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Fertility preservation for age-related fertility decline

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Cryopreservation of eggs or ovarian tissue to preserve fertility for patients with cancer has been studied since 1994 with R G Gosden's paper describing restoration of fertility in oophorectomised sheep, and for decades previously by others in smaller mammals. Clinically this approach has shown great success. Many healthy children have been born from eggs cryopreserved with the Kuwayama egg vitrification technique for non-medical (social) indications, but until now very few patients with cancer have achieved pregnancy with cryopreserved eggs. Often, oncologists do not wish to delay cancer treatment while the patient goes through multiple ovarian stimulation cycles to retrieve eggs, and the patient can only start using the oocytes after full recovery from cancer. Ovarian stimulation and egg retrieval is not a barrier for patients without cancer who wish to delay childbearing, which makes oocyte cryopreservation increasingly popular to overcome an age-related decline in fertility. Cryopreservation of ovarian tissue is an option if egg cryopreservation is ruled out. More than 35 babies have been born so far with cryopreserved ovarian tissue in patients with cancer who have had a complete return of hormonal function, and fertility to baseline. Both egg and ovarian tissue cryopreservation might be ready for application to the preservation of fertility not only in patients with cancer but also in countering the increasing incidence of age-related decline in female fertility.

Introduction

In the past decade, scientists have helped women to cryopreserve their gametes either through oocyte vitrification or ovarian cortex cryopreservation. The advent of these techniques offers hope to women confronted with the risk of iatrogenic gonadotoxicity (eg, due to chemotherapy) or to women with a genetic predisposition to primary ovarian insufficiency. In developed countries, which are characterised by a general trend among women to postpone childbearing, many anticipate an age-related decline in fertility. Healthy women are foreseeing pregnancy at a more advanced age and cryopreservation techniques are increasingly used to safeguard their future chances of reproductive success.^{1,2}

Widespread use of oocyte cryopreservation began after the introduction of the vitrification technique, and the birth of the first baby achieved using this method, in 1999.³ The oocyte vitrification method is likely to become more widespread because in 2013 the practice committees of the American Society for Reproductive Medicine (ASRM) and of the Society for Assisted Reproductive Technology removed its classification as an experimental procedure.⁴ With respect to the clinical application of elective cryopreservation to prevent age-related decline in fertility, both the ASRM⁴ and the European Society for Reproductive Medicine⁵ emphasise the importance of further follow-up with respect to the safety, cost-effectiveness, and psychological factors that might arise. Nonetheless, the European Society of Human Reproduction and Embryology (ESHRE) task force on ethics and law recommends that oocyte cryopreservation should be available for the prevention of age-related infertility and that a fertility specialist should refrain from passing judgment on a woman's motives to do so.⁵ Thus far hundreds of healthy babies have been born from eggs cryopreserved for non-medical reasons and

without an increase in the incidence of any birth defects or anomalies. Furthermore, several babies have been born using cryopreserved oocytes from patients with cancer who were otherwise sterile.⁶

An experimental approach to the prevention of age-related infertility is the cryopreservation of the ovarian cortex. In 2004 a livebirth after ovarian tissue cryopreservation for oncological reasons with auto-transplantation was described.⁷ This technique has since resulted in the birth of more than 35 healthy babies. The main advantages of this method are the large number of gametes that can be cryopreserved in one procedure and the absence of any need to delay the cancer treatment.⁸⁻¹²

In this Review we discuss various factors involved in oocyte and ovarian tissue cryopreservation, including the assessment of reproductive ageing, the various methods, and their clinical implications. Furthermore, we discuss the effects of delayed fertility on society and the demographic profiles of women who have embarked on such treatments.

Reproductive ageing

The fact that female fecundity decreases with increasing age was recognised in several demographic and epidemiological studies that consistently noted a decline in fertility beginning as early as the middle of the third decade.^{13,14} The incontrovertible effect of ageing on female reproductive function is most notable in the decline in ovarian function.^{15,16} Ovarian ageing causes a progressive loss of the finite pool of primordial follicles, ultimately resulting in menopause, and apart from this quantitative decline, an age-dependent decline in the quality of oocytes mainly as a result of increased chromosomal aneuploidy.

Artificial reproductive techniques provide an opportunity to increase fecundity for couples who are hypofertile, and of course couples who are infertile, to

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This is the third in a *Series* of three papers about fertility preservation

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have a child. However, artificial reproductive techniques cannot compensate fully for the natural decline in fertility with age¹⁷ because the age-related decline in the chance of a natural conception is reflected in an age-related decrease in the chances of fertility with artificial reproductive techniques.¹⁸ Nevertheless, more than 50% of all in-vitro fertilisation and intracytoplasmic sperm injection cycles done in Europe are in women aged 35 years or older.¹⁹ Many women, therefore, run the risk of age-related infertility and many might never get pregnant with their own oocytes.

A report¹⁸ on artificial reproductive techniques in 2009 from ESHRE registered 21604 women treated with donor oocytes, which is almost double the 11475 donor oocyte treatments in 2005.¹⁹ The mean age of oocyte recipients was 40 years or more in 56.2% of patients across Europe.¹⁸ Apart from a reversal of this trend towards women having children at a young age, the only preventive measure to avoid this increasing dependency on oocyte donation seems to be the timely cryopreservation of oocytes or ovarian tissue.

Oocyte cryopreservation

Cryopreservation process

Female gametes can survive the cryopreservation process when handled carefully. Survival is largely determined by the architecture, size, and shape of the oocyte, and by the risk of ice formation. The oocyte is a cell with high water content, which makes this gamete one of the most cryosensitive cells in the human body. Any cryopreservation process involves dehydration, together with the diffusion of cryoprotectants into the cytoplasm as a result of osmotic exchange across the oolemma. Mobilisation of water out of the oocyte and replacement with the cryoprotectant is complex in a large cell such as the oocyte and creates a high risk of crystallisation. Formation of ice in the cytoplasm, but also in the extracellular media, is the main source of cryo-injury to the oocyte (appendix).

Mazur and coworkers²⁰ extensively analysed the probability of ice formation and water loss in relation to the cooling rate of oocytes with slow-freezing procedures. Crystallisation occurs between -5°C and -80°C , causing severe mechanical effects on cell structures, and might be due to insufficient dehydration.²⁰ The probability of water crystallisation occurring in large cells such as oocytes is greater than in smaller, somatic cells and causes difficulty in reaching phase equilibrium across the cell membrane. Vitrification procedures for the cryopreservation of oocytes do not rely on establishing a chemical equilibrium; instead a higher concentration of cryoprotectant is used than in slow-freezing methods, and oocytes are rapidly frozen in liquid nitrogen, to avoid the formation of ice crystals whereby chilling injury occurs. When oocytes are ready to be used they are then gently warmed to room temperature and then to 37°C to enable rehydration.

Outcomes of slow freezing

Since Chen,²¹ claimed the first pregnancy achieved with frozen oocytes, results from slow freezing of oocytes show that conception is particularly difficult with this method. Difficulties arise mainly because of the substantial heterogeneity in the freezing protocol used, cryoprotectant concentration, small number of oocytes used in different studies, plus their nature (eg, failure to fertilise, in-vitro maturation, or germinal vesicle oocytes). Previously, especially in the 1990s, several major modifications were introduced into the protocol, which were related mainly to the mixture of cryoprotectants. Two studies^{22,23} involving larger series reported survival rates of around 75%, but with a pregnancy rate of 10–12% per patient per embryo transfer and an implantation rate of almost 5%. A meta-analysis²⁴ published in 2006 of the efficiency of oocyte cryopreservation as a potential strategy for fertility preservation concluded that the outcomes in terms of livebirth rate per oocyte achieved with slow-frozen oocytes are significantly lower compared with vitrified oocytes. The authors of a review²⁵ published a year later reached similar conclusions in terms of survival, fertilisation, and clinical outcomes. According to these authors, concerns surrounding the safety of this technology are alleviated by the studies done in the 1990s. Notably, the first babies born to patients with cancer after oocyte cryopreservation were achieved with slow-freezing procedures.^{26,27}

Outcomes of vitrification

The history of the clinical application of oocyte vitrification is shorter than that of slow freezing. Vitrification protocols have also been vastly modified over the years, more so than those for slow freezing. Changes to vitrification protocols have consisted of modification of the sample volume surrounding the oocyte, ratio and type of cryoprotectants, and the cooling and warming rates used. Nowadays, most standard methods include all or some of these modifications.

A comprehensive review²⁸ of the clinical application of oocyte vitrification in both ovum donation programmes and infertile patients undergoing autologous oocytes cycles was published. This review highlights the current state of oocyte vitrification in clinical practice. Another review²⁹ focuses on slow cooling versus the vitrification of oocytes and embryos, and concludes that vitrification procedures offer better outcomes in terms of oocyte survival and embryological development of the vitrified and warmed oocyte, which seem to be compromised after slow freezing. Additionally, the reviewers concluded that improved slow freezing methods of embryo cryopreservation yield high survival rates, and embryos show similar implantation potential to unfrozen oocytes.²⁹ A systematic review and meta-analysis³⁰ of randomised controlled trials evaluated the ongoing pregnancy rate after oocyte vitrification versus slow freezing and fresh oocytes. Only randomised controlled

See Online for appendix

trials using human oocytes and reporting data for at least one outcome measure (primary outcome: ongoing pregnancy rate; secondary outcomes: clinical pregnancy rate, implantation rate, and fertilisation rate) were selected, and open system vitrification (ie, direct contact with liquid nitrogen) was used in four of the five studies. The increasingly generalised use of open systems for oocyte vitrification in clinical practice was shown in another review.³¹ The survival rate was around 75% when a closed-system was used, but was 97% in studies that used open-system vitrification. When vitrification was compared with slow freezing in a meta-analysis³⁰ the fixed-effects model showed that the odds were in favour of vitrification (odds ratio [OR] 2.46, 95% CI 1.82–3.32). Interestingly, in the analysis of survival, sample heterogeneity was most probably due to the use of closed-system vitrification, according to a study by Smith and coworkers.³² The clinical pregnancy rate for the embryos developed from vitrified oocytes was about 38% when oocytes were derived from autologous oocyte retrieval cycles,^{32,33} and about 60% when oocytes used were from donors.^{34,35} The OR for fertilisation was in favour of vitrification compared with slow freezing (OR 1.50, 95% CI 1.07–2.11), and was similar when compared with fresh oocytes (1.02, 0.91–1.13).³⁰

Embryos developed after oocyte vitrification compared with those from fresh oocytes generated concomitantly in an ovum-donation programme showed no difference in quality in another study.³⁴ These findings were later confirmed in a randomised controlled study of sibling oocytes conducted in autologous cycles.³³ Cumulative pregnancy rates have also been calculated in a clinical programme that used fresh embryo transfers, plus additional frozen embryo transfers originating from vitrified oocytes. It showed the usefulness of this strategy, as well as the relation between cumulative pregnancy rates and patient age.³⁵

In a large randomised controlled trial,³⁵ including 600 recipients of donor oocytes and more than 6000 oocytes, investigators assessed the efficacy of cryopreserved donor oocytes from an egg-bank versus fresh donor oocytes in terms of pregnancy rate. The study showed no difference in ongoing pregnancy rates between cryopreserved and fresh oocytes.³⁵

The consistency of the oocyte vitrification method was shown in a multicentre study³⁶ of infertile patients using their own oocytes. Overall survival was 85% per oocyte and delivery rate was 28% per embryo transfer. No significant differences were found, in survival and delivery rates between centres, which shows the reproducibility of the technique. Additionally, a logistic regression model showed that patient age, number of oocytes thawed, and the developmental stage of the transferred embryos were directly related to the delivery rate, which led to interesting conclusions that might be useful for patient counselling. Cil and colleagues³⁷ did an individual patient analysis of infertile patients (figure 1).

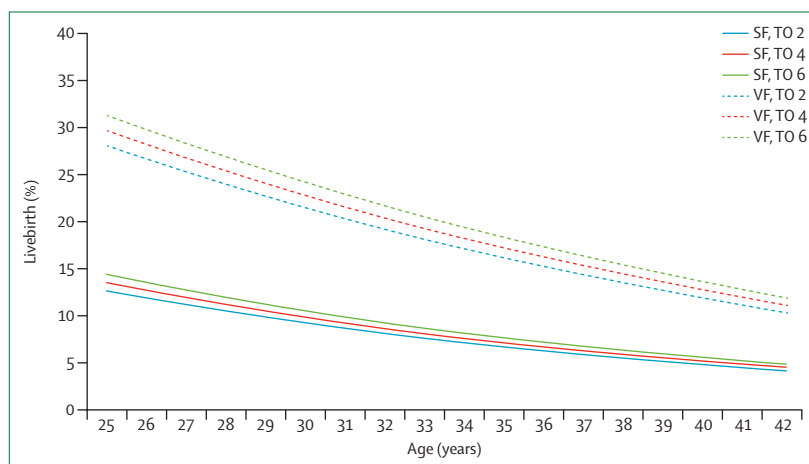


Figure 1: Predicted age-specific number of livebirths based on oocyte cryopreservation method and the number of oocytes thawed

Adapted from Cil and colleagues,³⁷ by permission of Elsevier. SF=slow freezing. VF=vitrification. TO=thawed oocytes.

Many requests for oocyte cryopreservation are from women aged 36–40 years.^{38,39} Follow-up of a cohort of 86 women with preventively cryopreserved oocytes at a mean age of 37 years showed that most women considered the process overwhelmingly positive; however, most would have preferred to have had the treatment at a younger age.⁴⁰ Most of these women had a high educational level and had partner-relationships in the past, but did not have children because they had not found the so-called right partner.³⁹

A publication by our group summarises our 5 years of experience in oocyte vitrification for fertility preservation in either patients with cancer or for other medical or non-medical reasons (table 1).⁴¹ However, information about outcomes for women who preserved their fertility through oocyte vitrification is still scarce, mainly because their gametes have not yet been used. Standardised methods have only been applied routinely in clinical practice since the second half of the past decade. Moreover, many older women who decide to preserve their fertility for social reasons could also face a long, hard road. Obviously, the large number of patients who voluntarily postpone motherhood implies a wait of several years. The period of time from when a woman opts for oocyte cryopreservation to when she decides to use them is indeterminate because she might experience fundamental changes in her life that motivate her to become a mother. The same report⁴¹ on data for 560 patients without cancer (mean age 36.7 years, SD 4.2) showed that 91% of these women decided to delay motherhood for social reasons, whereas the other 9% reported other medical conditions apart from cancer (eg, endometriosis, imminent adnexectomy) as the reason to delay motherhood. 26 (5%) of 560 patients without cancer came back to attempt pregnancy, 20 (77%) of these 26 women vitrified their oocytes because of age-related fertility decline, and the other six (23%)

	Non-oncological
Patients	26
Fresh embryo transfer procedures	37
Fresh embryo transfers	24
Mean number of embryos transferred (SD)	1.5 (0.6)
CPR per patient	11 (42.3%)
OPR per patient	8 (30.7%)
Patients with surplus embryos	17 (65.3%)
Surplus embryos vitrified [mean (SD)]	49 (2.8; 4.2)
Embryo cryotransfer procedures	15 (88.2%)
Embryo cryotransfer (SD)	2.3 (0.7)
CPR per patient	7 (46.6%)
OPR per patient	5 (33.3%)
Livebirths	5
Mean birthweight (g)	3150
Sex of the baby	
Girl	3 (60%)
Boy	2 (40%)

CPR=clinical pregnancy rate. OPR=ongoing pregnancy rate.

Table 1: Clinical outcome of the non-oncological fertility preservation



Figure 2: Transplantation of thawed and quilted ovarian slices

vitrified their oocytes because of endometriosis or oophorectomy. Accordingly, 191 oocytes were warmed (mean number of oocytes cryopreserved per patient 7.3 [SD 3.9]), and an 85% survival rate was achieved, which is similar to published values for patients who are infertile and opt for vitrification of their oocytes.^{42,43} Clinical outcomes are encouraging: 31% ongoing pregnancy rate and a 71% cumulative survival rate of both frozen and fresh embryo transfers, with five healthy babies born in this treatment group.

Ovarian cortex cryopreservation

Cryopreservation and transplantation of ovarian tissue has a long history in animal studies^{44–51,52} and early human studies.^{7,9,53–59} In 1960 Parrott and colleagues⁵⁶ showed that ovarian tissue could be successfully frozen and autografted in mice, and similar studies by Gunasena

and colleagues⁵⁰ 37 years later, verified livebirths of mice after autologous transplantation of cryopreserved mouse ovaries, originally shown in rats in 1954.⁶⁰ Others have shown that mice have a normal reproductive lifespan after autografts of fresh tissue.⁴⁶ Researchers in the 1990s showed that in both mice⁵⁰ and sheep^{44,48} frozen ovarian tissue could be successfully thawed and autotransplanted leading to normal ovarian function and livebirths. Oktay and colleagues in 2000⁵⁴ and 2004⁵⁵ showed normal embryological development in human beings after frozen ovarian tissue autografts, and Donnez and colleagues⁷ reported what is deemed to be the first human livebirth from orthotopic transplantation of human tissue in 2004, with another successful livebirth achieved by Meirov in 2005.⁹

Thus by the time S Silber entered the specialty in 2005 with a report⁶¹ of a livebirth from fresh ovarian tissue transplanted between identical twins discordant for premature ovarian failure, the way had already been prepared by more than 50 years of research by others. Fresh or frozen ovarian cortex transplantation might be more efficient than freezing ovarian tissue and not transplanting it back into the patient until 10 or even 20 years later.^{8,10–12,61–65} A large series⁶³ of 11 fresh ovary transplants resulted in 14 pregnancies and 11 healthy babies, and a remarkably consistent return of menstrual cycling and normal day 3 follicle-stimulating hormone concentrations by 4 to 5 months in all patients, which gives hope that a series of cryopreserved transplants might also provide robust results. The use of similar surgical techniques to cryopreserve ovarian tissue for patients with cancer led to four pregnancies from four cryopreserved transplants in addition to the 14 pregnancies after ovarian cortex transplants between twins (figure 2). We could, therefore, distinguish the egg loss due to transplant ischaemia from the egg loss due to cryopreservation. All studies were done with informed consent and ethics approval from the local institutional review board. Although the recipients of fresh ovary transplants prefer natural conception to in-vitro fertilisation with donor eggs, the question has to be raised of what effect ovary donation might have on the donor.

The potential effect of unilateral oophorectomy on both fertility and age of onset of menopause is controversial. Gosden and colleagues⁶⁶ in 1989 described a compensatory mechanism of follicle rescue in mice that prevented any major effect on fertility.⁶⁶ A later study in 1992⁶⁷ noted, “long term ovarian function is not substantially compromised by reducing as much as one-half of the ovarian mass”. Other more clinical papers also support a lack of serious effect on fertility by unilateral oophorectomy in human beings with menopause occurring only 1–2 years earlier than in controls.^{66,68–75} However, other studies⁷⁶ as recently as 2013 have disputed this view, and suggest a 7-year earlier onset of menopause after unilateral oophorectomy than in controls. However, if the traditional

view is correct, in which unilateral oophorectomy does not negatively affect fertility, this would support partial or complete oophorectomy and ovarian tissue cryopreservation to expand the reproductive lifespan of normal women who wish to delay childbearing, but do not want to lose their current reproductive potential. Thus we felt comfortable in undertaking a series of fresh ovary transplants, which led the way toward improving our ovarian freezing and transplantation methods.^{66,68–76}

Transplantation techniques

Several techniques have been described for transplantation of the ovarian cortex.^{7,9,63,77,78} In mice, Parrott,⁵⁶ used sliced little pieces of ovarian cortex. Others prepared peritoneum near the ovary⁷ but then switched to a technique similar to that described for fresh ovarian tissue.^{7,63,77,78} Ovarian cortical slices can also be transplanted under the surface of the cortex in the non-functional ovary.⁹ All these techniques have resulted in babies and there is no consensus on which is best.

Although almost all these pregnancies have been achieved with orthotopic ovarian tissue transplantation and most women had spontaneous pregnancies, a human patient with a heterotopic ovarian tissue transplant with in-vitro fertilisation and pregnancy has been reported in Australia⁷⁹ and even 10 years ago success with a heterotopic ovarian tissue transplant was reported in a rhesus monkey.⁸⁰ This success indicates that despite the popularity of orthotopic ovarian tissue transplants, heterotopic ovarian tissue transplantation has advantages (easier access for in-vitro fertilisation and to monitor potential tumour recurrence) and could become more popular.

Clinical outcomes

Initially there were only a few case reports, some very recently, of successful cryopreserved ovary transplantation but no unified single series.^{7,9,11,81–85} Long-term function of the transplant has been noted in only one report.⁸ However, more recently, investigators have accumulated data for 24 livebirths, and in tables 2 and 3 we present a worldwide livebirth rate of around 30% with more than 35 babies.^{86–88} New methods of grafting ovarian cortex are not limited only to a few centres.⁸⁷ Robust results are seen in the USA, Brussels, Paris, Spain, Denmark, and Israel, with successes also in Japan, Italy, Germany, and Australia (table 2 and 3). Cryopreserved ovarian tissue grafts with the slow-freezing method used in Denmark are functional for more than 5 years and many spontaneous pregnancies have been reported with no need for in-vitro fertilisation or other ancillary treatment. At the time of writing, 35 healthy babies have been born from cryopreserved ovarian tissue grafting, and 12 from fresh ovarian grafting resulting in more than 49 babies. Most pregnancies were achieved without the need for in-vitro fertilisation, and resulted instead from regular intercourse with no other treatment.

	Cryopreserved	Fresh	Country
Andersen et al (2008) ⁸²	6	..	Denmark
Demeestere et al (2007) ⁴	2	..	Belgium
Donnez et al (2004, 2013) ^{7,86}	6	1	Belgium
Meirow et al (2005) ⁹	3	..	Israel
Revel et al (2011) ³	3	..	Israel
Burmeister et al (2013) ³	1	..	Australia
Stern et al (2013) ⁸¹	2	..	Australia
Donnez et al (2011) ^{80*}	4	..	Spain
Donnez et al (2011) ^{80†}	3	..	France
Silber et al (2010) ^{10‡}	1	..	Japan
Revelli et al (2013) ⁸⁴	1	..	Italy
Dittrich et al (2012) ⁸⁵	1	..	Germany
Silber and Gosden (2007) ⁶³	4	11	USA
Total	35	12	10

Babies born worldwide from cryopreserved ovarian tissue (as of October, 2014).

*A Pellicer treating physician. †P Piver treating physician. ‡M Kuwayama treating physician.

Table 2: Livebirths after ovarian cortical tissue transplantation

	Number of transplants	Number of pregnancies (%)	Number of babies (%)
Andersen et al (2013) ⁸² Denmark	39	10 (26%)	6 (15%)
Demeestere et al (2007) ⁴ Belgium	6	3 (50%)	2 (33%)
Donnez et al (2004, 2013) ^{7,86} Belgium	13	6 (46%)	6 (46%)
Meirow et al (2005) ⁹ Israel	11	6 (55%)	3 (27%)
Burmeister et al (2013) ³ Australia	2	1 (50%)	1 (50%)
Stern et al (2013) ⁸¹ Australia	1	1 (100%)	2 (100%)
Donnez et al (2011) ^{80*} Spain	22	4 (18%)	4 (18%)
Donnez et al (2011) ^{80†} France	9	4 (33%)	3 (33%)
Silber et al (2010) ^{10‡} Japan	8	2 (25%)	1 (13%)
Silber and Gosden (2007) ⁶³	6	5 (83%)	4 (67%)
Total	121	45	35

Ovary transplants after ovary tissue cryopreservation (as of September, 2014). *A Pellicer treating physician. †P Piver treating physician. ‡M Kuwayama treating physician.

Table 3: Pregnancy and livebirth rates after ovarian cortical tissue transplantation

The most common benefit of ovarian transplantation was previously thought to be the preservation of fertility and future endocrine function in young women undergoing cancer treatment. However, in the absence of pelvic irradiation for cancer treatment, why not use ovarian tissue cryopreservation in otherwise healthy women who wish to preserve their fertility for non-medical reasons? With vitrification methods there is no difference in the viability or integrity of cryopreserved ovarian tissue compared with fresh ovarian tissue or that cryopreserved with the slow-freezing method.^{10,88,89} Furthermore, with cryopreserved ovarian tissue transplantation, hormonal function is restored in addition to fertility.

Societal and ethical issues

Couples continue to postpone a family until later in life for various economic, educational, and social reasons. This trend towards postponement of the first pregnancy has an effect on both family size and the risk of permanent biological childlessness. Consequently, the total fertility rate of most developed countries has dropped below the replacement level, usually deemed to be 2.1 children per woman. Low fertility rate and the resulting demographic ageing is increasingly regarded as a threat to the future welfare of these societies⁹⁰ in which artificial reproductive techniques have become part of the national strategy to address these demographic and reproductive challenges.⁹¹ For instance, one study⁹² showed that artificial reproductive techniques have the potential to substantially contribute to the total fertility rate after the authors assessed the demographic and economic effect of artificial reproductive techniques in Denmark and the UK. Furthermore, an increasing number of these techniques can only be done with eggs donated from a younger woman because the average female age of a first pregnancy and the average age that women desire a pregnancy is increasingly too advanced to offer a reasonable chance of pregnancy with a woman's own eggs. Cryopreservation of autologous oocytes and autologous tissue transplantation now offer women a realistic technological solution that could reduce the need for third party involvement in artificial reproductive techniques. Additionally, if these women no longer needed their cryopreserved oocytes in the future, they could decide to donate them to an oocyte donation programme.⁹³ How many women will embark on such preventive treatments is unclear. An electronic survey of more than 1000 women of reproductive age in Belgium showed that 3.4% of these women would consider such treatment and that 28% of them could be potential oocyte bankers.⁹⁴

Cryopreservation of both ovarian tissue and oocytes exposes women to medical risks in obtaining the oocytes or ovarian cortex. However, these risks are substantially less than those associated with pregnancy and childbirth, something that all these women envisage. Moreover, these risks are deemed acceptable for oocyte donors who might actually decrease the need for future oocyte donations. Ovarian stimulation has become much safer in recent years owing to optimisation of protocols. The ovarian hyperstimulation syndrome due to fertility treatment can now be prevented with a gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist.⁹⁵ Studies of oocyte donors have also noted that repeated ovarian stimulation does not seem to affect anti-müllerian hormone concentrations⁹⁶ or the donor's future chances of reproductive success.⁹⁷

In an analysis of the welfare of children conceived with artificial reproductive techniques, the focus should be not only on the inherent risks involved with the

treatment, but also the risks to the would-be parent or parents. Preventive cryopreservation of oocytes or ovarian tissue theoretically enables pregnancy at any age. However, this increase in the upper age-range for pregnancy is no different from that with oocyte donation, and available data suggest that women embarking on such treatment do not envisage pregnancies beyond the age of the physiological menopause.⁹⁹

Accurate estimates of treatment success rates and cost are imperative not only for patient counselling, but also for allocation of societal resources. van Loendersloot and colleagues⁹⁸ did a cost-effectiveness analysis to establish whether oocyte cryopreservation at age 35 years and the use of these oocytes at age 40 for in-vitro fertilisation is cost-effective compared with either in-vitro fertilisation at age 40 years with the use of fresh oocytes, or delayed natural conception without treatment. This study noted that oocyte cryopreservation is more cost-effective than in-vitro fertilisation, if at least 61% of the women return to use their oocytes and are willing to pay €19 560 extra per each additional livebirth. A second cost-based analysis⁹⁹ done in a US setting included the option of ovarian tissue cryopreservation. Conversely, these authors reported that neither oocyte cryopreservation (\$135 520 per additional livebirth) nor ovarian tissue cryopreservation seemed to be cost-effective for otherwise healthy women planning to delay childbearing. Irrespective of the cost-efficiency discussion, one can argue that the fertilisation of oocytes and transfer of embryos for anticipated gamete exhaustion¹⁰⁰ should be covered in those countries or states where patients undergoing in-vitro fertilisation receive several free cycles of ovarian stimulation.

Future prospects

Conventional oocyte donation is the common treatment for patients with premature ovarian failure who want to become pregnant, and oocyte vitrification is becoming the common method to preserve fertility against ageing of the ovary. Nevertheless, the robust results for ovary transplantation, either fresh or frozen, suggest that this could be an alternative strategy for preservation of fertility for some women. Cryopreservation and transplantation of ovarian tissue is more robust than previously thought in the past decade.

Although the transplantation surgery might seem more burdensome than oocyte retrieval, it is a straightforward and uneventful outpatient procedure. Hormonal function is restored in every case, and enables spontaneous pregnancies in most cases as long as no pelvic irradiation was used in the treatment of patients with cancer. After ovarian transplantation, patients are able to attempt natural conception without medical assistance and patients always prefer natural conception.¹⁰¹⁻¹⁰⁴ In Denmark, demographers predict that 50% of women today will live to age 100 years. They might not want to be menopausal for half of their

lifetime, although this is very speculative.^{8,105} Thus, aside from freezing eggs for these so-called social reasons, which more and more young women are doing, there is a possible endocrine benefit from ovary freezing.

The future prospects for oocyte and ovarian tissue cryopreservation to enable an expanded reproductive lifespan for women who wish to delay childbearing and even menopause might provoke argument and controversy. However, oocyte cryopreservation in young women, although not widely pursued, has a clear future. Yet there is still a great risk for women who take the success of any of these techniques for granted.

Contributors

All authors conceived the outline of the review and cowrote the body of the text.

Declaration of interests

We declare no competing interests.

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Fertility: progress and uncertainty

Reproductive medicine can boast many fertility milestones in its relatively short history: the arrival of in-vitro fertilisation (IVF) in the late 1970s; the development of intracytoplasmic sperm injection in the early 1990s; the first ovarian transplant a decade ago; and next week we will hear details of the first livebirth after uterine transplantation. No-one can be in doubt that reproductive medicine is characterised by remarkable scientific progress on a very fundamental question—the very matter of life itself.

A three-part Series about fertility preservation in this issue highlights the options available in developed countries to men and women whose fertility is compromised for medical reasons, notably in the case of cancer therapy. For boys and men, it is well known that exposure to alkylating agents and whole-body radiation can lead to infertility. Herman Tournaye and colleagues outline how sperm cryopreservation is an effective, but underused, method to safeguard spermatozoa, and comment how advances have been made in prepubertal germ cell storage aimed at later transplantation of testicular tissue and associated stem cells, although these approaches remain experimental.

Michel De Vos and colleagues discuss how recent advances in reproductive medicine and cryobiology are of particular relevance for girls and young women with cancer. Oocyte storage is a tried and tested method of fertility preservation, but is often thwarted with a fundamental practical problem—the need to avoid delaying the onset of cancer therapy being rightly prioritised over the logistics to stimulate oocyte production and retrieval. Cryopreservation of ovarian tissue, still in its infancy as a therapeutic option, could offer a more accessible solution. De Vos and colleagues also emphasise the importance—and relative paucity—of fertility preservation counselling, with around only half of women receiving it at present.

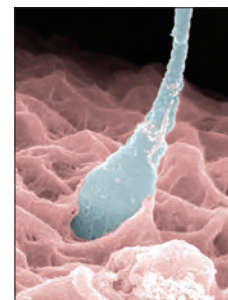
Fertility preservation for women in the wider population is a logical and intriguing consequence of these developments, discussed by Dominic Stoop and colleagues in the final Series paper. Why not, they propose, offer fertility preservation to women who want to delay pregnancy until later in life? A fair question, given the social and financial pressures often encountered in many societies today in the developed

world, such as relative decreases in earnings (a result of economic austerity), combined with spiralling costs in housing, food, energy, and child care. For many women, delaying motherhood and not having to worry about “the biological clock” is an attractive proposition.

Of course, the scientific opportunities afforded by reproductive medicine need to be balanced against the social and ethical questions that such progress raises. Is it right that society is seemingly putting pressure on the naturally fertile period of a woman's life by presenting an opportunity to delay motherhood? The average age of first childbirth in the UK, for example, has just passed 30 years for the first time. Some may view this with concern, with the statistics for safe pregnancy and delivery being more favourable for younger mothers. Others may see this as an inevitable result of inexorable change in the lifecycle; given that we are living longer, perhaps it is reasonable that we are starting families later.

However, if assisted reproductive techniques are sought, a look at the success rates of IVF on the UK's Human Fertilisation and Embryo Authority's website should go some way to managing the expectations of older women or couples struggling with fertility. The low success rate of 13.6% for women in their early 40s drops to a meagre 1.9% after the age of 45 years. IVF remains a fraught and expensive venture that often results in failure.

Next month, England will launch a national sperm bank, a collaboration between the NHS-funded National Gamete Donation Trust and Birmingham Women's Hospital. Its aim will be to increase donor recruitment, screening, and banking of sperm to the benefit of fertility programmes across the UK. For the first time, people from particular ethnic backgrounds will be able to choose sperm from culturally matched donors. This modernising and rebranding of sperm donor services is to be welcomed, along with secondary aims to demystify the often covert and taboo nature of sperm donation. A good mark of a society is how well it serves its citizens who need help. When it comes to fertility, science and society have a key part to play, to help shape the right conditions for the creation of life; however, nothing in, or about, life is ever certain, or can be taken for granted. ■ *The Lancet*



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See [Series](#) pages 1295, 1302, and 1311

For more on the [Human Fertility and Embryo Authority's IVF data](#) see <http://www.hfea.gov.uk/ivf-figures-2006.html>

For more on the [National Gamete Donation Trust](#) see <http://www.ngdt.co.uk/media-centre/launch-national-sperm-bank>

Fertility preservation: challenges and opportunities

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See [Series](#) pages 1295, 1302,
and 1311

Subfertility is a major health issue worldwide, and one that is growing because of an increasing number of subfertile couples, various causes of decreased fertility, and poorly understood mechanisms. In *The Lancet*, three Series papers on fertility preservation¹⁻³ discuss the effects of physiological and pathological factors on human fertility, and collectively show that fertility preservation is a potential strategy to combat this predicament.

Fertility preservation can play a pivotal part in reproductive medicine for three reasons. First, fertility preservation is the only option for patients with cancer hoping to conserve their fertility. Advancements in early diagnoses and new treatments have greatly lowered the death rate of young (aged 20–39 years) patients with cancer. For example, cancer mortality decreased by 1.8% per year in men and 1.4% per year in women in the USA between 2006 and 2010.⁴ Evidence suggests that most patients surviving cancer who are younger than 40 years expect their fertility to be maintained, or endocrine function to be restored.⁵ Second, fertility preservation is attractive for healthy couples who wish to postpone childbearing. According to the China's 6th National Census in 2010,^{6,7} the ratio of Chinese women giving birth at an advanced reproductive age (35–49 years) showed a 10% increase compared with the ratio in 2000. The phenomenon of delayed childbearing is also evident in Australia, New Zealand, the USA, and western Europe,⁸ and brings a risk of age-related

subfertility. In dealing with this consequence of socioeconomic forces, fertility preservation at a young age could reduce the risk of fertility loss in later life; indeed, donated oocytes from young women showed better outcomes in assisted reproductive technology than did the use of older women's own oocytes.⁹ Finally, fertility preservation can do a great service to reproductive medicine by development of new techniques such as pluripotent stem cells, with the hope of restoring lost fertility in various diseases, including reproductive cancers.

Although fertility preservation shows potential value, barriers exist for technique development and implementation. For patients with cancer, a personalised preservation scheme is needed that takes into account age, marital status, status of illness, classification of the patient's tumour, and genetic considerations. Normally, cryopreservation of gonadal tissue is preferred in patients with terminal cancer or preadolescents, and germ cells and embryo cryopreservation are conventionally used in patients who are at risk of or have cancer early in life.¹⁰ For the population with normal fertility, is it reasonable for such people to request fertility preservation? What is the paramount consideration in decision making—ethics, personal willingness, or medical indications? Fully informed consent is important for such persons because of continuing debates about the risks of cryopreservation and in-vitro culture.

To overcome these difficulties and help with fertility preservation in the clinic, several strategies have been proposed.¹ First, the establishment of uniform clinical guidelines is needed, including indication and contraindication for treatment, provision of informed consent, and duties of ethics committees. As a logical step to achieve this goal, a fertility preservation society should be formed, composed of oncologists, reproductive medicine clinicians, and embryologists, with the responsibility of drafting standard operating procedures, and building up an accessible system of clinical practice approved by the International Standardization Organization.² Second, active education networks should be developed, for better communication of the latest advances among professionals, explanation of the fundamentals to



Louise Olligny/BSP/Science Photo Library

the public, and development of new techniques by collaborative workshops, as discussed by Dominic Stoop and colleagues.³ Finally, technical advances will need integrated efforts by scientists and clinicians in basic research and medicine. Accordingly, regulations should be drafted and implemented to guarantee effective and efficient assessment and translation of techniques. On the basis of our own experiences, clinical research institutions affiliated with universities are well positioned for this endeavour with their strong capabilities in organisation and integration of research, clinical practice, translational medicine, and other professional activities.

Potential strategies have been proposed for applications of stem cells in reproductive medicine, including isolation and storage of stem cells derived from ovarian tissue or spermatogonia, and establishment and differentiation of pluripotent stem cells, as elaborated by Herman Tournaye and Michel De Vos and their respective colleagues in this issue.^{1,2} In 2014, the possible use of stem cells for human artificial gamete production, especially for eggs, is far from a mature clinical technique, and research is needed to establish efficacy and safety.

The clear message from the three Series papers¹⁻³ is about the importance of prevention of fertility loss. To achieve this vital goal for human health, collective efforts need to be made to educate the public as well as professionals to protect fertility by raising vigilance

about risk factors, undertaking early detection, valuing doctors' advice, pursuing early treatment, and considering fertility preservation and fertility restoration where feasible.

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