques, such as vitrification. The first live birth in non-human primates after ovarian tissue transplantation was reported in 2004.⁷⁵

Human ovarian cryopreservation

A method for cryopreservation of human ovarian tissue was first reported in 1996.⁷⁶ Cryopreservation of ovarian tissue for fertility preservation is indicated for adult women when ovarian stimulation for oocyte or embryo freezing is not feasible or not desired. For prepubertal girls, the cryopreservation of ovarian tissue is their only option to spare their eggs. As most eggs are within primordial follicles in the ovarian cortex, a significant number of eggs may be preserved by freezing pieces of ovarian cortex, even if they are small.

Ovarian biopsies may be harvested by minimal invasive surgery, such as laparoscopy. Ideally, this procedure should be carried out before starting cytotoxic treatment. Nevertheless, it may still be worth carrying out ovarian cortex harvesting after cytotoxic treatments in girls and young women previously unable to undergo the procedure, because girls and young women normally have high follicle counts in their ovaries.⁷⁷ Consensus is lacking about how much ovarian tissue should be harvested for cryopreservation, and some programmes for fertility preservation propose unilateral oophorectomy as standard procedure.^{78,79}

Several cryoprotectants for slow freezing of human ovarian tissue have been investigated, such as glycerol, ethylene glycol, DMSO and propanediol. Follicle survival has been evaluated after thawing pieces of frozen ovarian cortex with those cryoprotectants. The highest follicle survival rates have been obtained with ethylene glycol, whereas glycerol has been associated with the poorest results.⁷²

Studies investigating the most favourable cooling rates and dehydration times have also been conducted. After evaluating the tissue by electron microscopic morphology, slow

programmed freezing with a relatively long dehydration time became a standard cryopreservation method for human ovarian tissue from the end of the 1990s.⁸⁰

Vitrification of ovarian tissue

A concern of slow-programmed freezing has been the relatively poor survival of the ovarian stroma,⁸¹ which has been demonstrated by transmission-electron microscopy, a method that accurately evaluates cryoinjury of membranes, mitochondria and other organelles not seen in detail with any other methods.⁸² The introduction of vitrification techniques for cryopreservation of ovarian tissue has been shown to improve the viability of all compartments of the tissue, with a survival rate of follicles similar to that after slow freezing, much better integrity of ovarian stroma and undamaged morphology of blood vessels.⁸²

Vitrification of ovarian tissue used in combination with orthotopic autotransplantation has been successful in rodents⁸³ and sheep.⁸⁴ Human studies comparing slow-freezing protocols with vitrification of ovarian tissue have produced conflicting results, which may be explained by differences in the protocols and the medium used.^{85,86}

As suggested by transmission-electron microscopy, vitrification could be more effective than slow-programmed freezing when cryopreserving ovarian tissue.⁸² In one study,⁸⁷ thawing of vitrified ovarian tissue resulted in improved oocyte survival compared with slow freezing (89% v 42%, respectively).⁸⁷ The methods for vitrification have further developed, achieving a clinical grade closed cGMP-compatible system, in which the ovarian tissue sample will not be in any direct contact with liquid nitrogen during vitrification or storage, thus meeting the requirements of international tissue directives.⁸⁸

Whole-organ freezing and transplantation

Whole-ovary freezing has also been investigated, with the aim of achieving vascular anastomoses and a functioning organ after transplantation. The method has been shown to restore fertility in rats⁸⁹ and sheep,⁹⁰ but a high rate of follicle loss is still a concern.⁹⁰ Directional freezing combined with microvascular anastomosis have improved outcomes, and long lasting ovarian function has thus been obtained in sheep.⁹¹

In humans, research of whole-ovary freezing and transplantation is still at the initial stages. Transplantation of the whole fresh ovary has been reported between monozygotic twins when premature ovarian failure has occurred in the recipient twin.⁹²

Use of experimental human ovarian transplantation in the xeno-transplantation model

As the investigation of transplantation of human ovarian tissue in humans is not feasible, the xeno-transplantation of human ovarian tissue into immune-incompetent severe combined immunodeficiency (SCID) mice was initially proposed.⁹³ The SCID mouse presents with T- and B-cell immune deficiency owing to a genetic mutation,⁹⁴ and it has been used for the investigation of various xenografts, as they revascularise and survive without being rejected. The experimental model of human ovarian transplant on SCID mice has also allowed the investigation of transplant survival, xenograft's hormone production and the induction of follicle development experimentally by gonadotropin stimulation.^{95,96} In the xeno-transplant model, the thawed tissue transplanted under the kidney capsule of the SCID mouse has also allowed the efficacy of various freezing protocols and cryoprotectants to be compared⁹⁷ and optimal size of cortical pieces for freezing to be investigated.⁹⁸ Xenograft models have, in addition, been used to investigate the histological and molecular mechanism

of the effect of chemotherapy agents in ovarian follicle loss after administration of chemotherapy agents in similar doses to those used for clinical treatments.⁹⁹

Studies in the xenograph model aimed at improving ovarian transplant survival

It has been shown that the survival of an ovarian transplant is greatly dependent on the revascularisation process, as the ischaemic phase that follows immediately after transplanting is associated with an initial massive follicle loss.^{100,101} In the SCID mouse model, experiments allow the investigation of pharmacologically active substances in xenographs. Antiapoptotic substances, such as sphingosine-1-phosphate, have previously been investigated in rodents, resulting in inhibition of oocyte apoptosis induced by cytotoxic treatments.¹⁰² Recently, in the xenograph model, sphingosine-1-phosphate has been shown to increase vascular density after transplantation in the human ovarian graft, accelerated neo-angiogenesis and reduced necrosis and tissue hypoxia, resulting in significant improvement of oocyte survival compared with vehicle-treated controls.¹⁰³

Transplantation of cryopreserved ovarian tissue for recovering ovarian function and fertility

The successful development of cryopreservation techniques for human ovarian tissue stimulated the initiation of ovarian tissue freezing as an option to preserve female fertility from the late 1990s.

In the year 2000, the first case of auto-transplantation of cryopreserved and thawed ovarian tissue by laparoscopy in a woman was reported.¹⁰⁴ She had undergone oophorectomy at age 28 years owing to intractable dysfunctional bleeding, and her ovarian tissue was frozen by using a slow-freezing protocol with propanediol as cryoprotectant. Because of serious menopausal symptoms, the woman requested autotransplantation of the ovarian tissue, and the procedure was carried out 8 months after cryostorage. The histological analysis of one piece of cortex revealed normal stromal cellularity and presence of follicles with one or two layers of granulosa cells. Six pieces of cortex were cultured *in vitro* for 6 days in the presence of gonadotropins, and increased amounts of oestradiol, progesterone and testosterone could be measured. After investigating the quality of the frozen and thawed cortical tissue, 80 pieces of cortex measuring 2 by 2 mm to 5 by 10 mm were thawed using stepwise cryoprotectant dilution, as previously described.¹⁰⁵

The ovarian cortical tissue was grafted by laparoscopy.¹⁰⁶ After thawing, the cortical pieces were strung with a 6-0 delayed absorbable suture to two absorbable cellulose membrane frames and loaded retrogradely into a 13-mm trocar (Fig. 1). The graft was thereafter placed in a pocket created in the pelvic sidewall (ovarian fossa) posterior to the broad ligament (Fig. 2) flattened and sutured (Fig. 3) and covered by peritoneum (Fig. 4).¹⁰⁶ The grafts could be seen immediately after the surgery, and blood flow to the transplant was confirmed by Doppler ultrasonography.

Hormone stimulation with daily administration of gonadotropins 15 weeks after transplantation resulted in follicle development and ovulation, which was corroborated by increases of oestradiol and subsequently progesterone. A corpus luteum in the transplant confirmed ovulation by ultrasonography. The endometrium also presented follicular and luteal phase changes, and the woman menstruated 2 weeks after ovulation.

Since 2004, several centres worldwide have reported cases of orthotopic transplantation of cryopreserved ovarian tissue in cancer survivors, resulting in live births.^{107–110} A recent

review of the first 10 cases of successful orthotopic transplantation, which has resulted in the birth of 13 children, indicates that age at cryopreservation may be an important prognostic factor, as all except one woman in that report were younger than 30 years, and six of the women were younger than 25 years.¹¹¹ As noted by the American Society of Clinical Oncology recommendations on fertility preservation, the benefit of ovarian cryopreservation for women older than 40 years of age is uncertain because of their age-reduced ovarian reserve of primordial follicles.¹⁷

In reports of successful ovarian transplantation, restoration of ovarian function has been shown by the rise of oestrogen levels and decrease of gonadotropin levels between 3.5 and 6.5 months after ovarian grafting.¹¹¹ Ovarian transplants in those cases have been shown to become functional after grafting in a peritoneal window close to the ovarian hilus and on the ovarian medulla, as proved by follicle development. In all cases, slow-programmed freezing was used to cryopreserve the ovarian tissue, and both large strips of 8–10 mm × 5 mm or small pieces of 2 × 2 mm of tissue were shown to restore ovarian endocrine function effectively. More than 50% of the women in that report conceived naturally, which argues in favour of orthotopic transplantation; only a few women required IVF to become pregnant.¹¹¹ A recent report of preparation of thin ovarian fragments for transplantation (less than 350 µm thickness) led to a successful pregnancy after failure of ovarian strip transplantation on two previous occasions.¹¹²

Heterotopic ovarian transplantation

Subcutaneous heterotopic transplantation of ovarian tissue have been proposed in the forearm¹¹³ and in the lower abdominal wall.¹¹⁴ Both techniques have been associated with reports of restoration of hormonal function, follicle development and oocyte retrieval.^{113,114} In addition, embryo generation has been reported after IVF of oocytes recovered from heterotopic transplants,^{114,115} nevertheless no live births have yet been reported.

Spontaneous pregnancies after heterotopic ovarian transplant have been reported;¹¹⁶ in one case, four pregnancies and three live births resulted within a lapse of 5 years after a subcutaneous transplantation on the lower abdominal wall in a woman having being menopausal until the time of transplant (Fig. 5).¹¹⁷ Several case reports have described spontaneous pregnancies resulting in women who have undergone ovarian graft transplants simultaneously at orthotopic and heterotopic locations.^{118–120}

It has been hypothesised that the transplant of ovarian tissue containing a healthy niche may provide endocrine or paracrine signals, which could activate the remaining atrophic cytotoxic-treated ovary by mechanisms still unknown. The true origin of pregnancies after ovarian transplantation has been questioned.¹¹⁷

The advantages of a subcutaneous heterotopic transplantation include the simplicity and non-invasiveness of the surgical technique, as well as the potential benefit of closely monitoring the tissue for an eventual recurrence of malignancy in the graft. The disadvantages of heterotopic transplantation include cosmetic issues, potential adverse effects of an environment different from the pelvic intra-abdominal to the growing follicles, and the presumed requirement for assisted reproduction to achieve a pregnancy.

In a recent experimental study in baboons,¹²¹ four different heterotopic sites for ovarian transplantation were compared. Ovarian biopsies taken after a second-look laparoscopy 3–6 months after transplantation revealed that an omental location was associated with better follicle survival and development of large antral follicles compared with grafts transplanted in the abdominal wall or into the pouch of Douglas; these did not result in follicle development.¹²¹

Ovarian tissue cryopreservation and transplantation has been shown to not interfere with proper genomic imprinting in mice pups;¹²² additional studies in other animal models, however, are needed.

Safety concerns with ovarian tissue transplantation

Autotransplantation of frozen and thawed ovarian tissue is only possible if absence of cancer cells in the graft is confirmed and there is a legitimate concern for the reseeding of malignant cells when carrying out ovarian transplantation. In a mouse model, the potential for reintroduction of malignant cells has been shown by grafting fresh and frozen ovarian tissue from mice with an aggressive type of lymphoma.¹²³ The transplant of human ovarian tissue, however, from women with Hodgkin's lymphoma in SCID mice did not transfer the disease to the recipients.¹²⁴

Methods for detecting cancer cells in the ovarian tissue of women having suffered from haematological malignancies are under development, including immunohistochemistry or the polymerase chain reaction applied to the tissue.¹²⁵ The investigation of residual malignant cells in the ovarian tissue, with the aim of grafting, may also be carried out by xenotransplantation to an immunodeficient SCID mouse before transplant. Autotransplantation of ovarian tissue in women who have suffered from systemic haematological malignancies is not recommended because of the high risk of retransmission of malignancy. Only women with cancer diagnosis associated with a negligible or no risk of ovarian compromise should be considered for future autotransplantation.¹²⁶

Follicles cultured isolated, or within a piece of thawed tissue, will be the option for women with haematological and ovarian malignancies. Although many improvements have been reported on the in-vitro culture of follicles at early stages, with the aim of developing them into competent mature follicles, those methods are still in development.^{127–129}

Conclusion

Cryopreservation of oocytes and ovarian tissue seem to hold much promise for fertility preservation of women undergoing gonadotoxic treatments. Cryopreservation of oocytes has reached promising standards, and more than 1000 children are born worldwide after fertilisation of frozen and thawed oocytes. Nevertheless, that technique is still considered experimental.

Ovarian-tissue harvesting seems to be safe, but the experience with ovarian transplantation is still limited owing to low utilisation. Autotransplantation of ovarian tissue in women having suffered from systemic haematological malignancies is not recommended, owing to the high risk of retransmission of malignancy. Only women with cancer diagnosis associated with a negligible or no risk of ovarian compromise should be considered for future autotransplantation.

Acknowledgments

Dr Rodriguez-Wallberg is supported by grants from The Swedish Society of Medical Research (SSMF). Dr Oktay is supported by NIH grants R01 HD 53112 (NICHD and NCI) and R21 HD 061259 from NICHD.

References

1. Hoagland H, Pincus G. Revival of mammalian sperm after immersion in liquid nitrogen. *J Gen Physiol.* 1942; 25:337–344. [PubMed: 19873277]

2. Polge C, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature*. 1949; 164:666. [PubMed: 18143360]
3. Sherman JK. Research on frozen human semen: past, present, and future. *Fertil Steril*. 1964; 15:485–499. [PubMed: 14208752]
4. Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilization in vitro of human oocytes matured in vitro. *Nature*. 1969; 221:632–635. [PubMed: 4886881]
5. Edwards RG, Steptoe PC, Purdy JM. Fertilization and cleavage in vitro of preovulatory human oocytes. *Nature*. 1970; 227:1307–1309. [PubMed: 4916973]
6. Edwards RG, Steptoe PC. Induction of follicular growth, ovulation and luteinization in the human ovary. *J Reprod Fertil Suppl*. 1975; 22:121–163. [PubMed: 1058970]
7. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet*. 1978; 2:366. [PubMed: 79723]
8. Edwards, RG.; Steptoe, PC. The relevance of the frozen storage of human embryos; Ciba Found Symp; 1977. p. 235-250.
9. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. *Nature*. 1983; 305:707–709. [PubMed: 6633637]
10. Gosden R. Cryopreservation: a cold look at technology for fertility preservation. *Fertil Steril*. 2011; 96:264–268. [PubMed: 21718989]
11. Oktay K, Rodriguez-Wallberg KA, Sahin G. Fertility preservation by ovarian stimulation and oocyte cryopreservation in a 14-year-old adolescent with Turner syndrome mosaicism and impending premature ovarian failure. *Fertil Steril*. 2010; 94:753.e15–753.e19. [PubMed: 20188362]
12. Oktay K, Buyuk E, Davis O, et al. Fertility preservation in breast cancer patients: IVF and embryo cryopreservation after ovarian stimulation with tamoxifen. *Hum Reprod*. 2003; 18:90–95. [PubMed: 12525446]
13. Oktay K, Buyuk E, Libertella N, et al. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. *J Clin Oncol*. 2005; 23:4347–4353. [PubMed: 15824416]
14. Oktay K, Hourvitz A, Sahin G, et al. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. *J Clin Endocrinol Metab*. 2006; 91:3885–3890. [PubMed: 16882752]
15. Oktay K, Turkcuoglu I, Rodriguez-Wallberg RA. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. *Reprod Biomed online*. 2010; 20:783–788. [PubMed: 20382080]
16. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. *J Clin Oncol*. 2008; 26:2630–2635. [PubMed: 18509175]
17. Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J Clin Oncol*. 2006; 24:2917–2931. [PubMed: 16651642]
18. Noyes N, Knopman J, Labella P, et al. Oocyte cryopreservation outcomes including pre-cryopreservation and post-thaw meiotic spindle evaluation following slow cooling and vitrification of human oocytes. *Fertil Steril*. 2010; 94:2078–2082. [PubMed: 20188356]
19. Werner M, Reh A, Labella PA, et al. Laboratory evaluation in oocyte cryopreservation suggests retrieved oocytes are comparable whether frozen for medical indications, deferred reproduction or oocyte donation. *J Assist Reprod Genet*. 2010; 27:613–617. [PubMed: 20665237]
20. Van den Abbeel E, Schneider U, Liu J, et al. Osmotic responses and tolerance limits to changes in external osmolalities, and oolemma permeability characteristics, of human in vitro matured MII oocytes. *Hum Reprod*. 2007; 22:1959–1972. [PubMed: 17428880]
21. Benson CT, Critser JK. Variation of water permeability (L_p) and its activation energy (E_a) among unfertilized golden hamster and ICR murine oocytes. *Cryobiology*. 1994; 31:215–223. [PubMed: 8050267]

22. Ruffing NA, Steponkus PL, Pitt RE, et al. Osmometric behavior, hydraulic conductivity, and incidence of intracellular ice formation in bovine oocytes at different developmental stages. *Cryobiology*. 1993; 30:562–580. [PubMed: 8306705]
23. Leibo SP, Pool TB. The principal variables of cryopreservation: solutions, temperatures, and rate changes. *Fertil Steril*. 2011; 96:269–276. [PubMed: 21782053]
24. Zenzes MT, Bielecki R, Casper RF, et al. Effects of chilling to 0 degrees C on the morphology of meiotic spindles in human metaphase II oocytes. *Fertil Steril*. 2001; 75:769–777. [PubMed: 11287033]
25. Mandelbaum J, Anastasiou O, Lévy R, et al. Effects of cryopreservation on the meiotic spindle of human oocytes. *Eur J Obstet Gynecol Reprod Biol*. 2004; 113(Suppl 1):S17–S23. [PubMed: 15041125]
26. Songsasen N, Yu IJ, Ratterree MS, et al. Effect of chilling on the organization of tubulin and chromosomes in rhesus monkey oocytes. *Fertil Steril*. 2002; 77:818–825. [PubMed: 11937140]
27. Pickering SJ, Johnson MH. The influence of cooling on the organization of the meiotic spindle of the mouse oocyte. *Hum Reprod*. 1987; 2:207–216. [PubMed: 3597741]
28. Almeida PA, Bolton VN. The effect of temperature fluctuations on the cytoskeletal organisation and chromosomal constitution of the human oocyte. *Zygote*. 1995; 3:357–365. [PubMed: 8730901]
29. Gomes CM, Silva CA, Acevedo N, et al. Influence of vitrification on mouse metaphase II oocyte spindle dynamics and chromatin alignment. *Fertil Steril*. 2008; 90(4 Suppl):1396–1404. [PubMed: 18359482]
30. Cobo A, Rubio C, Gerli S, et al. Use of fluorescence in situ hybridization to assess the chromosomal status of embryos obtained from cryopreserved oocytes. *Fertil Steril*. 2001; 75:354–360. [PubMed: 11172839]
31. Lassalle B, Testart J, Renard JP. Human embryo features that influence the success of cryopreservation with the use of 1,2 propanediol. *Fertil Steril*. 1985; 44:645–651. [PubMed: 4054343]
32. Chen C. Pregnancy after human oocyte cryopreservation. *Lancet*. 1986; 1:884–886. [PubMed: 2870356]
33. Al-Hasani S, Diedrich K, van der Ven H, et al. Cryopreservation of human oocytes. *Hum Reprod*. 1987; 2:695–700. [PubMed: 3437048]
34. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*. 1992; 340:17–18. [PubMed: 1351601]
35. Porcu E, Fabbri R, Seracchioli R, et al. Birth of a healthy female after intracytoplasmic sperm injection of cryopreserved human oocytes. *Fertil Steril*. 1997; 68:724–726. [PubMed: 9341619]
36. Borini A, Sciajno R, Bianchi V, et al. Clinical outcome of oocyte cryopreservation after slow cooling with a protocol utilizing a high sucrose concentration. *Hum Reprod*. 2006; 21:512–517. [PubMed: 16239316]
37. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. *Fertil Steril*. 2006; 86:70–80. [PubMed: 16818031]
38. Fabbri R, Porcu E, Marsella T, et al. Human oocyte cryopreservation: new perspectives regarding oocyte survival. *Hum Reprod*. 2001; 16:411–416. [PubMed: 11228204]
39. Bianchi V, Cotichio G, Fava L, et al. Meiotic spindle imaging in human oocytes frozen with a slow freezing procedure involving high sucrose concentration. *Hum Reprod*. 2005; 20:1078–1083. [PubMed: 15760952]
40. Rienzi L, Martinez F, Ubaldi F, et al. Polscope analysis of meiotic spindle changes in living metaphase II human oocytes during the freezing and thawing procedures. *Hum Reprod*. 2004; 19:655–659. [PubMed: 14998966]
41. Bianchi V, Cotichio G, Distratis V, et al. Differential sucrose concentration during dehydration (0.2 mol/l) and rehydration (0.3 mol/l) increases the implantation rate of frozen human oocytes. *Reprod Biomed Online*. 2007; 14:64–71. [PubMed: 17207333]
42. Martino A, Songsasen N, Leibo SP. Development into blastocysts of bovine oocytes cryopreserved by ultra-rapid cooling. *Biol Reprod*. 1996; 54:1059–1069. [PubMed: 8722627]

43. Kuleshova L, Gianaroli L, Magli C, et al. Birth following vitrification of a small number of human oocytes: case report. *Hum Reprod.* 1999; 14:3077–3079. [PubMed: 10601099]
44. Yoon TK, Kim TJ, Park SE, et al. Live births after vitrification of oocytes in a stimulated in vitro fertilization-embryo transfer program. *Fertil Steril.* 2003; 79:1323–1326. [PubMed: 12798878]
45. Katayama KP, Stehlik J, Kuwayama M, et al. High survival rate of vitrified human oocytes results in clinical pregnancy. *Fertil Steril.* 2003; 80:223–224. [PubMed: 12849831]
46. Kuwayama M, Vajta G, Kato O, et al. Highly efficient vitrification method for cryopreservation of human oocytes. *Reprod Biomed Online.* 2005; 11:300–308. [PubMed: 16176668]
47. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. *Theriogenology.* 2007; 67:73–80. [PubMed: 17055564]
48. Nagy ZP, Chang CC, Shapiro DB, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. *Fertil Steril.* 2009; 92:520–526. [PubMed: 18692830]
49. Cobo A, Meseguer M, Remohí J, et al. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. *Hum Reprod.* 2010; 25:2239–2246. [PubMed: 20591872]
50. Cobo A, Kuwayama M, Pérez S, et al. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertil Steril.* 2008; 89:1657–1664. [PubMed: 17889865]
51. Cutting R, Barlow S, Anderson R. Human oocyte cryopreservation: evidence for practice. *Hum Fertil.* 2009; 12:125–136.
52. Cobo A, Romero JL, Pérez S, et al. Storage of human oocytes in the vapor phase of nitrogen. *Fertil Steril.* 2010; 94:1903–1907. [PubMed: 20138272]
53. Parmegiani L, Cognigni GE, Filicori M. Ultra-violet sterilization of liquid nitrogen prior to vitrification. *Hum Reprod.* 2009; 24:2969. [PubMed: 19749194]
54. Parmegiani L, Accorsi A, Cognigni GE, et al. Sterilization of liquid nitrogen with ultraviolet irradiation for safe vitrification of human oocytes or embryos. *Fertil Steril.* 2010; 94:1525–1528. [PubMed: 19591992]
55. Tucker MJ, Morton PC, Wright G, et al. Clinical application of human egg cryopreservation. *Hum Reprod.* 1998; 13:3156–3159. [PubMed: 9853874]
56. Mandelbaum J, Junca AM, Plachot M, et al. Cryopreservation of immature and mature hamster and human oocytes. *Ann N Y Acad Sci.* 1988; 541:550–561. [PubMed: 3195933]
57. Baka SG, Toth TL, Veeck L, et al. Evaluation of the spindle apparatus of in-vitro matured human oocytes following cryopreservation. *Hum Reprod.* 1995; 10:1816–1820. [PubMed: 8582988]
58. Boiso I, Martí M, Santaló J, et al. A confocal microscopy analysis of the spindle and chromosome configurations of human oocytes cryopreserved at the germinal vesicle and metaphase II stage. *Hum Reprod.* 2002; 17:1885–1891. [PubMed: 12093855]
59. Stachecki JJ, Munne S, Cohen J. Spindle organization after cryopreservation of mouse, human, and bovine oocytes. *Reprod Biomed Online.* 2004; 8:664–672. [PubMed: 15169583]
60. Cha KY, Koo JJ, Ko JJ, Choi DH, et al. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. *Fertil Steril.* 1991; 55:109–113. [PubMed: 1986950]
61. Tucker MJ, Wright G, Morton PC, et al. Birth after cryopreservation of immature oocytes with subsequent in vitro maturation. *Fertil Steril.* 1998; 70:578–579. [PubMed: 9757897]
62. Kan A, Kilani S, Tilia L, et al. Pregnancy from intracytoplasmic injection of a frozen-thawed oocyte. *Aust N Z J Obstet Gynaecol.* 2004; 44:262–263. [PubMed: 15191455]
63. Oktay K, Buyuk E, Rodriguez-Wallberg KA, et al. In vitro maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation. *Reprod Biomed Online.* 2010; 20:634–638. [PubMed: 20219430]
64. Borini A, Bianchi V, Bonu MA, et al. Evidence-based clinical outcome of oocyte slow cooling. *Reprod Biomed Online.* 2007; 15:175–181. [PubMed: 17697493]
65. Chian RC, Huang JY, Tan SL, et al. Obstetric and perinatal outcome in 200 infants conceived from vitrified oocytes. *Reprod Biomed Online.* 2008; 16:608–610. [PubMed: 18492361]

66. Noyes N, Porcu E, Borini A. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. *Reprod Biomed Online*. 2009; 18:769–776. [PubMed: 19490780]
67. Wennerholm UB, Söderström-Anttila V, Bergh C, et al. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. *Hum Reprod*. 2009; 24:2158–2172. [PubMed: 19458318]
68. Wikland M, Hardarson T, Hillensjö T, et al. Obstetric outcomes after transfer of vitrified blastocysts. *Hum Reprod*. 2010; 25:1699–1707. [PubMed: 20472913]
69. Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technologies. Ovarian tissue and oocyte cryopreservation. *Fertil Steril*. 2008; 90:134–135.
70. Practice Committee of the American Society for Reproductive Medicine. ASRM Practice Committee response to Rybak and Lieman: elective self-donation of oocytes. *Fertil Steril*. 2009; 92:1513–1514. [PubMed: 19836735]
71. Parrot D. The fertility of mice with orthotopic ovarian grafts derived from frozen tissue. *J Reprod Fertil*. 1960; 1:230–241.
72. Newton H, Aubard Y, Rutherford A, et al. Low temperature storage and grafting of human ovarian tissue. *Hum Reprod*. 1996; 11:1487–1491. [PubMed: 8671490]
73. Gosden RG, Baird DT, Wade JC, Webb R, et al. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at –196 degrees C. *Hum Reprod*. 1994; 9:597–603. [PubMed: 8046009]
74. Baird DT, Campbell B, de Souza C, Telfer E, et al. Long-term ovarian function in sheep after ovariectomy and autotransplantation of cryopreserved cortical strips. *Eur J Obstet Gynecol Reprod Biol*. 2004; 113(Suppl 1):S55–S59. [PubMed: 15041133]
75. Lee DM, Stouffer RL, Zelinski-Wooten MB, et al. Live birth after ovarian tissue transplant. *Nature*. 2004; 428:137–138. [PubMed: 15014485]
76. Hovatta O, Silye R, Krausz T, et al. Cryopreservation of human ovarian tissue using dimethylsulphoxide and propanediol-sucrose as cryoprotectants. *Hum Reprod*. 1996; 11:1268–1272. [PubMed: 8671438]
77. Wallberg KA, Keros V, Hovatta O. Clinical aspects of fertility preservation in female patients. *Pediatr Blood Cancer*. 2009; 53:254–260. [PubMed: 19340856]
78. Rosendahl M, Andersen CY, Ernst E, et al. Ovarian function after removal of an entire ovary for cryopreservation of pieces of cortex prior to gonadotoxic treatment: a follow-up study. *Hum Reprod*. 2008; 23:2475–2483. [PubMed: 18635528]
79. Harel S, Ferme C, Poirot C. Management of fertility in patients treated for Hodgkin's lymphoma. *Haematologica*. 2011; 96:1692–1699. [PubMed: 21828120]
80. Gook DA, Edgar DH, Stern C. Effect of cooling rate and dehydration regimen on the histological appearance of human ovarian cortex following cryopreservation in 1, 2-propanediol. *Hum Reprod*. 1999; 14:2061–2068. [PubMed: 10438427]
81. Hreinsson J, Zhang P, Swahn ML, et al. Cryopreservation of follicles in human ovarian cortical tissue. Comparison of serum and human serum albumin in the cryoprotectant solutions. *Hum Reprod*. 2003; 18:2420–2428. [PubMed: 14585896]
82. Keros V, Xella S, Hultenby K, et al. Vitrification versus controlled-rate freezing in cryopreservation of human ovarian tissue. *Hum Reprod*. 2009; 24:1670–1683. [PubMed: 19359339]
83. Sugimoto M, Maeda S, Manabe N, et al. Development of infantile rat ovaries autotransplanted after cryopreservation by vitrification. *Theriogenology*. 2000; 53:1093–1103. [PubMed: 10798487]
84. Bordes A, Lornage J, Demirci B, et al. Normal gestations and live births after orthotopic autograft of vitrified-warmed hemi-ovaries into ewes. *Hum Reprod*. 2005; 20:2745–2748. [PubMed: 15980012]
85. Isachenko V, Isachenko E, Weiss JM. Human ovarian tissue: vitrification versus conventional freezing. *Hum Reprod*. 2009; 24:1767–1768. author reply 1768–1769. [PubMed: 19386631]

86. Isachenko V, Isachenko E, Kreienberg R, et al. Human ovarian tissue cryopreservation: quality of follicles as a criteria of effectiveness. *Reprod Biomed Online*. 2010; 20:441–442. [PubMed: 20153256]
87. Silber S, Kagawa N, Kuwayama M, et al. Duration of fertility after fresh and frozen ovary transplantation. *Fertil Steril*. 2010; 94:2191–2196. [PubMed: 20171622]
88. Sheikhi M, Hultenby K, Niklasson B, et al. Clinical grade vitrification of human ovarian tissue: an ultrastructural analysis of follicles and stroma in vitrified tissue. *Hum Reprod*. 2011; 26:594–603. [PubMed: 21217141]
89. Yin H, Wang X, Kim SS, et al. Transplantation of intact rat gonads using vascular anastomosis: effects of cryopreservation, ischaemia and genotype. *Hum Reprod*. 2003; 18:1165–1172. [PubMed: 12773441]
90. Courbiere B, Caquant L, Mazoyer C, et al. Difficulties improving ovarian functional recovery by microvascular transplantation and whole ovary vitrification. *Fertil Steril*. 2009; 91:2697–2706. [PubMed: 18440531]
91. Arav A, Gavish Z, Elami A, et al. Ovarian function 6years after cryopreservation and transplantation of whole sheep ovaries. *Reprod Biomed Online*. 2010; 20:48–52. [PubMed: 20158987]
92. Silber SJ. Fresh ovarian tissue and whole ovary transplantation. *Semin Reprod Med*. 2009; 27:479–485. [PubMed: 19806517]
93. Gosden RG, Boulton MI, Grant K, et al. Follicular development from ovarian xenografts in SCID mice. *J Reprod Fertil*. 1994; 101:619–623. [PubMed: 7966017]
94. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. *Nature*. 1983; 301:527–530. [PubMed: 6823332]
95. Oktay K, Newton H, Mullan J, et al. Development of human primordial follicles to antral stages in SCID/hpg mice stimulated with follicle stimulating hormone. *Hum Reprod*. 1998; 13:1133–1138. [PubMed: 9647533]
96. Weissman A, Gotlieb L, Colgan T, et al. Preliminary experience with subcutaneous human ovarian cortex transplantation in the NOD-SCID mouse. *Biol Reprod*. 1999; 60:1462–1467. [PubMed: 10330106]
97. Oktay K, Newton H, Gosden RG. Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice. *Fertil Steril*. 2000; 73:599–603. [PubMed: 10689020]
98. Nisolle M, Casanas-Roux F, Qu J, et al. Histologic and ultrastructural evaluation of fresh and frozen-thawed human ovarian xenografts in nude mice. *Fertil Steril*. 2000; 74:122–129. [PubMed: 10899508]
99. Oktem O, Oktay K. A novel ovarian xenografting model to characterize the impact of chemotherapy agents on human primordial follicle reserve. *Cancer Res*. 2007; 67:10159–10162. [PubMed: 17974956]
100. Baird DT, Webb R, Campbell BK, et al. Long-term ovarian function in sheep after ovariectomy and transplantation of autografts stored at -196 C. *Endocrinology*. 1999; 140:462–471. [PubMed: 9886858]
101. Liu J, Van der Elst J, Van den Broecke R, et al. Early massive follicle loss and apoptosis in heterotopically grafted newborn mouse ovaries. *Hum Reprod*. 2002; 17:605–611. [PubMed: 11870111]
102. Morita Y, Perez GI, Paris F, et al. Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. *Nat Med*. 2000; 6:1109–1114. [PubMed: 11017141]
103. Soleimani R, Heytens E, Oktay K. Enhancement of neoangiogenesis and follicle survival by sphingosine-1-phosphate in human ovarian tissue xenotransplants. *PLoS One*. 2011; 6:e19475. [PubMed: 21559342]
104. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. *N Engl J Med*. 2000; 342:1919. [PubMed: 10877641]
105. Oktay K, Newton H, Aubard Y, et al. Cryopreservation of immature human oocytes and ovarian tissue: an emerging technology? *Fertil Steril*. 1998; 69:1–7. [PubMed: 9457923]

106. Oktay K, Aydin BA, Karlikaya G. A technique for laparoscopic transplantation of frozen-banked ovarian tissue. *Fertil Steril*. 2001; 75:1212–1216. [PubMed: 11384651]
107. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet*. 2004; 364:1405–1410. [PubMed: 15488215]
108. Meirow D, Levron J, Eldar-Geva T, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *N Engl J Med*. 2005; 353:318–321. [PubMed: 15983020]
109. Demeestere I, Simon P, Buxant F, et al. Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: case report. *Hum Reprod*. 2006; 21:2010–2014. [PubMed: 16585122]
110. Andersen CY, Rosendahl M, Byskov AG, et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. *Hum Reprod*. 2008; 23:2266–2272. [PubMed: 18603535]
111. Donnez J, Silber S, Andersen CY, et al. Children born after autotransplantation of cryopreserved ovarian tissue. a review of 13 live births. *Ann Med*. 2011; 43:437–450. [PubMed: 21226660]
112. Revel A, Laufer N, Ben Meir A, et al. Micro-organ ovarian transplantation enables pregnancy: a case report. *Hum Reprod*. 2011; 26:1097–1103. [PubMed: 21421665]
113. Oktay K, Economos K, Kan M, Rucinski J, et al. Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm. *JAMA*. 2001; 286:1490–1493. [PubMed: 11572742]
114. Oktay K, Buyuk E, Veeck L, et al. Embryo development after heterotopic ransplantation of cryopreserved ovarian tissue. *Lancet*. 2004; 363:837–840. [PubMed: 15031026]
115. Rosendahl M, Loft A, Byskov AG, et al. Biochemical pregnancy after fertilization of an oocyte aspirated from a heterotopic autotransplant of cryopreserved ovarian tissue: case report. *Hum Reprod*. 2006; 21:2006–2009. [PubMed: 16684840]
116. Oktay K. Spontaneous conceptions and live birth after heterotopic ovarian transplantation: is there a germline stem cell connection? *Hum Reprod*. 2006; 21:1345–1348. [PubMed: 16459347]
117. Oktay K, Turkcuoglu I, Rodriguez-Wallberg KA. Four spontaneous pregnancies and three live births following subcutaneous transplantation of frozen banked ovarian tissue: what is the explanation? *Fertil Steril*. 2011; 95:804 e7–804 e10. [PubMed: 20801441]
118. Demeestere I, Simon P, Emiliani S, et al. Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease. *Oncologist*. 2007; 12:1437–1442. [PubMed: 18165621]
119. Demeestere I, Simon P, Moffa F, et al. Birth of a second healthy girl more than 3 years after cryopreserved ovarian graft. *Hum Reprod*. 2010; 25:1590–1591. [PubMed: 20395218]
120. Ernst E, Bergholdt S, Jørgensen JS, et al. The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue. *Hum Reprod*. 2010; 25:1280–1281. [PubMed: 20172869]
121. Diaz-Garcia C, Milenkovic M, Groth K, et al. Ovarian cortex transplantation in the baboon: comparison of four different intra-abdominal transplantation sites. *Hum Reprod*. 2011; 26:3303–3311. [PubMed: 21954279]
122. Sauvat F, Capito C, Sarnacki S, et al. Immature cryopreserved ovary restores puberty and fertility in mice without alteration of epigenetic marks. *PLoS One*. 2008; 3:e1972. [PubMed: 18414667]
123. Shaw JM, Bowles J, et al. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. *Hum Reprod*. 1996; 11:1668–1673. [PubMed: 8921114]
124. Kim SS, Radford J, et al. Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation. *Hum Reprod*. 2001; 16:2056–2060. [PubMed: 11574491]
125. Meirow D, Hardan I, et al. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. *Hum Reprod*. 2008; 23:1007–1013. [PubMed: 18344563]
126. Ajala T, Rafi J, et al. Fertility preservation for cancer patients: a review. *Obstet Gynecol Int*. 2010; 2010:160386.

127. Telfer EE, McLaughlin M, Ding C, et al. A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin. *Hum Reprod.* 2008; 23:1151–1158. [PubMed: 18326514]
128. Smits J, Dolmans MM, Donnez J, et al. Current achievements and future research directions in ovarian tissue culture, in vitro follicle development and transplantation: implications for fertility preservation. *Hum Reprod Update.* 2010; 16:395–414. [PubMed: 20124287]
129. Telfer EE, McLaughlin M. In vitro development of ovarian follicles. *Semin Reprod Med.* 2011; 29:15–23. [PubMed: 21207331]

Practice points

- Oocyte cryopreservation by vitrification techniques is highly developed, and overall pregnancy rates are improving; however, freezing of oocytes is still considered experimental.
- Oocyte cryopreservation can be offered to women without a partner or to women who have ethical or religious objections to freeze embryos.
- Ovarian tissue cryopreservation and transplantation are investigational methods for fertility preservation and should be offered under institutional review board approved protocols.
- Ovarian tissue cryopreservation may be an option for preserving fertility for adult women who cannot undergo hormonal stimulation for egg freezing or when there is not time for that treatment.
- Ovarian tissue cryopreservation is the only fertility preservation option for prepubertal girls.
- Ovarian tissue harvesting can easily be performed by laparoscopy.
- Survival of follicles after ovarian transplant is still low and has to be improved.

Research agenda

- Long-term perinatal outcomes of children born after oocyte cryopreservation and ovarian tissue cryopreservation.
- Predictive variables of success of cryopreservation methods for fertility preservation.
- Effectiveness of transplanted ovarian tissue on recovery of fertility.
- Best place and technique for transplantation of ovarian tissue.
- Whether ovarian transplants can play a role on the recovery of the damaged cytotoxic-treated ovary.
- Effect of fertility preservation by cryopreservation of oocytes and ovarian tissue on patients' disease recurrence and survival.

Fig. 1. Retrograde loading of the graft that was reconstructed by stringing the ovarian tissue between two strips of Surgicel®. Published with permission.¹⁰⁶

Fig. 2.
Placement of the leading suture in the pelvic pocket and through the lower peritoneal edge.
Published with permission.¹⁰⁶

Fig. 3.
Placement of the base suture through the upper peritoneal edge. By pulling on this suture, the graft is flattened against the vascular pelvic wall. Published with permission.¹⁰⁶

Fig. 4. Closure of peritoneum with interrupted sutures. Note the placement of two grafts side by side. Published with permission.¹⁰⁶

Fig. 5. Heterotopic ovarian transplant. FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; IVF, in-vitro fertilization; LH, luteinising hormone; Published with permission.¹¹⁷