Review Article

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Oocyte cryopreservation: a narrative review

Carina CW CHAN, MBBS, FHKAM (O&G), FRCOG, FHKCOG, Cert RCOG (Reprod Med), Cert HKCOG (Reprod Med)
Hong Kong Reproductive Health Centre
William WK SO, MBBS, FHKAM (O&G), FRCOG, FHKCOG, Cert HKCOG (Reprod Med)
Premier Medical Centre

Oocyte cryopreservation is a method to preserve fertility for young cancer patients. Its indications have been extended to include the quarantine and storage of donor oocytes in egg donation programs, women with medical conditions that may culminate in premature ovarian insufficiency, and women who wish to safeguard fertility decline associated with ageing. In this review, we discuss the history of oocyte cryopreservation and its various clinical applications, with a focus on the safety of the procedure for cancer patients, especially those with hormone-dependent cancers such as breast cancers. We also discuss ethical considerations for women who are cryopreserving their oocytes to protect against age-related fertility loss, the optimal age to undergo oocyte cryopreservation, and the optimal number of oocytes to freeze. The risks associated with the procedure and potential risks to children born from cryopreserved oocytes are also addressed.

Keywords: oocyte cryopreservation; fertility preservation; cancer patients; ethical considerations; safety

History

Early attempts to cryopreserve human oocytes were hindered by a high incidence of aneuploidy and digynic polyploidy in the cryopreserved mammalian oocytes because of damage of the sensitive meiotic spindle at the metaphase II stage when mature oocytes were frozen. In 1986, a breakthrough was made using oocytes cryopreserved with the slow-freezing method[1]. However, oocyte survival on thawing was low, and safety of the children born from cryopreserved oocytes was a concern. In 2004, the ban on zygote and embryo cryopreservation in Italy provided an incentive to optimise oocyte cryopreservation. The first baby born from vitrified oocytes was reported in 1999[2]. The major obstacle restricting the clinical application of oocytes vitrification was the lack of an appropriate carrier. The introduction of Cryotop enabled an extremely rapid-cooling rate with minimal fluid volume and achieved an oocyte survival rate of >90% and the establishment of live births[3-5]. Vitrification is preferred to slow freezing, with higher rates of oocyte survival, fertilisation, embryo cleavage, and clinical pregnancy[6]. Comparisons between fresh and vitrified oocytes showed comparable oocyte survival and clinical pregnancy rates[7-9]. In view of the growing evidence on efficacy, the European Society for Human Reproduction and Embryology[10] and the American Society for Reproductive Medicine[11] affirmed that oocyte cryopreservation should no longer be considered as experimental in 2012 and 2013, respectively.

Oocyte cryopreservation

Controlled ovarian stimulation is the first step. Among the various protocols adopted from in vitro fertilisation (IVF) treatment cycles, the antagonist protocol is preferred because of its flexibility and shorter duration. Treatment is initiated in the early follicular phase of a spontaneous or combined oral contraceptive pill-induced menstrual cycle with daily injections of follicle-stimulating hormone. Daily gonadotropin releasing hormone (GnRH) antagonist injections are added on day 6 (fixed protocol) or when the leading follicles are ≥14 mm in diameter (flexible protocol), in order to prevent premature luteinising hormone surge. An ovulation trigger injection is administered when the leading follicle exceeds 18 mm in diameter. An GnRH agonist is almost always used to minimise the risk of ovarian hyperstimulation syndrome (OHSS). The oocytes are then retrieved by aspirating these follicles under transvaginal ultrasound guidance.

Clinical applications

In the early days, oocyte cryopreservation was largely reserved for women undergoing gonadotoxic chemotherapy or radiotherapy. As the technology evolves, it has become the standard protocol for cryopreserving donor oocytes to establish egg banks. Indications for oocyte cryopreservation have further been extended to include medical conditions other than cancers and to those who wish to delay childbearing for various reasons – often known as social reasons, elective oocyte cryopreservation,

Correspondence to: Dr Carina CW CHAN
Email: carinacwchan@gmail.com

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age-related fertility loss, or elective fertility preservation (EPP).

Fertility preservation for cancer patients

Cancer treatments often have detrimental effects on female fertility when involving irradiation to the pelvic organs, surgical removal of the ovaries, or systemic gonadotoxic agents. The extent of damage to the ovarian function depends on the type and dose of chemotherapeutic agent used, the patient's age, and the ovarian reserve at baseline. Alkylating agents such as cyclophosphamide are the most gonadotoxic, causing depletion of the primordial follicle pool, and thus compromising the ovarian reserve[12,13].

Advances in cancer treatments have substantially improved patient survival. Professional organisations such as the American Society for Reproductive Medicine[14] and the American Society of Clinical Oncology[15] recommend oncologists to discuss with their patients the impacts of chemotherapy on fertility during cancer treatment planning and refer patients to reproductive specialists to discuss the possibility of fertility preservation. Despite this, only 3036 (44%) of 6976 patients in the United States were counselled regarding the risk of infertility associated with chemotherapy[16].

In cancer patients undergoing ovarian stimulation for oocyte cryopreservation, there is a concern that supra-physiological levels of oestrogen during ovarian stimulation may stimulate growth of hormone-dependent cancers such as breast cancers. The addition of an aromatase inhibitor in combination with gonadotropins has been proposed. Letrozole is an aromatase inhibitor that effectively reduces the peak oestradiol level and does not affect the oocyte yield[17,18]. It is usually administered orally starting on the second or third day of a spontaneous cycle until the day of ovulation trigger, and then restarted after oocyte retrieval until menstruation returns[19]. Final egg maturation is achieved with a GnRH agonist instead of the conventional human chorionic gonadotropin. GnRH agonist triggering results in significantly decreased oestradiol level on the day of retrieval and a faster drop of oestradiol levels in subsequent days[20]. GnRH agonist also reduces the risk of OHSS.

Breast cancer patients who underwent combined letrozole-gonadotropin ovarian stimulation showed no significant difference with controls in terms of short-term recurrence rate and relapse-free survival[21], as well as longer term follow-up of 5 (range, 1-13) years[22] and 6.3 years (range, 3 months to 23.6 years)[23].

For cancer patients, there is a time constraint before the commencement of chemotherapy or radiotherapy. Novel ovarian stimulation protocols can shorten the time interval to oocyte retrieval. The random-start protocol[24] initiates ovarian stimulation at the time of patient presentation rather than waiting for spontaneous menstruation. It is equally effective as conventional start protocol in terms of the total number of mature oocytes retrieved, oocyte maturity rate and fertilisation rate, irrespective of whether the stimulation is started in the late follicular phase or the luteal phase[25-28].

The number of oocytes retrieved is important in determining the probability of the patient having a successful live birth in the future. Cancer patients do not have much time to undergo repeated ovarian stimulation and oocyte retrieval cycles. The double stimulation or DuoStim protocol combines conventional follicular phase stimulation together with luteal phase stimulation, so that two oocyte retrieval procedures can be performed within the same ovarian cycle, maximising the total number of oocytes that can be retrieved for an individual patient[28-33]. The oocytes collected from the luteal phase stimulation have comparable rates of fertilisation, blastulation, euploid embryo, and pregnancy after embryo transfer, compared with oocytes collected from the follicular phase stimulation in IVF patients[33]. Because of the low utilisation rate of cryopreserved oocytes in cancer patients, data regarding pregnancy and live birth rates from these two novel ovarian stimulation protocols are scarce and inconclusive. Nonetheless, the European Society of Human Reproduction and Embryology recognised these as options when there is urgency in cryopreserving oocytes[34].

Other medical indications

Other medical and iatrogenic conditions causing premature ovarian insufficiency include autoimmune, genetic and epigenetic, environmental, metabolic, and gynaecological conditions (Table). The impact of endometriotic cysts (both the occurrence and removal) on the ovarian reserve is often overlooked. Women with endometriomas have a faster depletion of ovarian follicles and early (premature) ovarian insufficiency. It is pertinent that doctors looking after these women discuss or refer these women to an appropriate specialist who can offer the option of fertility preservation including oocyte or embryo cryopreservation.

Oocyte donation programmes

Oocyte donation is an alternative to adoption for women with premature ovarian failure who desire to bear

Table. Medical conditions other than cancer for oocyte cryopreservation

Iatrogenic
Surgery
Radiotherapy
Chemotherapy
Chromosomal and genetic aberrations
BRCA gene carriers before prophylactic oophorectomy
X chromosome abnormality: 45X, 47XXX
Fragile X premutation
Autoimmune ovarian damage
Autoimmune diseases requiring chemotherapy
Systemic lupus erythematosus
Rheumatoid arthritis
Environmental factors
Viruses
Chemical agents
Radiation
Metabolic diseases
Diabetes type 1
Galactosaemia
17-OH deficiency
21-OH deficiency
Endometriosis
Endometrioma
Endometrioma surgery

children[35]. It can be used to treat women with age-related infertility owing to the reduction in the number and the quality of oocytes or simply diminished ovarian reserve. Women who are carriers of a known genetic disease who wish to avoid passing the abnormality to the next generation can also benefit from oocyte donation.

In the early days, fresh donor oocytes were used, and the menstrual cycles of the donor and the recipient had to be synchronised to allow transfer of the resultant (fresh) embryo in the same cycle. In addition, donor oocytes cannot be quarantined for infectious diseases such as HIV. Cryopreserved oocytes can be quarantined for the incubation period and kept in an oocyte bank[8]. Recipients can have the embryos replaced at their 'convenience'. Oocytes from a pool of donors can be allocated to more than one recipient, potentially improving the efficiency and reducing the cost and waiting time of oocyte donation programmes.

Oocyte donation programmes enable study of the efficacy of the oocyte cryopreservation process, as frozen and fresh oocytes can be compared with regard to their capacity to be fertilised, cleaved, implant, and ultimately the live birth rate. The oocyte survival rate was reported to be 92% on thawing, with a comparable ongoing pregnancy rate between vitrified and fresh oocytes (43.7% vs 41.7%) in a single-centre prospective randomised study[8]. However, the Society for Assisted Reproductive Technology reported a lower live birth rate in recipients of cryopreserved donor oocytes (43.2% vs 49.6%)[36]. A follow-up study reporting two additional years (2103-2015) of the national outcome data using the same database[37] confirmed a lower live birth rate per recipient cycle started (39.7% vs 51.1%) and per embryo transfer (45.3% vs 56.4%), despite a similar number of embryos transferred. Reasons for the lower live birth rates in the US remain speculative. It may be attributable to the allotment of oocytes from one donor to several recipients rather than giving the entire cohort to a single recipient in order to reduce cycle costs for each recipient. However, it is impossible to evenly divide a cohort based on quality. This explains cancellation in recipient cycles where all allocated oocytes fail to survive the thawing process. Although this may lower the live birth rate per recipient cycle started, it should not affect the live birth rate per embryo transfer. Another possible explanation may be related to the freezing and thawing process in each centre. It can negatively affect the developmental potential of an oocyte and that of the subsequent embryo. Vitrification technique is difficult to master, as is the thawing process[7-9,36-38]. Embryologists in different centres may not have the same level of skill and experience. Finally, donor selection by commercial donor oocyte banks may not be as rigid as selection for fresh donor cycles by fertility centres.

Cryopreserved donor oocytes offer advantages over fresh ones. These include simplified access to a larger pool of oocytes particularly for ethnic minorities, ability to transport frozen oocytes over long distances thereby reducing the need for reproductive tourism and lowering the cost per treatment cycle. Although cryopreservation of donor oocytes has become a routine practice for some IVF centres, the American Society for Reproductive Medicine does not recommend routine donor oocyte banking until clinical data on safety and equivalent efficacy of oocyte cryopreservation become available[39].

Elective fertility preservation

Women's fertility declines with age[40,41], and the decline accelerates after the age of 35 years. The decline is due to reduction in oocyte quantity and quality[42],

reflected by an increased miscarriage rate[43] and a higher risk of carrying a fetus with chromosomal abnormalities[44]. However, a study using shared oocytes between the donors and recipients showed that oocyte recipients had comparable pregnancy and delivery rates to their donors[45], indicating that uterine or endometrial factors do not seem to be reduced in women of advanced reproductive age, and that the age-related decline in fertility can be largely overcome by using younger oocytes.

When women of advanced reproductive age fail to conceive, they have the option to undergo IVF with donor oocytes. If they have previously cryopreserved their own oocytes, they can use their own oocytes for IVF and have their own genetic offspring. Compared with using donor oocytes, elective oocyte cryopreservation may potentially reduce the cost of multiple cycles of IVF. Thus, EPP may offer a solution to prevent unavoidable age-related infertility.

Ethical issues

Society is divided on whether oocyte cryopreservation should be made available to women who wish to postpone child-bearing. This issue can be examined from the perspectives of autonomy, beneficence, non-maleficence, and justice.

Oocyte cryopreservation enhances women's reproductive autonomy by enabling them to decide whether, when, and with whom they wish to start a family. It allows them to divert their energies towards alternative life goals such as education and career plans and not to rush to start a family because of the biological clock pressure. Reproductive autonomy is further enhanced by granting women, particularly single women, the control of their destiny. To generate embryos, the couple has to be legally married and those contributing the gametes will have a stake. Conflicts may ensue when the partner changes his mind and decides against having children, or when they separate, divorce, or posthumously. The Hong Kong Human Reproductive Technology Ordinance prohibits the transfer of embryos to persons who are not the parties to a marriage (Cap.561 Part III Section 15-5). It prohibits the posthumous use of embryos and any stored embryos have to be disposed of when the partner passes away. Furthermore, oocytes are generally not afforded the same status as embryos. The latter may conjure emotional or religious connotations upon disposal. Cryopreserving oocytes thus provides a more flexible option for single women and for those who prefer not to generate and then cryopreserve embryos[46].

Women have a narrower reproductive window than men; their optimal fecundity spans less than two decades and is drastically reduced 5 to 10 years before the menopause. Historically, women had to choose between childbearing or education and career development. This biological inequity can be partially offset by oocyte cryopreservation. Women can pursue other life goals or career plans without losing their natural reproductive potential and/or before they are able to find a suitable partner. Oocyte cryopreservation can thus foster gender equality[47].

In regard to beneficence, oocyte cryopreservation, strictly speaking, is not fertility preservation; rather it preserves gametes for future attempts at reproduction. There is no guarantee that one or more live births will result from the cryopreserved oocytes. In fact, it may do harm by giving women a false sense of security so that they may delay childbearing until it is too late.

The process is not without risks (non-maleficence)[47]. Controlled ovarian stimulation can lead to severe OHSS and its attendant complications. Oocyte retrieval is painful and invasive; it can be complicated by substantial internal haemorrhage and pelvic infection. These can result in infertility and even mortality. Nonetheless, if society accepts oocyte donors to undergo a medical intervention for no personal benefit, there is no reason why the same risks become unacceptable when a woman chooses to cryopreserve her own oocytes. Current adoption of the antagonist (stimulation) protocol, use of a GnRH agonist ovulation trigger and withholding embryo transfer can practically reduce the risk of severe OHSS to near zero[48].

Delaying childbearing until women are in their fifth decade or beyond may also do more harm than good, because older women have more obstetric and neonatal complications[49]. Their offspring may face negative psychosocial consequences of being born to a mother of advanced age, and may lose a parent relatively early in his/her life. Children as caregivers are more likely to suffer from depression and behavioural problems, and they have less time for school activities and to make friends. They live in constant fear of losing one or both parents[50]. The long-term impact of early (before the age of 18 years) parental death has shown a negative impact in adulthood with regards to trust, relationships, self-esteem, loneliness, and isolation[51].

In terms of justice, oocyte cryopreservation is expensive and often not covered by health insurance and thus not every woman has access to this option, although Apple and Facebook offer EPP to their women employees

as health benefits. There are social, racial and ethnic disparities in women's access to this option[52]. It is also important that women are not pressured into delaying childbearing just because their company is providing insurance coverage for oocyte cryopreservation, and that they will not be considered as non-committal to their career if they choose to have children early rather than cryopreserving their oocytes and defer motherhood to a later age[46], thereby undermining the whole concept of reproductive autonomy.

Optimal age for EPP

The conception rate, natural or via reproductive technology treatment, diminishes rapidly with advancing maternal age. This is largely due to the age-related decline in the quantity and quality of oocytes in a woman's ovaries. In a study of associations between maternal age and the prevalence of embryonic aneuploidy in over 15000 consecutive trophectoderm biopsies, the lowest risk was seen in women in their mid to late twenties, and the risk of having no euploid embryo was lowest in women aged 26 to 37 years[53].

In a retrospective analysis of IVF patients, the chance of having a live birth for each fresh oocyte reduced gradually from 8.67% for women aged <30 years to 7.33% for those aged 35 to 37 years, and then rapidly to 1.06% for those aged ≥43 years. In women who used their autologous cryopreserved oocytes, the chance of having a live birth showed a similar downward trend[54]. In another retrospective study of women who underwent EPP, the live birth rate was significantly higher at the age cutoff of 35 years (50% [95% CI=32.7-67.3] vs 22.9% [95% CI=14.9-30.9])[55].

Younger women may be able to maximise the number of 'good quality' oocytes for storage, but they may be less likely to use these oocytes in the future. The procedure and expense of oocyte cryopreservation may become unnecessary if they never have to use these oocytes. Cryopreserving oocytes at a later age may yield fewer and poorer-quality oocytes per cycle, and women may need multiple cycles to bank an adequate number of oocytes to have a reasonable chance of a live birth and this increases the cost. Using a mathematical model, in women who plan to delay childbearing until the age of 40 years, oocyte cryopreservation before the age of 38 years reduces the cost to achieve a live birth[56]. In a decision-tree model, the highest probability of live birth is seen when oocyte cryopreservation is performed at the age of <34 years (>74%), and that oocyte cryopreservation versus no action has the largest benefit at the age of 37 years and is most cost-effective[57]. However, there is little benefit to cryopreserve oocytes for younger women aged 25 to 30 years, because they may not need to use these oocytes in the end. Nonetheless, young women at risk of premature ovarian insufficiency should be counselled of the option of oocyte cryopreservation at an earlier age.

In Hong Kong, the Council on Human Reproductive Technology specifies that "the maximum storage period for gametes or embryos stored for patients' own use in a reproductive technology procedure should not exceed 10 years" (Chapter X, Para 10.7). This means that for women younger than 32 years, their cryopreserved oocytes would have to be disposed of before the age of 42 years, thereby defeating the intention of EPP. Therefore, the optimal age for EPP – at least in Hong Kong – is between 33 and 37 years of age.

Optimal number of oocytes to freeze

Every vitrified-warmed oocyte has a 5% to 7.4% chance of a live birth with an overall efficiency of 6.4%[54]. The number of oocytes required varies with the woman's age at the time of cryopreservation. In a study of IVF outcome using vitrified oocytes, in women aged ≤35 years, the cumulative live birth rate increased sharply from five (15.4%) to eight (40.8%) oocytes, with an 8.4% gain for each additional oocyte banked, and the rate of increase plateaued at 10 to 15 oocytes (85.2%)[55]. This contrasted with a milder increase for women aged >36 years, their cumulative live birth rate was 5.1% (5 oocytes) and 19.9% (8 oocytes), reaching a plateau of 35.6% with 11 oocytes[55]. For women aged <38 years, 15 to 20 oocytes should be frozen to produce a 70% to 80% chance of having at least one live birth; and 25 to 30 oocytes should be frozen for women aged 38 to 40 years to produce a 65% to 75% chance of having at least one live birth[54]. Based on a mathematical model, women aged 34, 37, or 42 years, each with 20 mature oocytes frozen, are expected to have a 90%, 75%, and 37% chance of having at least one live birth, respectively; and 10, 20, and 61 oocytes should be frozen to produce a 75% likelihood of having at least one live birth[58]. All these studies are of single-centre, retrospective, and have not been validated or reproduced. In a study from Reprogenetics regarding the euploidy rates in donor egg cycles among 42 fertility clinics in the United States, the average euploidy rate per centre ranged from 39.5% to 82.5%, whereas the mean expected rate of euploidy was 68.4%. The implication of these findings is that centre-specific assisted reproductive technology practices and outcomes can vary considerably, including oocyte cryopreservation[59].

Safety for women

The risks associated with oocyte cryopreservation involve controlled ovarian stimulation and oocyte retrieval. The risks of oocyte retrieval include pelvic infection, internal bleeding, inadvertent damage to other intra-abdominal organs, and ovarian torsion. OHSS is the most serious complication. OHSS can be classified as mild, moderate, severe, and critical[60]. Mild and moderate OHSS is characterised by abdominal pain, enlarged ovaries, and weight gain, with an incidence of 3% to 6%; it can be managed conservatively, as it is self-limiting and generally resolves upon resumption of menstruation. Severe and critical OHSS can occur in 1% to 3% of IVF cycle. These women have fluid retention in the form of ascites and sometimes pleural effusion, massive ovarian enlargement, haemoconcentration and oliguria, and venous thrombo-embolism. It is potentially life-threatening and worsens by an ensuing pregnancy following fresh embryo transfer. The rise in endogenous human chorionic gonadotropin can exacerbate its symptoms and duration. Elective cryopreservation of embryos can prevent OHSS[61,62]. Women who are cryopreserving oocytes will not have embryo transfer, and hence late-onset OHSS can be avoided. A systematic review and meta-analysis demonstrated a significantly lower incidence of OHSS in the GnRH antagonist protocol compared with the GnRH agonist protocol (OR=0.59, 95% CI=0.42-0.82)[63]. The risk can be further reduced with the concomitant use of a GnRH agonist for final oocyte maturation instead of the traditional human chorionic gonadotropin. A Cochrane review showed that the incidence of moderate to severe OHSS was significantly lower in the GnRH agonist trigger group compared with the human chorionic gonadotropin group (OR=0.10, 95% CI=0.01-0.82)[64]. Therefore, the antagonist stimulation protocol coupled with GnRH agonist trigger is recommended, as it minimises the risk of OHSS.

Safety for children

In the early days of oocyte cryopreservation, there were concerns about the risk of meiotic spindle damage leading to an increased aneuploidy and digynic triploidy in the subsequent embryos derived from cryopreserved oocytes. The aneuploidy rate by fluorescence in situ hybridisation showed that the percentage of embryos with aneuploidy in the cryopreservation group was comparable to that observed in the controls[65]. A review of over 900 live births derived from cryopreserved oocytes also showed no increased risk of congenital anomalies, compared with the general population[66]. In a retrospective study of 2252 live babies born from cryopreserved oocyte in the Italian National Register, only 0.9% had congenital malformations reported[67].

Despite these, the safety of long-term cryopreservation of oocytes is lacking. Cryopreserving oocytes for up to 4 years did not seem to affect the IVF success outcomes[68]. The euploid rate of blastocysts was similar to those derived from fresh oocytes, after a median of 3.5 years (maximum, 6 years)[69]. There is no study reporting the long-term follow-up of children born with oocyte cryopreservation, especially when the oocytes are cryopreserved for a prolonged period.

Conclusion

Oocyte cryopreservation is an option for women with various medical conditions to preserve fertility. It is also widely applied in oocyte donation and has been extended to women who wish to preserve their fertility against age-related fertility loss. As the number and quality of oocytes decrease with advanced reproductive age, women who wish to cryopreserve oocytes should preferably consider this procedure before 37 years of age. The procedures of ovarian stimulation and egg retrieval are generally safe for women. The safety of children born from cryopreserved oocytes is reassuring, but the long-term outcome is lacking.

Declaration

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