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Oocyte cryopreservation: where are we now?

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INTRODUCTION: Since the first live birth from oocyte cryopreservation three decades ago, oocyte cryopreservation has become an important component of ART. Cryopreservation techniques have evolved, leading to higher success rates and the introduction of oocyte cryopreservation into IVF clinics worldwide. Concurrently, there has been an increase in patient demand, especially for so-called 'social egg freezing' that allows women to preserve their fertility in anticipation of age-related fertility decline. This review addresses a need to evaluate the current status of oocyte cryopreservation. It explores current techniques and success rates, clinical applications, the rise of elective oocyte cryopreservation, and future implications.

METHODS: A search was performed using Web of Science and PubMed databases for publications between January 1980 and December 2015. Keywords used included 'egg freezing', 'oocyte freezing', 'oocyte cryopreservation', 'oocyte vitrification', and 'fertility preservation'.

RESULTS: The success rate of oocyte cryopreservation has risen, and the increasing use of vitrification offers has improved outcomes, with IVF pregnancy rates now similar to those achieved with fresh oocytes. There are conflicting opinions about the comparative success rates of open and closed vitrification. Patients are accessing and receiving oocyte cryopreservation for a wide range of indications, and there has been a marked increase in patient numbers and oocyte cryopreservation cycles. Oocyte cryopreservation for circumventing age-related infertility is becoming more widely accepted.

CONCLUSION: Oocyte cryopreservation is an established component of ART, with vitrification now being the cryopreservation technique of choice. Increasing numbers of women undergo oocyte cryopreservation for both medical and social reasons. It is important to continue auditing outcomes and reporting long-term follow-up of children born from frozen–thawed oocytes.

Key words: oocyte cryopreservation / oocyte vitrification / fertility preservation / egg freezing / ART

Introduction

The scope of assisted reproductive technology (ART) has widened greatly since the birth of Louise Brown in 1978 (Steptoe and Edwards, 1978). Both embryo and sperm cryopreservation are well-established procedures, but it was not until relatively recently that oocyte cryopreservation has become available as an additional fertility preservation option for women. Significantly, oocyte cryopreservation challenges the ethical, legal and religious concerns surrounding embryo cryopreservation, making it preferable in many situations.

Since the first birth from a frozen oocyte was achieved in Australia in 1986 (Chen, 1986), many advances have been made in the field. Research has been accelerated because of legal restrictions and ethical concerns relating to embryo storage. Now, improved techniques and success rates have led to the application of oocyte cryopreservation for many different indications, including age-related fertility decline.

Methods

This literature review will discuss the current status of oocyte cryopreservation, considering its history, clinical indications, and the efficacy of techniques. Web of Science and PubMed databases were used to search for relevant publications between January 1980 and December 2015. Keywords used included 'egg freezing', 'oocyte freezing', 'oocyte cryopreservation', 'oocyte vitrification', and 'fertility preservation'.

A history of oocyte cryopreservation

Successful pregnancies from frozen oocytes were first achieved in the late 1980s, using slow-freeze and rapid-thaw cryopreservation techniques (Chen, 1986, 1988; Van Uem et al., 1987). However, there was a lack of progress in the field owing to technical concerns and low success rates (Bernard and Fuller, 1996). Oocytes are notoriously difficult cells to cryopreserve, due to their low surface area to volume ratio and high susceptibility to intracellular ice formation (Paynter *et al.*, 1999). Early papers highlighted difficulties in predicting the membrane permeability characteristics of human oocytes along with other biophysical parameters (Fuller *et al.*, 1992; Hunter *et al.*, 1992a, b). Studies also revealed the negative effects of cryopreservation on the stability of microtubules and microfilaments in mammalian oocytes (Pickering and Johnson, 1987; Sathananthan *et al.*, 1988; Vincent *et al.*, 1989; Pickering *et al.*, 1990; Joly *et al.*, 1992), which are vital for normal chromosomal segregation (Glenister *et al.*, 1987). Hardening of the zona pellucida, and subsequent low fertilization rates, were further difficulties associated with cryopreservation (Johnson *et al.*, 1988; Vincent *et al.*, 1990). Later papers produced more promising results, suggesting that human oocytes had the potential to retain their morphology and chromosomal integrity post cryopreservation (Gook *et al.*, 1994; Van Blerkom and Davis, 1994). However, a reliable protocol for the clinical cryopreservation of oocytes was still lacking.

Research into oocyte cryopreservation was accelerated by legislative restrictions surrounding the storage of embryos, particularly those in Italy that prevented the cryopreservation of excess embryos (Benagiano and Gianaroli, 2004). The introduction of vitrification as an alternative to slow freezing reduced damage to internal structures and led to superior success rates (Antinori *et al.*, 2007; Cao *et al.*, 2009; Fadini *et al.*, 2009; Smith *et al.*, 2010). Further, the use of intracytoplasmic sperm injection (ICSI) as an insemination method for vitrified oocytes was found to rectify fertilization issues due to zona pellucida hardening (Kazem *et al.*, 1995; Porcu *et al.*, 1997; Fabbri *et al.*, 1998).

The Human Fertilisation and Embryology Authority (HFEA) has allowed the use of frozen oocytes for infertility treatment in the UK since 2000 (Wise, 2000). HFEA regulations allow the storage of gametes for a standard 10-year period, which can be extended under certain circumstances (HFEA, 2015). In 2013, the American Society for Reproductive Medicine (ASRM) lifted the experimental label applied to oocyte freezing, following four randomized controlled trials (Cobo *et al.*, 2008, 2010a, Rienzi *et al.*, 2010; Parmegiani *et al.*, 2011) that suggested that in-vitro fertilization (IVF) using vitrified/warmed oocytes could produce similar fertilization and pregnancy rates to IVF with fresh oocytes (ASRM, 2013). This has prompted the introduction of oocyte cryopreservation into a growing number of IVF clinics worldwide.

Cryopreservation techniques

Cryopreservation involves the preservation of cells and tissues for extended periods of time at sub-zero temperatures. Cryoprotective additives (CPAs) are used in order to reduce cryodamage by preventing ice formation. They are classified into permeating or non-permeating CPAs, depending on their ability to cross the cell membrane. Various combinations of permeating (e.g. dimethyl sulfoxide (DMSO), glycerol, 1,2 propanediol (PrOH)) and non-permeating (e.g. sucrose, glucose, fructose, trehalose) CPAs can be used.

There are two basic techniques applied to the cryopreservation of human oocytes: controlled slow freezing, which was favoured in early protocols, and ultrarapid cooling by vitrification, which is now well-established. Slow freezing results in a liquid changing to a solid state whereas vitrification results in a non-crystalline amorphous solid.

During slow freezing, cells are exposed to a low concentration of CPAs and slow decreases in temperature. Oocytes are first cooled to a temperature of −5°C to −7°C, at which equilibration and seeding take place. Oocytes are then cooled at a slow rate of 0.3–0.5°C/minute, until a temperature of between −30°C and −65°C has been reached, before being added to liquid nitrogen for storage (Saragusty and Arav, 2011). Multiple studies have demonstrated success using slow freeze techniques to cryopreserve oocytes (Porcu *et al.*, 1997; Tucker *et al.*, 1998; Fabbri *et al.*, 2001; Winslow *et al.*, 2001; Boldt *et al.*, 2003; Borini *et al.*, 2004; Bianchi *et al.*, 2007; Grifo and Noyes, 2010). Although various alterations to slow freeze protocols (e.g. Boldt *et al.*, 2006; Borini

et al., 2006) have contributed to higher success rates, there are still concerns surrounding the clinical efficiency of this technique. Comparisons between success rates using slow frozen and fresh human oocytes have suggested poorer outcomes with frozen/thawed oocytes (Levi Setti et al., 2006; Borini et al., 2010; Magli et al., 2010).

Vitrification requires higher concentrations of CPAs, lowering the risk of ice nucleation and crystallization, and cooling rates of 100s to 10 000°C per minute before submersion into liquid nitrogen (Saragusty and Arav, 2011). The first live birth following vitrification was achieved in 1999 (Kuleshova et al., 1999), whilst Kuwayama et al. developed the widely used 'Cryotop' vitrification method in 2005 (Kuwayama et al., 2005).

There are two main classes of vitrification protocol: open and closed vitrification. Open vitrification involves direct contact between oocytes and liquid nitrogen, using low volume devices such as capillary glasses, cooper devices, pulled straws, and loops (Glujovsky et al., 2014). In contrast, closed vitrification involves indirect contact between oocytes and liquid nitrogen using tubing systems (Glujovsky et al., 2014).

Open versus closed vitrification

The randomized controlled trials supporting similar success rates between IVF with vitrified/warmed oocytes and IVF with fresh oocytes (Cobo et al., 2008, 2010a; Rienzi et al., 2010; Parmegiani et al., 2011) have all used open vitrification systems. Despite their proven proficiency, concerns have been raised over the sterility of open systems due to potential cross-contamination between the vitrification sample and liquid nitrogen. Accordingly, studies have highlighted the potential of liquid nitrogen sterilization using ultraviolet light (Parmegiani et al., 2010) or oocyte storage in vapour phase liquid nitrogen, which contains a lower density of environmental airborne contaminants (Cobo et al., 2010b).

The introduction of closed vitrification has offered a solution to contamination concerns (Vajta et al., 2015). After exposure to contaminated liquid nitrogen, Criado et al. (2011) found that closed devices were bacteria free, whereas bacteria were present in 45% of open devices. However, the use of closed systems raises new concerns about the efficiency of oocyte vitrification, due to their decreased cooling rates. One study suggested that the use of closed vitrification might not preserve the oocyte ultrastructure as well as in open systems (Bonetti et al., 2011). Another study, comparing an open and closed vitrification system, found reduced fertilization, cleavage, and clinical pregnancy rates in the latter (Paffoni et al., 2011).

Contrarily, other studies have suggest that closed vitrification can lead to excellent clinical outcomes whilst also providing an aseptic environment (Smith et al., 2010; Stoop et al., 2012; Papatheodorou et al., 2013). A cross-section of UK clinics in 2011 suggested that 75% used closed containers rather than open containers for vitrification (Brison et al., 2012). Whichever vitrification protocol is favoured, it is important to consider the warming rate. Observations have indicated that the warming rate is, in fact, more important than the cooling rate for oocyte survival in vitrification protocols (Seki and Mazur, 2009; Leibo and Pool, 2011; Mazur and Seki, 2011). It has also been noted that optimal outcomes are more likely to be achieved if vitrification and warming are carried out in the same clinic or by matched protocols (Brison et al., 2012).

The trend towards vitrification

Studies in the past few years have suggested the superiority of vitrification compared to slow freeze protocols. In 2006, a meta-analysis of oocyte cryopreservation implied that pregnancy rates with cryopreserved oocytes could be improved with the use of vitrification, although at this point few pregnancies had been recorded (Oktay et al., 2006). Subsequently, comparisons of IVF outcomes from slow-frozen and vitrified oocytes have demonstrated that vitrification leads to better survival, fertilization, and pregnancy rates (Cao et al., 2009; Fadini et al., 2009; Smith et al., 2010), although only Fadini et al. (2009) reported pregnancy rates that were significantly higher (18.2 versus 7.6%). Increasing evidence on the efficacy of IVF with vitrified oocytes has suggested that it could achieve similar outcomes to IVF using fresh oocytes, with oocyte survival rates of over 84% (Nagy et al., 2009; Almodin et al., 2010; Ubaldi et al., 2010; Rienzi et al., 2012). Whereas in 1999, close to 100 cryopreserved oocytes were needed to achieve one pregnancy (Porcu et al., 1999), now only 20 vitrified oocytes are required (Donnez and Dolmans, 2013), although this is highly dependent on the age of the oocyte (Cobo et al., 2015a, b). Randomized controlled trials have produced clinical pregnancy rates (CPRs) per transfer ranging between 35.5 and 65.2% (Cobo et al., 2008, 2010a; Rienzi et al., 2010; Parmegiani et al., 2011). A recent meta-analysis of five studies found that the rates of fertilization, embryo cleavage, high quality embryos and ongoing pregnancy did not differ between vitrification and fresh oocyte groups (Cobo and Diaz, 2011).

Many IVF programmes now favour vitrification as a technique to cryopreserve oocytes (Rudick et al., 2010; Brison et al., 2012; Glujovsky et al., 2014), and a 2013 update to the National Institute for Health and Care Excellence (NICE) guidelines states: 'In cryopreservation of oocytes and embryos, use vitrification instead of controlled-rate freezing if the necessary equipment and expertise is available' (NICE, 2013).

Outcomes of oocyte cryopreservation

Studies have considered the longer term obstetric and perinatal outcomes associated with vitrification. An analysis of 165 pregnancies and 200 infants found that the mean birthweight and incidence of congenital abnormalities (2.5%) were similar in infants born following oocyte vitrification to those born from spontaneous conceptions or through regular IVF (Chian et al., 2008). Another review of 936 infants, born following either slow freezing or vitrification of oocytes, also found a comparable incidence of congenital abnormalities (1.3%) (Noyes et al., 2009). Cobo et al. (2014) have reported that vitrification does not increase adverse obstetric and perinatal outcomes. No long-term follow-up of these children has yet been published.

Despite the reassurances surrounding the efficacy and safety of oocyte cryopreservation, it is important to continue auditing the data whilst the number of cycles increases worldwide. Fresh concerns were highlighted in a recent review that compared outcomes of fresh and cryopreserved donation cycles in the USA in 2013. Live birth rates per recipient cycle were found to be significantly lower when cryopreserved oocytes were used (43.3 versus 49.6%) (Kushnir et al., 2015). However, it must be emphasized that this study did not adjust for important confounding factors such as donor and recipient ages or cryopreservation protocols.

Clinical applications of oocyte cryopreservation

Whilst the technique was initially reserved for women with medical indications who had no other fertility options (European Society of Human

Reproduction and Embryology (ESHRE) Task Force on Ethics and Law, 2004; ASRM, 2008), oocyte cryopreservation now plays a role in many different circumstances. Perhaps most notable is the introduction of 'social egg freezing', as a means to preserve fertility in anticipation of age-related fertility decline (Baldwin *et al.*, 2014; Stoop *et al.*, 2014). Other applications of oocyte cryopreservation include donation programmes and storage of 'spare' gametes during IVF.

In the UK, the HFEA reports that up to 2012, around 18 000 oocytes have been stored for the patients' own use, while 160 thawing/warming cycles have been performed, resulting in 20 live births. A large proportion of IVF centres worldwide offer 'social egg freezing'.

Oocyte versus embryo cryopreservation

Embryo cryopreservation is an established ART technique with many advantages. The ability to store surplus embryos can reduce the number of embryos transferred during a fresh cycle and thus minimize the risk of multiple pregnancy, reduce the need for repeated stimulation cycles, and increase cumulative pregnancy rates. However, embryo cryopreservation is not an option for all couples, because of personal religious or moral objections, or restrictive legislation in certain countries. Decisions over the fate of stored embryos can also lead to major disagreements or legal disputes, particularly in the case of divorce or separation (Pennings, 2000; Lee *et al.*, 2006). Oocyte cryopreservation may be a feasible alternative to embryo cryopreservation for many couples undergoing IVF (ASRM and Society for Assisted Reproductive Technology, 2013). There tends to be less moral sensitivity surrounding the storage and fate of gametes, and since the genetic material originates from one individual, there are fewer issues surrounding ownership.

Oocyte donation

Oocyte donation has become an integral part of ART. It was initially accepted in the treatment of women with premature ovarian insufficiency (Kawwass *et al.*, 2013). In recent years the demand for oocyte donation has increased, as it has become a treatment option for large numbers of women experiencing age-related infertility. Owing to the careful selection of young and healthy oocyte donors, pregnancy rates in donation cycles often surpass those achieved by conventional IVF (Sauer and Kavic, 2006). However, traditional fresh donation cycles present some difficulties (Cobo *et al.*, 2011). One is the requirement for menstrual cycle synchronization between donor and recipient, so that oocytes can be collected and transferred within a certain time frame. Another concern is the absence of proper quarantine measures which are adhered to during sperm donation, using semen cryostorage (Cobo *et al.*, 2011). As the demand for oocytes increases (Kawwass *et al.*, 2013), recipient waiting times also lengthen. This is not helped by the fact that in fresh donation cycles, all donor oocytes tend to be allocated to a single recipient (Cobo *et al.*, 2011).

The introduction of oocyte cryopreservation into donation programmes overcomes these challenges. It simplifies the logistics of ART cycles as there is no need for menstrual cycle synchronization between donor and recipient; it allows for the testing of donors for infectious diseases during quarantine; and it potentially reduces cost through the efficient allocation of oocytes from a pool of donors to many recipients (Cobo *et al.*, 2011). Oocyte cryopreservation has also led to the development of donor oocyte banks, which allow recipients to review a list of donors, and minimize waiting times (Cobo *et al.*, 2011; Quaas *et al.*, 2013). As well as cryopreserved oocytes from individual donors, surplus oocytes from infertile couples may be cryopreserved and subsequently donated, which proves particularly significant in countries where embryo donation is not allowed (Li *et al.*, 2005).

A growing proportion of donor oocyte cycles now utilize cryopreserved oocytes (Kawwass *et al.*, 2013). As of 2013, according to a study in the USA, 3130 oocytes from 294 donors were in storage in commercial oocyte banks. Six out of seven oocyte banks were using vitrification rather than slow freezing and all were expanding their donor databases (Quaas *et al.*, 2013).

Oocyte donation programmes are particularly useful for auditing the success of oocyte cryopreservation. The potential utility of cryostoring oocytes for donation was demonstrated by preliminary results from a donor oocyte bank that used slow freezing, achieving a pregnancy rate of 50% per cycle (Akin *et al.*, 2007), and a case series of four donor-recipient cycles using slow freezing yielded a clinical pregnancy rate of 75% (Barritt *et al.*, 2007). Nagy *et al.* (2009) compared the outcomes of fresh and vitrified oocyte donation cycles using the same 20 donors; the cumulative pregnancy rates were 78 and 85% respectively. Oocyte donation cycles in paired patients receiving fresh or vitrified oocytes from the same donor have led to similar ongoing pregnancy rates per embryo transfer: fresh 43.9% versus vitrified 47.2% (Trokoudes *et al.*, 2011). Solé *et al.* (2013) found comparable live birth rates (fresh, 38.4% versus vitrified, 43.4%, $P > 0.05$) in their study on sibling oocytes from 99 donors. A larger randomized study compared success rates in recipients of fresh and vitrified oocytes; each group comprised 300 couples (Cobo *et al.*, 2010a). Ongoing pregnancy rates were 41.7 and 43.7% in the fresh and vitrification groups, with an odds ratio of 0.921 (95% CI 0.667–1.274), showing no significant difference.

The largest reported series to date of oocyte recipients undergoing treatment using vitrified donor oocytes (3610 warming procedures) demonstrated an oocyte survival rate of 90%; it was not possible to develop a predictive model for oocyte survival. The reported clinical pregnancy rate of 48% represents an 'oocyte-to-baby' rate of 6.5% (Cobo *et al.*, 2015a).

In summary, comparisons of fresh and frozen cycles have demonstrated the efficacy of oocyte 'cryobanking'; satisfactory pregnancy rates are achieved, and oocyte donation programmes are increasingly relying on vitrified oocytes. Of course, new ethical concerns have been raised by the development of commercial oocyte banks and the ease with which oocytes can be shipped between countries with varying or inadequate regulation, (Quaas *et al.*, 2013). However, with careful management, oocyte banks may change the dynamic of oocyte donation for the better.

Fertility preservation in cancer patients

Cancer treatment regimes can have a detrimental effect on female fertility, due to the removal of reproductive organs or the use of radiation therapy and cytotoxic agents. The extent of damage depends on follicular reserve, patient age, and the type and dose of treatment, with alkylating agents being particularly gonadotoxic (Knopman *et al.*, 2010b; Fleischer *et al.*, 2011).

Around 10% of cancers occur in women under the age of 45 (Donnez and Dolmans, 2013), and the demand for fertility preservation amongst these patients has increased with improving cancer survival rates (Fleischer *et al.*, 2011; Noyes *et al.*, 2011). Embryo cryopreservation has been an option for some time (Ethics Committee of the ASRM, 2005; Lobo, 2005; Lee *et al.*, 2006; Kim, 2006), but has disadvantages as explained above, with the risk of dispute if couples separate. It is also not appropriate for single women or young patients without a stable partner (Noyes *et al.*, 2011).

Advances in oocyte cryopreservation mean that it can now be offered routinely for fertility preservation. In a study of 108 breast cancer patients undergoing fertility preservation treatment between 2005 and 2010, 16.7% opted for oocyte cryopreservation (Kim *et al.*, 2012). In another study, 71.6% of cancer patients enquiring about fertility preservation underwent oocyte cryopreservation (Garcia-Velasco *et al.*, 2013). At present, there are few reports of cancer patients returning to use their oocytes, but successful live births have been achieved (Martinez *et al.*, 2004; Kim *et al.*, 2011b; Garcia-Velasco *et al.*, 2013).

The disadvantage of cryopreserving mature, metaphase II (MII), oocytes in cancer patients is the need for ovarian stimulation. This can delay cancer treatment by several weeks, and may carry particular risk for those patients with a hormone receptive cancer (Kim *et al.*, 2011a). These problems can be reduced by the introduction of 'anytime start' protocols (Cakmak *et al.*, 2013), to reduce delay, and the use of anti-estrogens during stimulation for women with breast cancer (Reddy and Oktay, 2012). Alternatives include the cryopreservation of oocytes at the germinal vesicle (GV) stage (Toth *et al.*, 1994) or retrieval of immature oocytes followed by in-vitro maturation (IVM) (Rao *et al.*, 2004; Huang *et al.*, 2008; Oktay *et al.*, 2010), neither of which require ovarian stimulation.

An alternative to oocyte cryopreservation, which does not delay cancer treatment, is ovarian tissue banking. An ovarian cortical biopsy or oophorectomy is performed as a day-case surgical procedure, independent of the menstrual cycle. The disadvantages are that the patient must be fit for surgery, and that the tissue must subsequently be regrafted, with potential risk of reintroducing malignant cells (Hoekman *et al.*, 2015). In-vitro maturation of oocytes from human ovarian tissue has not yet been achieved. To date, however, relatively few births have been reported after regrafting frozen ovarian tissue (Donnez and Dolmans, 2015). A combination of oocyte cryopreservation with ovarian tissue banking may be the most effective fertility preservation technique in cancer patients (Donnez and Dolmans, 2013).

Now that oocyte cryopreservation for oncology patients is available, it is crucial that women are referred quickly to fertility specialists in order to discuss their options and initiate treatment (Kim *et al.*, 2011a). Lee *et al.* (2010) found that both fertility preservation cycles and chemotherapy could be started earlier in breast cancer patients referred before breast surgery. Additionally, 9 of 35 patients referred before surgery were able to undergo two fertility preservation cycles, compared to only 1 of 58 patients referred after surgery. Multiple cycles are advantageous as more oocytes (or embryos) can be cryopreserved, potentially leading to greater success rates. Papers which have assessed the characteristics of oncological patients receiving fertility counselling (Lee *et al.*, 2011) and undergoing fertility preservation (Kim *et al.*, 2012) have highlighted the need to improve overall patient access.

Elective oocyte cryopreservation

In almost all countries, an increasing number of women are postponing motherhood, resulting in rising numbers experiencing childlessness which they had not necessarily intended (Kneale and Joshi, 2008). Now that the cryopreservation of oocytes for age-related fertility decline is considered acceptable (ESHRE Task Force on Ethics and Law, 2012), 'social egg freezing' has become a popular subject within the media, and demand for the procedure has increased rapidly (Garcia-Velasco *et al.*, 2013). Although the procedure is relatively new, there is already a growing debate surrounding its use.

The use of younger oocytes can reduce the risk of fetal loss and aneuploidies associated with ageing oocytes (Goold and Savulescu, 2009). The use of cryopreserved, autologous oocytes also allows the mother to have a genetic relation to her child that could not be achieved through oocyte donation (Dondorp and De Wert, 2009; Goold and Savulescu, 2009), and will provide a higher chance of pregnancy than the use of standard IVF at an older age (Sauer *et al.*, 1990). A recent report of the largest series to date, 1468 women undergoing elective oocyte cryopreservation for non-oncologic reasons, of whom 137 returned to use them, showed that pregnancy rates were age-dependent and the optimal number of stored MII oocytes was at least 8–10 (Cobo *et al.*, 2015b).

Age-related fertility decline

The fertility decline experienced by women, which accelerates after the age of 35, is a well-known phenomenon (Dunson *et al.*, 2004; Sozou and Hartshorne, 2012). This decline is largely attributable to a decrease in follicular number and oocyte quality (Faddy *et al.*, 1992). If older women do conceive, they are at a significantly higher risk of fetal chromosomal abnormalities (Hook, 1981) and fetal loss (Nybo Anderson *et al.*, 2000). Reproductive potential can be extended by the use of donated oocytes from younger women (Sauer *et al.*, 1990; Navot *et al.*, 1991)

A recent study reviewed the relationship of age-related fertility decline and the chance of realizing a desired family size (Habbema *et al.*, 2015). Using a computer simulation that took into account natural fertility rates and current IVF success rates, it was suggested that women should start trying to conceive at 35 for a 90% chance of a one-child family, and at 28 for a 90% chance of a three-child family. In the absence of IVF, these ages dropped to 32 and 23 respectively. Studies have highlighted that many women are unaware of the effect of age on fertility (Lampic *et al.*, 2006; Hashiloni-Dolev *et al.*, 2011; Daniluk *et al.*, 2012), and are thus compromising their reproductive future.

Older women accessing ART experience lower success rates, and therefore often undergo multiple cycles when using their own oocytes (Leridon, 2004) before considering different options. With the use of donated oocytes, they can experience similar success rates to their younger counterparts (Sauer *et al.*, 1990). However, offspring from oocyte donation are not genetically related, and the process can be psychologically, emotionally, and financially challenging. A recent survey of 183 women found that 15% would rather remain childless than embark on oocyte donation (Hodes-Wertz *et al.*, 2013).

For those women who choose gamete donation, there may be further hurdles; there is a worldwide shortage of oocyte donors (Trokoudes *et al.*, 2011). By electing to undergo oocyte cryopreservation in anticipation of age-related fertility decline, women can effectively become their own oocyte donor (Knopman *et al.*, 2010a), avoiding the challenges described above.

The rise of 'social' oocyte cryopreservation: social and ethical implications

Oocyte cryopreservation has been described as a 'breakthrough for reproductive autonomy' (Harwood, 2009) and an 'emancipation' for women (Homburg *et al.*, 2009). There is an obvious gender inequality in reproduction, since men are able to reproduce at much older ages than women (Dondorp and De Wert, 2009). However, there are various reasons for which women may wish to delay motherhood, for example to focus on their career, to find a suitable partner or because they simply do not feel 'ready' (Goold and Savulescu, 2009). Oocyte cryopreservation can give women the ability to make more reproductive choices; to decide when and with whom they wish to have children.

Many concerns have also been raised in response to oocyte cryopreservation. There are risks involved in the process of ovarian stimulation and oocyte retrieval themselves (Goold and Savulescu, 2009; Bayliss, 2015). Additionally, when older women come back to use oocytes, they are also more likely to experience pregnancy complications such as pre-eclampsia, hypertension and gestational diabetes and to be delivered by Caesarean section (Ziadeh and Yahaya, 2001; Joseph *et al.*, 2005). However, it has been highlighted that these risks are the same as those in older women using conventional IVF (Goold and Savulescu, 2009). Another concern is whether the availability of oocyte cryopreservation encourages women to become complacent about their declining fertility (Goold and Savulescu, 2009). It is paramount that women receive the correct information about oocyte cryopreservation and its success rates, and do not accept it as an 'insurance policy' as it is often portrayed (Lockwood, 2011). Further, the decision by companies such as Apple and Facebook to offer 'social egg freezing' to their employees, may lead to women feeling pressured to delay childbirth (Bayliss, 2015), undermining the concept of reproductive autonomy. Whilst it has been emphasized that oocyte cryopreservation should be providing women with a back-up for conception into their 40s (Dondorp and De Wert, 2009), there are concerns that women will delay motherhood until their late 50s or 60s.

There are also worries about the high cost of oocyte cryopreservation. A study by Hirshfeld-Cytron *et al.* (2012) determined that if women were to cryopreserve their oocytes at the age of 25 and return to them at 40, this would be a less cost-effective strategy than simply undertaking ART at the age of 40 if difficulties were found trying to conceive naturally. More recently, a study suggested that oocyte cryopreservation before the age of 38 reduces the cost of achieving a live birth aged 40 and beyond (Devine *et al.*, 2015). A similar study found that oocyte cryopreservation was a more cost-effective strategy than IVF (Van Loendersloot et al., 2011). In a response to high costs and a shortage of oocyte donors, some clinics now provide a 'freeze and share' programme, whereby women can donate a proportion of their frozen oocytes in order to receive free or discounted treatment. It could be argued, however, that women should be encouraged to have children early, rather than to invest in an expensive technique with no guarantee of pregnancy.

Attitudes surrounding 'social' oocyte cryopreservation

Although it is still unclear exactly how many women will access elective oocyte cryopreservation (Stoop *et al.*, 2014), there is increasing research to indicate the perceptions of reproductive-age women. A survey of 1049 Belgian women found that 77.6% had been previously aware of the technique and 31.5% of women would potentially cryopreserve their oocytes (Stoop *et al.*, 2011). A smaller survey of 129 medical students in Singapore found that 36.4% of respondents had heard of the technique and 26.4% would consider it (Tan *et al.*, 2014). Hodes-Wertz *et al.* (2013) surveyed 183 patients who had undergone elective oocyte cryopreservation in the USA. The main reason stated for delaying childbirth (88%) was the lack of a partner. Interestingly, 84% of those surveyed were over the age of 35. This is significant, since age at time of oocyte cryopreservation is an important determinant of outcome. Of these women, 79% of the women wished that they had frozen their oocytes earlier and 83% of patients believed that the media had given them a false impression of the reproductive lifespan. Following oocyte cryopreservation, 53% of women stated that they felt more secure about their reproductive future (Hodes-Wertz *et al.*, 2013). In two further studies, women banking oocytes were of a similar age (means of 36.7 and 36.9 years) (Baldwin *et al.*, 2015; Stoop *et al.*, 2015). In one cohort of 65 women, 65% reported that they were motivated to cryopreserve their oocytes as an 'insurance' against future infertility, and 49% wanted more time to find a partner (Stoop *et al.*, 2015).

Follow-up of 'social-freezers'

Information about the outcomes of elective oocyte cryopreservation is scarce, as there are few data about women returning to use them (Stoop *et al.*, 2014). In the survey by Hodes-Wertz *et al.* (2013), only 6% of women (11 respondents) had used their oocytes during the 6-year timeframe. Of these, three patients reported having subsequently achieved a pregnancy from a thaw cycle, whilst five were unsuccessful and three others failed to comment. Stoop *et al.* (2015) found that 50.8% of women who cryopreserved their oocytes anticipated using them at some point. Interestingly, when their relationship and reproductive choices following oocyte cryopreservation were compared with those who did not cryopreserve their oocytes, no significant differences were found. In a follow-up of 23 women who had cryopreserved their oocytes, two had returned to use them with one successful pregnancy (Baldwin *et al.*, 2015).

Other indications

Alongside oocyte donation, fertility preservation for cancer patients and 'social egg freezing', there are a growing number of other indications for oocyte cryopreservation.

Oocyte cryopreservation can be an important fertility option for women with a range of medical conditions other than cancer (Donnez and Dolmans, 2013) for example, women with endometriosis who may experience reduced ovarian reserve post surgery (Elizur *et al.*, 2009), women with autoimmune diseases requiring gonadotoxic treatment (Elizur *et al.*, 2008), and women with genetic aberrations leading to subfertility or risk of early menopause (Goswami and Conway, 2005). Oocyte cryopreservation can also provide an option for fertility preservation in gender reassignment surgery.

In addition, now that assessment of ovarian reserve is widely available using biophysical (antral follicle count) and biochemical (Anti-Mullerian hormone, early follicular FSH) measures, many women who are asymptomatic are identified as being at risk of early menopause. Although

ovarian reserve measurement has not been shown to have predictive value for spontaneous pregnancy, it is a reasonable strategy for these women to consider elective oocyte cryopreservation.

Another useful application of oocyte cryopreservation arises in the situation where a male partner fails to produce a sperm sample on the day of oocyte retrieval for IVF (Emery *et al.*, 2004). The efficacy of 'emergency' oocyte cryopreservation was demonstrated in cases during which sperm extraction from male partners with non-obstructive azoospermia had failed (Song *et al.*, 2011). Following emergency vitrification, 15 couples chose to warm the oocytes and use donor sperm for insemination, resulting in a clinical pregnancy rate of 53.3%. Vitrification has also been proposed as an efficient strategy for managing low-responders, allowing the storage and accumulation of oocytes from multiple ovarian stimulation cycles before IVF and embryo transfer (Cobo *et al.*, 2012).

Where are we now?

Oocyte cryopreservation is now an established technology with a wide range of indications. As the number of oocyte cryopreservation cycles increases, there is a real need to monitor why it is being done, the techniques being used, and the success rates achieved. Ideally national and international registries should be established to monitor the process. The increase in patients and oocyte cryopreservation cycles should be an advantage for carrying out future research. This should include the long-term follow-up of children born following oocyte vitrification. As with all ART techniques, there are ethical questions that need to be considered, particularly in the case of 'social egg freezing'. Oocyte cryopreservation is an exciting technique that will prove beneficial to many patients in the future.

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C.E.A.: preformed literature searches and drafted the manuscript. J.C.H.: initiated the review and contributed to the manuscript. M.C.D.: contributed to writing and reviewing of the manuscript.

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