Review Article

# Female Fertility: Is it Safe to "Freeze?"

**Lu Zhang, Li-Ying Yan, Xu Zhi, Jie Yan, Jie Qiao**

Department of Obstetrics and Gynecology, Center for Reproductive Medicine, Peking University Third Hospital, Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproduction, Beijing 100191, China

## Abstract

**Objective:** To evaluate the safety and risk of cryopreservation in female fertility preservation.

**Data sources:** The data analyzed in this review were the English articles from 1980 to 2013 from journal databases, primarily PubMed and Google scholar. The criteria used in the literature search show as following: (1) human; embryo; cryopreservation/freezing/vitrification, (2) human; oocyte/immature oocyte; cryopreservation/ freezing/vitrification, (3) human; ovarian tissue transplantation; cryopreservation/ freezing/vitrification, (4) human; aneuploidy/DNA damage/epigenetic; cryopreservation/freezing/vitrification, and (5) human; fertility preservation; maternal age.

**Study selection:** The risk ratios based on survival rate, maturation rate, fertilization rate, cleavage rate, implantation rate, pregnancy rate, and clinical risk rate were acquired from relevant meta-analysis studies. These studies included randomized controlled trials or studies with one of the primary outcome measures covering cryopreservation of human mature oocytes, embryos, and ovarian tissues within the last 7 years (from 2006 to 2013, since the pregnancy rates of oocyte vitrification were significantly increased due to the improved techniques). The data involving immature oocyte cryopreservation obtained from individual studies was also reviewed by the authors.

**Results:** Vitrifications of mature oocytes and embryos obtained better clinical outcomes and did not increase the risks of DNA damage, spindle configuration, embryonic aneuploidy, and genomic imprinting as compared with fresh and slow-freezing procedures, respectively.

**Conclusions:** Both embryo and oocyte vitrifications are safe applications in female fertility preservation.

**Key words:** Embryo Cryopreservation; Oocyte Cryopreservation; Ovarian Tissue Cryopreservation

## Introduction

In recent decades, more and more women have infertility problems due to declining oocyte quality, commonly related to the maternal age,[1] invasive cancer treatment,[2] premature ovarian failure (POF),[3] and polycystic ovary syndrome (PCOS).[3] For example, in USA, 805,500 women were diagnosed as new cases of invasive cancer in 2013, and about 10% of them were <45 years old.[4] The fertility of survivors who underwent cancer treatments such as chemotherapy,[5] radiotherapy[6] and bone marrow transplantation[7] was impaired by the treatments. Thus, it is imperative to reserve these cancer survivors' fertility before cancer treatments by cryopreserving their ovaries, oocytes or embryos.

Furthermore, some nononcological diseases such as POF and PCOS may also lead to the ovarian dysfunction.[3] Moreover, it is increasingly common for women in many countries to delay childbearing until the age of 35 years or older.[8] Therefore, fertility preservation in women is not only a medical problem, but also a current social issue to be resolved.

Cryopreservation is a crucial option for people seeking fertility preservation, which refers to freezing cells and tissues to sub-zero temperatures in order to stop all biologic activity and preserve them for future use.[9] Thus, a woman could bear children even after chemotherapy or menopause, as well as store eggs extracted for an *in vitro* fertilization (IVF) cycle.[10] Ever since the first baby born from a frozen embryo in 1983,[11] and the first pregnancy achieved from a frozen oocyte in 1986,[12] interests in cryopreservation techniques are growing tremendously. Over the past 30 years, techniques for cryopreservations of human embryos, oocytes, and ovarian tissues have been developed, with two main techniques commonly being used: the traditional, slow-freezing method, and vitrification, a novel technique combining ultra-rapid cooling time with high cryoprotectant concentration, engendering glass-like formation to avoid damaging ice crystals.[13]

Numerous individual studies investigating the different

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**Address for correspondence:** Prof. Jie Qiao, Department of Obstetrics and Gynecology, Center for Reproductive Medicine, Peking University Third Hospital, Beijing 100191, China
E-Mail: jie.qiao@263.net

reproductive outcomes of cryopreservation on human embryos, immature or mature oocytes, or ovarian tissues have been reported. Currently, embryo and mature oocyte cryopreservation following IVF are the only methods endorsed by the American Society of Reproductive Medicine (ASRM)[9] and recommended by the American Society of Clinical Oncology (ASCO) for fertility preservation for patients with cancer.[2] However, the overall safety and risks for cryopreservation techniques in female fertility preservation are still uncertain. The aim of this article was to review the current knowledge on the possible effects of freezing methods on human embryos, oocytes, and ovarian tissues, and provide recommendations for clinical applications.

## Clinical Outcome Assessments

### Better clinical outcomes: frozen embryos versus fresh ones

Human embryo freezing is the initial method to preserve the fertility for women at reproductive age. In addition, it is the first freezing method of fertility preservation for women endorsed by ASRM.[14] This technique has been investigated by a respectable number of research teams, and hereby we summarized the latest systematic studies to discuss the clinical effects of cryopreservation on human embryos.[15]

In Roque *et al*.[16] systematic meta-analysis, three clinical trials accounting for 633 cycles in women aged 27–33 years old showed that frozen embryo transfer resulted in a statistically significant increase in the ongoing pregnancy rate [the relative risk (*RR*) and 95% confidence intervals (*CI*) was 1.32 (1.10-1.59)] and clinical pregnancy rate [*RR* (95% *CI*) was 1.31 (1.10-1.56)] compared with the fresh transfer group.[16-19] Interestingly, the fresh group showed a higher miscarriage rate [*RR* (95% *CI*) was 0.83 (0.43-1.60)], but no statistical difference was found when compared with the frozen group.[16-19] Moreover, Maheshwari *et al*.[20] systematically quantified the obstetric and perinatal risks for singleton pregnancies after frozen embryo transfer and compared it with those after fresh embryo transfer. Eleven studies included in this meta-analysis indicated better perinatal outcomes in singleton pregnancies after the transfer of frozen-thawed embryos when compared to those after fresh IVF embryos. This was, because the *RR*s (95% *CI*) of: antepartum hemorrhage [0.67 (0.55-0.81)], very preterm birth (delivery at < 32 weeks) [0.73 (0.50-1.08)], preterm delivery (delivery at < 37 weeks) [0.84 (0.78-0.90)], small for gestational age [0.45 (0.30-0.66)], low birth weight (birth weight < 2500 g) [0.69 (0.62-0.76)], and perinatal mortality [0.68 (0.48-0.96)] was significantly lower in women who received frozen embryos than those transferred with fresh embryos.[20-31] The *RR* differences of very low birth weight (birth weight < 1500 g) [0.72 (0.50-1.04)], congenital anomalies [1.05 (0.81-1.35)], and transfer rate to neonatal intensive care unit [1.0 (0.92-1.08)] between frozen and fresh groups were not available.[20-31] However, pregnancies from frozen-thawed embryos had an increased risk of cesarean section [1.1 (1.05-1.15)] compared with those after fresh embryos.[20]

The reason for improved clinical outcomes with frozen embryos is not clear. It might be associated with having a well-balanced embryo-endometrium interaction in frozen cycle and lacking controlled ovarian hyperstimulation, which may adversely affect endometrial receptivity during fresh IVF cycle.[19] In addition, when hormone replacement cycles were applied in frozen embryo transfers, estrogen and progesterone were given in physiological doses to mimic natural cycles while supraphysiological doses of gonadotropins were given in fresh cycles.[17,19] Another feasible explanation is that freezing and thawing may screen the good quality embryos to survive, resulting in better fetal growth.[22]

### Equivalent outcomes: frozen mature oocytes versus fresh ones

Mature oocyte cryopreservation is a freezing method, which was just approved by ASRM in 2012.[9] Before 2005, the survival rates and pregnancy rates of freezing oocyte were very low because of limited freezing techniques and oocyte fragility.[15] Recently, oocyte vitrification, a novel, and well-developed freezing method, improved the clinical outcomes. Hence, we reviewed the current relevant studies to evaluate the efficacy and safety of mature oocyte cryopreservation.

Five randomized controlled trials were systematically analyzed to assess the efficacy of metaphase II (MII) oocyte vitrification in clinical pregnancy outcomes from 2005 to 2009.[32] These studies concluded that there were no differences between the vitrified MII oocytes and the fresh oocytes in terms of ongoing pregnancy [*RR* (95% *CI*) was 1.03 (0.73-1.45)], top-quality embryo [*RR* (95% *CI*) was 0.91 (0.83-1.01)], embryo cleavage [*RR* (95% *CI*) was 1.0 (0.90-1.11)], fertilization [*RR* (95%*CI*) was 1.02 (0.91-1.13)], and implantation [*RR* (95%*CI*) was 0.96 (0.75-1.24)].[32-37] Furthermore, the incidence of congenital anomalies was not increased in live born babies from oocyte cryopreservation, when compared with natural conception.[38] Thus, on the basis of the evidence provided by Cobo and Diaz's meta-analysis,[32] oocyte vitrification seemed to be an efficient method of fertility preservation, appearing to have similar clinical outcomes as fresh mature oocytes.

### Reduced oocyte viability: frozen immature oocytes versus fresh ones

Theoretically, immature oocytes, such as oocytes at the germinal vesicle (GV), GV breakdown, and MI stages, should be more tolerant of cooling, since they do not have a spindle apparatus and are absent during the ovarian hyperstimulation. Thus, immature oocyte cryopreservation seemed to be a safer option for women to reserve the fertility.[39,40] Unfortunately, attempts to optimize protocols were hindered by the scarcity of clinical materials and the restriction of *in vitro* maturation (IVM).[41] To date, although normal infants were successfully obtained from embryos

derived from immature oocytes,[42,43] only a handful of global centers are using immature oocyte cryopreservation as an alternative option for women with cancer.[44-47]

In 1997, Park *et al*.[48] reported that human oocytes matured *in vitro* after cryopreservation at the GV stage showed an increased incidence of chromosomal and spindle abnormalities, as well as lower maturation rates. Sixteen years later, both maturation rate and oocyte viability rate are still lower in frozen-thawed immature oocytes than fresh immature oocytes.[49-52] With respect to this problem, it was presumably related to the reduced cortical granules at the cortex of cryopreserved GV oocytes, as well as the appearance of vacuoles and small mitochondria-smooth endoplasmic reticulum complexes in the ooplasm.[53] However, the survival rate of frozen-thawed immature oocytes was better than that of mature MII oocytes.[54] Considering the above situation, it appeared that cryopreservation of immature oocytes after IVM to MII stage is a superior strategy over freezing immature oocytes directly.[51,52,55] Although the survival rates seemed to have been improved, many problems still need to be solved, including the fertilization and pregnancy outcome of immature oocyte freezing. Therefore, immature oocyte cryopreservation is still an investigated technique used in fertility preservation and requires further exploration before its clinical application.

## Better clinical outcomes: vitrification versus slow-freezing

There are two cryopreservation methods: slow-freezing and vitrification. Slow-freezing has the advantages of using low concentrations of cryoprotectants, but induces the risk of intracellular ice formation. On the other hand, vitrification is a rapid method and requires high concentrations of cryoprotectants, which is associated with chemical toxicity and osmotic shock yet reducing chilling sensitivity and crystallization damage to gametes.[56] To compare these two methods, we reviewed newly published literatures and found two meta-analysis reviews concerned with human embryos, one meta-analysis review referring to human mature oocytes, and several studies of human immature oocytes.

### Embryo cryopreservation

In meta-analysis reported by Loutradi *et al*.,[57] the survival rates of the two methods were compared in four eligible studies, including three randomized controlled trials from 1980 to 2006. Furthermore, in systematic review reported by AbdelHafez *et al*.,[58] the primary and secondary clinical outcomes of the two methods were examined in six randomized controlled trials from 1980 to 2008 (among those, two original papers were unavailable, one study was overlapping in both meta-analyses).[59-64] The information summarized by Loutradi *et al*.[57] indicated that the survival rates of both cleavage stage embryos [*RR* (95% *CI*) was 15.57 (3.68-65.82)] and blastocysts [*RR* (95% *CI*) was 2.2 (1.50-3.16)] after vitrification were significantly higher when compared with slow-freezing.[57,63,65-67] In addition, AbdelHafez *et al*.[58] consistently showed that the survival rate [*RR* (95% *CI*) was 4.87 (3.01-7.88)], clinical pregnancy rate [*RR* (95% *CI*) was 1.55 (1.03-2.32)], ongoing pregnancy rate [*RR* (95% *CI*) was 1.82 (1.04-3.20)], and implantation rate [*RR* (95% *CI*) was 1.49 (1.03-2.15)] of vitrified embryo transfers were statistically higher than those from slow-freezing embryos.[59-64] There were no differences in multiple pregnancy rate [*RR* (95% *CI*) was 2.11 (0.99-4.52)], a miscarriage rate [*RR* (95% *CI*) was 0.57 (0.16-2.03)], and live-birth rate [*RR* (95% *CI*) was 0.87 (0.36-2.12)] between vitrification and slow-freezing.[59-64] Vitrification is superior to slow-freezing in human embryo cryopreservation.

### Mature oocyte cryopreservation

Cobo and Diaz's [32] meta-analysis analyzed five randomized controlled trials between 2005 and 2009 to compare the clinical efficacy of mature (MII phase) oocyte vitrification with slow-freezing. The survival rate [*RR* (95% *CI*) was 2.46 (1.82-3.32)], fertilization rate [*RR* (95% CI) was 1.5 (1.07-2.11)], top-quality embryo rate [*RR* (95% *CI*) was 3.32 (1.37-8.02)], embryo cleavage rate [*RR* (95% *CI*) was 2.25 (1.32-3.85)], and pregnancy rate [*RR* (95% *CI*) was 3.18 (1.06-9.52)] were remarkably higher in vitrified mature oocyte group when compared with those in slow-freezing group.[32-37] The strength of the evidence provided by these randomized trials proved vitrification to be an efficient method to cryopreserve mature oocytes.

### Immature oocyte cryopreservation

In 2007, Sarajari *et al*.[68] compared the survival rate and the resumption rate of meiosis of immature oocytes (GV, MI phases) using vitrification and slow-freezing methods. The data from two groups showed that the survival rate was much higher in the vitrification group when compared with the slow-freezing group, but the maturation rate after IVM was no different between the two groups. Similar to Sarajari *et al*.'s results, the vitrification protocol of immature oocytes yielded better primary outcomes in survival rate, maturation rate, and cleavage rate as compared with the slow-freezing procedure.[69] Additionally, Combelles *et al*.[70] investigated the cytoskeletal and chromosome organization of immature oocytes that were either vitrified or slow-frozen, and the results indicated that the survival and maturation rates were comparable between the two groups, with similar rates of spindle morphometrics and chromosome organization; however, fewer oocytes underwent spontaneous activation or appeared to have spindle abnormalities, and more oocytes showed a bipolar spindle after IVM following vitrification when compared with slow-freezing.

## Assessments of DNA integrity, spindle configuration, aneuploidy and genomic imprinting after cryopreservation

In spite of the excellent clinical outcome of cryopreservation, there are still many more questions about cryo-injury, such as DNA damage, spindle deformation, chromosome abnormalities, and aberrant genomic imprinting when cells are exposed to mechanical, thermal, and chemical stresses during cryopreservation.[71] Multiple centers in the world have analyzed the possible damages to subcellular structures of mature oocytes using slow-freezing and vitrification

protocols. In general, it has been demonstrated that mature oocytes are less tolerant to cooling than embryos, most probably due to the spindle sensitivity to cryoprotectants and low temperature.[72]

Studies showed that the meiotic spindle was a dynamically sensitive structure, which disappeared after freezing and reforms after thawing during cryopreservation.[73-76] This phenomenon was regulated by precise polymerization and depolymerization of tubulin, and shown to be in a very delicate equilibrium.[77,78] The chromosomes were aligned tightly on the spindle even when the spindle was disassembled.[79] About 3–5 hours after thawing, the spindle reformed completely.[73,74] Interestingly, no DNA fragmentation was observed by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling assay in both freezing and fresh oocytes.[80,81] Concomitantly, a randomized controlled trial demonstrated that oocyte vitrification did not increase the risk of embryonic aneuploidy, verified by DNA fingerprinting.[82]

Given the complications of epigenetic stability during oocyte and early embryo development, only one study investigated the cryogenic effects on the imprinted methylation of a human oocyte. As shown in Al-Khtib *et al*. study,[83] vitrified GV oocytes followed by IVM to M II acquired full imprint at Kv differentially methylated region 1 (a maternal imprinting center) and generally retained the unmethylated state of H19DMR (a paternal imprinting center) with the same efficiency as fresh GV oocytes in human, which suggested that freezing does not alter the genomic imprinting regardless of IVM. Unquestionably, the genomic imprinting of cryopreserved oocytes was affected by a complex relationship among the impaired fertility, assisted reproductive technologies and cryopreservation.[84,85] Further studies on imprinting establishment in human oocyte cryopreservation are necessary to reach definitive conclusions. In addition, some studies have compared vitrification of oocytes with slow-freezing by examining morphological appearance, meiotic spindle, DNA integrity, and oocyte configuration. The results proved that vitrification was more effective than slow-freezing, offering higher survival rate, faster cellular volume recovery, faster spindle recovery, and similar DNA fragmentation.[77,81,86]

### Decreased ovarian function and increased recurrent ovarian failure: cryopreserved ovarian tissue transplantation versus fresh ovarian tissue transplantation

Cryopreservation of ovarian tissue may be the only available option for prepubertal girls with cancer to reserve the fertility.[87] One theoretic option, *in vitro* growth of isolated immature follicles before or after ovarian tissue freezing, may avoid the transmission of malignant cells, but it is still at a laboratorial stage.[88] Alternatively, ovarian tissue cryopreservation with subsequent transplantation has been categorized as an experimental option for fertility preservation by ASRM.[89] Despite few clinically available applications, ovarian tissue banking has been offered to patients as a clinical service by many global centers.[90] As of now, only xenografting of ovarian tissue and cryopreservation with subsequent ovarian transplantation have been successfully applied in humans.[91] Due to the small number of patients, the difficulties with surgical techniques, and the duration of follow-up, it is hard to execute large studies on the clinical outcomes after ovarian tissue transplantation (OTT). So far, only one meta-analysis was reported on the reproductive function after cryopreserved and fresh ovarian tissue grafts.[92] In Bedaiwy *et al*.'s systematical review,[92] 25 individual studies, including 46 unique cases, were analyzed by the time of re-establishment of ovarian function, in which the short-term (<12 months) and long-term (>12 months) ovarian function as well as the pregnancy rate after freezing or fresh OTT were evaluated.[91-117] The precise data indicated that cryopreserved ovarian grafts had a decreased trend in the recovery of ovarian function [*RR* (95% *CI*) was 0.41 (0.15-1.09)] and an increased trend of recurrent ovarian failure [*RR* (95% *CI*) was 2.13 (0.89-5.56)] compared with fresh ovarian grafts.[91-117] The decreased reproductive potentiality of frozen OTT may be related with the ischemic injury to follicles, caused by the freeze-thaw procedure in cryopreserved ovarian grafts.[91-117] As for the long-term efficacy, there was insufficient evidence to tell the difference between fresh and cryopreserved OTT. According to the reported cases, the pregnancy rate of patients from frozen grafted ovaries was similar to those from fresh grafted ovaries.[91-117] Consequently, the efficacy of OTT using cryopreserved tissues is not yet equivalent to that of fresh grafts. The ovarian cryopreservation protocols are required to be improved and optimized in the future, supported by strong experimental evidence.

## Clinical Application Recommendation

1. Embryo vitrification is a well-established method for adult women who have a male partner or wish to use donor sperm for medical and social reasons. However, it is a cost-based procedure, requiring ovarian stimulation, oocyte collection, and the use of assisted reproduction techniques that may take 2–5 weeks, which is not feasible for some patients with hormone-sensitive tumors such as breast cancer or requiring urgent cancer treatment.[89]
2. Mature oocyte vitrification, an established method for fertility preservation recently endorsed by ASRM and ASCO, is an option for older postpubertal female children and adult women who are single or who have religious or ethical objection to embryo freezing for medical and social reasons.[118] However, it also requires a cycle of ovarian stimulation before the beginning of chemotherapy or radiation therapy, which could delay the cancer treatment and increase the risks of stimulating hormone-sensitive cancers.[89]
3. Immature oocyte vitrification is an experimental option for patients who need urgent cancer treatments or

have hormone-sensitive cancers, providing the benefit of avoiding both ovarian stimulation and expensive medications.[79] Unfortunately, this method is limited because of poor clinical results.

4. Ovarian tissue cryopreservation is considered as an investigational approach. Only subsequent transplantation or IVM offers the advantages of preserving thousands of primordial follicles at one time and potentially restoring temporary endocrine function to cancer survivors and patients with POF.[92] This procedure of cryopreservation may be the only practical option for prepubertal girls with cancer.[87] Additionally, this option currently emerges to serve other specific groups of patients, such as women with hormone-sensitive cancers or women who require an immediate cancer treatment.[90] The disadvantage of this technique is multiple challenges involved, including: oocyte atresia from both ischemia at the time of biopsy and the freeze-thaw process,[108] altered hormonal profiles compared to normal ovaries,[108] surgical trauma of transplantation, short duration of ovarian function (the mean time reported was 4 to 5 years[87,90]) and potential reintroduction of cancer cells.

## Novel Options for Healthy Women

Fertility cryopreservation is commonly applying for patients who had already developed cancer or aging women at risk of declining ovarian function, considered as an elective method to prevent deterioration of ovarian function. However, more and more women are recently choosing delayed child-bearing family pattern in the United State and some European countries,[8] who prefer to reserve their oocytes or embryos for nonmedical purposes, which is referred as "social freezing".[119] Although social freezing is legitimate in these countries, it is still a debated issue for many medical, ethical, and social reasons, such as decreasing pregnancy and increasing obstetric complications, questionable long-term effects of cryopreservation, controversial offspring quality from cryopreserved gametes, and the changing of family patterns. Further studies on the new problems after social freezing are urgently needed.

## Conclusions

Studies provided reassuring data on safety and efficacy of oocyte and embryo cryopreservations, and both embryo and oocyte vitrifications are excellent options for female patients to reserve reproductive fertility. Nonetheless, the risks of oocyte and embryo vitrifications are still retained, particularly from the unclear potential cytotoxic effects of ice crystals and the cryoprotectants, as well as whether oocytes and embryos are ready for long-term storage. With more cryopreservation applications, more problems will be raised, including its unresolved inherent medical risks and emerging social risks concerning the health and outcomes of freezing children and the changing of family patterns. In the future, large-scale studies should be executed to optimize the freeze-thaw protocol in order to reduce the clinical risks and social side-effects.

## References


1. Szafarowska M, Jerzak M. Ovarian aging and infertility. Ginekol Pol 2013;84:298-304.
2. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, *et al.* Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500-10.
3. Petríková J, Lazúrová I. Ovarian failure and polycystic ovary syndrome. Autoimmun Rev 2012;11:A471-8.
4. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.
5. Letourneau JM, Niemasik EE, McCulloch CE, Rugo HS, Katz PP, Cedars MI, *et al*. Temporary amenorrhea predicts future infertility in young women treated with chemotherapy. J Cancer Ther Res 2013;2:16.
6. Reinmuth S, Hohmann C, Rendtorff R, Balcerek M, Holzhausen S, Müller A, *et al.* Impact of chemotherapy and radiotherapy in childhood on fertility in adulthood: the FeCt-survey of childhood cancer survivors in Germany. J Cancer Res Clin Oncol 2013;139:2071-8.
7. Jadoul P, Donnez J. How does bone marrow transplantation affect ovarian function and fertility? Curr Opin Obstet Gynecol 2012;24:164-71.
8. Johnson J, Tough S. Delayed child-bearing. J Obstet Gynecol 2012;34:80-93.
9. Practice Committees of American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril 2013;99:37-43.
10. Husseinzadeh N, Husseinzadeh HD. Preservation of fertility in female cancer patients desiring future child bearing; what is available and what can be offered. World J Oncol 2013;4:1-7.
11. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature 1983;305:707-9.
12. Chen C. Pregnancy after human oocyte cryopreservation. Lancet 1986;1:884-6.
13. Cobo A, Domingo J, Pérez S, Crespo J, Remohí J, Pellicer A. Vitrification: an effective new approach to oocyte banking and preserving fertility in cancer patients. Clin Transl Oncol 2008;10:268-73.
14. Schattman G, Battaglia D, Damario M, Paulson R, Stovall D, Vernon M, *et al*. Guidelines for development of an emergency plan for *in vitro* fertilization programs. Fertil Steril 2008;89:793-5.
15. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil Steril 2006;86:70-80.
16. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, *et al.* Fresh embryo transfer versus frozen embryo transfer in *in vitro* fertilization cycles: a systematic review and meta-analysis. Fertil Steril 2013;99:156-62.
17. Aflatoonian A, Oskouian H, Ahmadi S, Oskouian L. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. J Assist Reprod Genet 2010;27:357-63.
18. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for *in vitro* fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders. Fertil Steril 2011;96:516-8.
19. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for *in vitro* fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril 2011;96:344-8.
20. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting

from the transfer of frozen thawed versus fresh embryos generated through *in vitro* fertilization treatment: a systematic review and meta-analysis. Fertil Steril 2012;98:368-77.e1.
21. Aflatoonian A, Mansoori Moghaddam F, Mashayekhy M, Mohamadian F. Comparison of early pregnancy and neonatal outcomes after frozen and fresh embryo transfer in ART cycles. J Assist Reprod Genet 2010;27:695-700.
22. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, *et al.* Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod 2008;23:1644-53.
23. Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish national cohort study 1995-2006. Fertil Steril 2010;94:1320-7.
24. Wada I, Macnamee MC, Wick K, Bradfield JM, Brinsden PR. Birth characteristics and perinatal outcome of babies conceived from cryopreserved embryos. Hum Reprod 1994;9:543-6.
25. Belva F, Henriet S, Van den Abbeel E, Camus M, Devroey P, Van der Elst J, *et al.* Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. Hum Reprod 2008;23:2227-38.
26. Wennerholm UB, Hamberger L, Nilsson L, Wennergren M, Wikland M, Bergh C. Obstetric and perinatal outcome of children conceived from cryopreserved embryos. Hum Reprod 1997;12:1819-25.
27. Wikland M, Hardarson T, Hillensjö T, Westin C, Westlander G, Wood M, *et al.* Obstetric outcomes after transfer of vitrified blastocysts. Hum Reprod 2010;25:1699-707.
28. Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hydén-Granskog C, *et al.* Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995-2006. Hum Reprod 2010;25:914-23.
29. Henningsen AK, Pinborg A, Lidegaard Ø, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. Fertil Steril 2011;95:959-63.
30. Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. Fertil Steril 2005;83:1650-8.
31. Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, *et al.* Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria Australia. Hum Reprod 2010;25:265-74.
32. Cobo A, Diaz C. Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril 2011;96:277-85.
33. Cobo A, Kuwayama M, Pérez S, Ruiz A, Pellicer A, Remohí J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril 2008;89:1657-64.
34. Cao YX, Xing Q, Li L, Cong L, Zhang ZG, Wei ZL, *et al.* Comparison of survival and embryonic development in human oocytes cryopreserved by slow-freezing and vitrification. Fertil Steril 2009;92:1306-11.
35. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, *et al.* Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod 2010;25:66-73.
36. Smith GD, Serafini PC, Fioravanti J, Yadid I, Coslovsky M, Hassun P, *et al*. Prospective randomized comparison of human oocyte cryopreservation with slow-rate freezing or vitrification. Fertil Steril 2010;94:2088-95.
37. Cobo A, Meseguer M, Remohí J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. Hum Reprod 2010;25:2239-46.
38. Noyes N, Porcu E, Borini A. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. Reprod Biomed Online 2009;18:769-76.
39. Gosden R. Cryopreservation: a cold look at technology for fertility preservation. Fertil Steril 2011;96:264-8.
40. Gosden RG. Memoir of fertility preservation. Adv Exp Med Biol 2013;761:85-94.
41. Anckaert E, De Rycke M, Smitz J. Culture of oocytes and risk of imprinting defects. Hum Reprod Update 2013;19:52-66.
42. Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, Yoon TK. Pregnancy after *in vitro* fertilization of human follicular oocytes collected from nonstimulated cycles, their culture *in vitro* and their transfer in a donor oocyte program. Fertil Steril 1991;55:109-13.
43. Cha KY, Do BR, Chi HJ, Yoon TK, Choi DH, Koo JJ, *et al*. Viability of human follicular oocytes collected from unstimulated ovaries and matured and fertilized *in vitro*. Reprod Fertil Dev 1992;4:695-701.
44. Demirtas E, Elizur SE, Holzer H, Gidoni Y, Son WY, Chian RC, *et al.* Immature oocyte retrieval in the luteal phase to preserve fertility in cancer patients. Reprod Biomed Online 2008;17:520-3.
45. Huang JY, Tulandi T, Holzer H, Tan SL, Chian RC. Combining ovarian tissue cryobanking with retrieval of immature oocytes followed by *in vitro* maturation and vitrification: an additional strategy of fertility preservation. Fertil Steril 2008;89:567-72.
46. Huang JY, Chian RC, Gilbert L, Fleiszer D, Holzer H, Dermitas E, *et al.* Retrieval of immature oocytes from unstimulated ovaries followed by *in vitro* maturation and vitrification: a novel strategy of fertility preservation for breast cancer patients. Am J Surg 2010;200:177-83.
47. Yang D, Brown SE, Nguyen K, Reddy V, Brubaker C, Winslow KL. Live birth after the transfer of human embryos developed from cryopreserved oocytes harvested before cancer treatment. Fertil Steril 2007;87:1469.e1-4.
48. Park SE, Son WY, Lee SH, Lee KA, Ko JJ, Cha KY. Chromosome and spindle configurations of human oocytes matured *in vitro* after cryopreservation at the germinal vesicle stage. Fertil Steril 1997;68:920-6.
49. Lee JA, Barritt J, Moschini RM, Slifkin RE, Copperman AB. Optimizing human oocyte cryopreservation for fertility preservation patients: should we mature then freeze or freeze then mature? Fertil Steril 2013;99:1356-62.
50. Yazdanpanah F, Khalili MA, Eftekhar M, Karimi H. The effect of vitrification on maturation and viability capacities of immature human oocytes. Arch Gynecol Obstet 2013;288:439-44.
51. Versieren K, Heindryckx B, O'Leary T, De Croo I, Van den Abbeel E, Gerris J, *et al.* Slow controlled-rate freezing of human *in vitro* matured oocytes: effects on maturation rate and kinetics and parthenogenetic activation. Fertil Steril 2011;96:624-8.
52. Wang H, Racowsky C, Combelles CM. Is it best to cryopreserve human cumulus-free immature oocytes before or after *in vitro* maturation? Cryobiology 2012;65:79-87.
53. Shahedi A, Hosseini A, Khalili MA, Norouzian M, Salehi M, Piriaei A, *et al.* The effect of vitrification on ultrastructure of human *in vitro* matured germinal vesicle oocytes. Eur J Obstet Gynecol Reprod Biol 2013;167:69-75.
54. Boiso I, Martí M, Santaló J, Ponsá M, Barri PN, Veiga A. A confocal microscopy analysis of the spindle and chromosome configurations of human oocytes cryopreserved at the germinal vesicle and metaphase II stage. Hum Reprod 2002;17:1885-91.
55. Fasano G, Demeestere I, Englert Y. *In-vitro* maturation of human oocytes: Before or after vitrification? J Assist Reprod Genet 2012;29:507-12.
56. Arav A, Natan Y. Vitrification of oocytes: from basic science to clinical application. Adv Exp Med Biol 2013;761:69-83.
57. Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, *et al.* Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil Steril 2008;90:186-93.
58. AbdelHafez FF, Desai N, Abou-Setta AM, Falcone T, Goldfarb J. Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. Reprod Biomed Online 2010;20:209-22.
59. Van den Abbeel E, Camus M, Van Waesberghe L, Devroey P, Van Steirteghem AC. A randomized comparison of the cryopreservation

of one-cell human embryos with a slow controlled-rate cooling procedure or a rapid cooling procedure by direct plunging into liquid nitrogen. Hum Reprod 1997;12:1554-60.
60. Mauri AL, Petersen CG, Baruffi RL, Ferreira RC, Franco JG Jr. Comparison of the cryopreservation of human embryos obtained after intracytoplasmic sperm injection with a slow cooling or an ultrarapid cooling procedure. J Assist Reprod Genet 2001;18:257-61.
61. Bernal DP, Chang CC, Colturato LF, Leef DM, Kort HI, Nagy ZP. Evaluation of blastocyst recuperation, implantation and pregnancy rates after vitrification/warming or slow freezing/thawing cycles. Fertil Steril 2008;90:S277-8.
62. Kim S, Lee J. Study on the vitrification of human blastocysts. II: effect of vitrification on the implantation and the pregnancy of human blastocysts. Korean J Fertil Steril 2000;27:67-74.
63. Rama Raju GA, Haranath GB, Krishna KM, Prakash GJ, Madan K. Vitrification of human 8-cell embryos, a modified protocol for better pregnancy rates. Reprod Biomed Online 2005;11:434-7.
64. Li Y, Chen ZJ, Yang HJ, Zhong WX, Ma SY, Li M. Comparison of vitrification and slow-freezing of human day 3 cleavage stage embryos: post-vitrification development and pregnancy outcomes. Chin J Obstet Gynecol 2007;42:753-5.
65. Kuwayama M, Vajta G, Ieda S, Kato O. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. Reprod Biomed Online 2005;11:608-14.
66. Zheng WT, Zhuang GL, Zhou CQ, Fang C, Ou JP, Li T, *et al.* Comparison of the survival of human biopsied embryos after cryopreservation with four different methods using non-transferable embryos. Hum Reprod 2005;20:1615-8.
67. Huang CC, Lee TH, Chen SU, Chen HH, Cheng TC, Liu CH, *et al.* Successful pregnancy following blastocyst cryopreservation using super-cooling ultra-rapid vitrification. Hum Reprod 2005;20:122-8.
68. Sarajari S, Manipalviram S, Surrey MW, Danzer HC, Briton-Jones C, Hill DL. Post-thaw survival and resumption of meiosis of immature oocytes using slow-freeze vs. vitrification methods of cryopreservation. Fertil Steril 2007;881:S354-5.
69. Zhang Z, Liu Y, Xing Q, Zhou P, Cao Y. Cryopreservation of human failed-matured oocytes followed by *in vitro* maturation: vitrification is superior to the slow freezing method. Reprod Biol Endocrinol 2011;9:156.
70. Combelles CM, Ceyhan ST, Wang H, Racowsky C. Maturation outcomes are improved following Cryoleaf vitrification of immature human oocytes when compared to choline-based slow-freezing. J Assist Reprod Genet 2011;28:1183-92.
71. Clark NA, Swain JE. Oocyte cryopreservation: Searching for novel improvement strategies. J Assist Reprod Genet 2013;30:865-75.
72. Friedler S, Giudice LC, Lamb EJ. Cryopreservation of embryos and ova. Fertil Steril 1988;49:743-64.
73. Bianchi V, Coticchio G, Fava L, Flamigni C, Borini A. Meiotic spindle imaging in human oocytes frozen with a slow freezing procedure involving high sucrose concentration. Hum Reprod 2005;20:1078-83.
74. Bromfield JJ, Coticchio G, Hutt K, Sciajno R, Borini A, Albertini DF. Meiotic spindle dynamics in human oocytes following slow-cooling cryopreservation. Hum Reprod 2009;24:2114-23.
75. Cobo A, Pérez S, De los Santos MJ, Zulategui J, Domingo J, Remohí J. Effect of different cryopreservation protocols on the metaphase II spindle in human oocytes. Reprod Biomed Online 2008;17:350-9.
76. Rienzi L, Martinez F, Ubaldi F, Minasi MG, Iacobelli M, Tesarik J, *et al.* Polscope analysis of meiotic spindle changes in living metaphase II human oocytes during the freezing and thawing procedures. Hum Reprod 2004;19:655-9.
77. Ciotti PM, Porcu E, Notarangelo L, Magrini O, Bazzocchi A, Venturoli S. Meiotic spindle recovery is faster in vitrification of human oocytes compared to slow freezing. Fertil Steril 2009;91:2399-407.
78. Smith GD, Silva E Silva CA. Developmental consequences of cryopreservation of mammalian oocytes and embryos. Reprod Biomed Online 2004;9:171-8.
79. Borini A, Bianchi V. Cryopreservation of mature and immature oocytes. Clin Obstet Gynecol 2010;53:763-74.
80. Gualtieri R, Iaccarino M, Mollo V, Prisco M, Iaccarino S, Talevi R. Slow cooling of human oocytes: ultrastructural injuries and apoptotic status. Fertil Steril 2009;91:1023-34.
81. Martínez-Burgos M, Herrero L, Megías D, Salvanes R, Montoya MC, Cobo AC, *et al.* Vitrification versus slow freezing of oocytes: effects on morphologic appearance, meiotic spindle configuration, and DNA damage. Fertil Steril 2011;95:374-7.
82. Forman EJ, Li X, Ferry KM, Scott K, Treff NR, Scott RT Jr. Oocyte vitrification does not increase the risk of embryonic aneuploidy or diminish the implantation potential of blastocysts created after intracytoplasmic sperm injection: a novel, paired randomized controlled trial using DNA fingerprinting. Fertil Steril 2012;98:644-9.
83. Al-Khtib M, Perret A, Khoueiry R, Ibala-Romdhane S, Blachère T, Greze C, *et al*. Vitrification at the germinal vesicle stage does not affect the methylation profile of H19 and KCNQ1OT1 imprinting centers in human oocytes subsequently matured *in vitro*. Fertil Steril 2011;95:1955-60.
84. Denomme MM, Mann MR. Genomic imprints as a model for the analysis of epigenetic stability during assisted reproductive technologies. Reproduction 2012;144:393-409.
85. El Hajj N, Haaf T. Epigenetic disturbances in *in vitro* cultured gametes and embryos: implications for human assisted reproduction. Fertil Steril 2013;99:632-41.
86. Chen SU, Yang YS. Slow freezing or vitrification of oocytes: their effects on survival and meiotic spindles, and the time schedule for clinical practice. Taiwan J Obstet Gynecol 2009;48:15-22.
87. Poirot CJ, Martelli H, Genestie C, Golmard JL, Valteau-Couanet D, Helardot P, *et al.* Feasibility of ovarian tissue cryopreservation for prepubertal females with cancer. Pediatr Blood Cancer 2007;49:74-8.
88. Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol 2013;9:735-49.
89. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, *et al.* American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 2006;24:2917-31.
90. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Sanchez Serrano M, Schmidt KT, *et al.* Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. Fertil Steril 2013;99:1503-13.
91. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. N Engl J Med 2000;342:1919.
92. Bedaiwy MA, El-Nashar SA, El Saman AM, Evers JL, Sandadi S, Desai N, *et al.* Reproductive outcome after transplantation of ovarian tissue: a systematic review. Hum Reprod 2008;23:2709-17.
93. Silber SJ, Gosden RG. Ovarian transplantation in a series of monozygotic twins discordant for ovarian failure. N Engl J Med 2007;356:1382-4.
94. Leporrier M, von Theobald P, Roffe JL, Muller G. A new technique to protect ovarian function before pelvic irradiation. Heterotopic ovarian autotransplantation. Cancer 1987;60:2201-4.
95. Oktay K, Aydin BA, Karlikaya G. A technique for laparoscopic transplantation of frozen-banked ovarian tissue. Fertil Steril 2001;75:1212-6.
96. Oktay K, Buyuk E, Rosenwaks Z, Rucinski J. A technique for transplantation of ovarian cortical strips to the forearm. Fertil Steril 2003;80:193-8.
97. Marconi G, Quintana R, Rueda-Leverone NG, Vighi S. Accidental ovarian autograft after a laparoscopic surgery: case report. Fertil Steril 1997;68:364-6.
98. Wølner-Hanssen P, Hägglund L, Ploman F, Ramirez A, Manthorpe R, Thuring A. Autotransplantation of cryopreserved ovarian tissue to the right forearm 4 (1/2) years after autologous stem cell transplantation. Acta Obstet Gynecol Scand 2005;84:695-8.
99. Rosendahl M, Loft A, Byskov AG, Ziebe S, Schmidt KT, Andersen AN, *et al.* Biochemical pregnancy after fertilization of an oocyte aspirated from a heterotopic autotransplant of cryopreserved ovarian tissue: case report. Hum Reprod 2006;21:2006-9.
100. Oktay K, Buyuk E, Veeck L, Zaninovic N, Xu K, Takeuchi T, *et al.* Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;363:837-40.
101. Oktay K, Economos K, Kan M, Rucinski J, Veeck L, Rosenwaks Z. Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm. JAMA 2001;286:1490-3.
102. Schmidt KL, Andersen CY, Loft A, Byskov AG, Ernst E,

Andersen AN. Follow-up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation. Hum Reprod 2005;20:3539-46.
103. Kiran G, Kiran H, Coban YK, Guven AM, Yuksel M. Fresh autologous transplantation of ovarian cortical strips to the anterior abdominal wall at the pfannenstiel incision site. Fertil Steril 2004;82:954-6.
104. Kim SS, Hwang IT, Lee HC. Heterotopic autotransplantation of cryobanked human ovarian tissue as a strategy to restore ovarian function. Fertil Steril 2004;82:930-2.
105. Von Theobald P, Roffé JL, Berrocal J, Le Porrier M, Lévy G, Muller G. Heterotopic autotransplantation of the ovary in women. Presse Med 1987;16:1239-41.
106. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, *et al.* Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;364:1405-10.
107. Tryde Schmidt KL, Yding Andersen C, Starup J, Loft A, Byskov AG, Nyboe Andersen A. Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer-follicular growth, steroid production and oocyte retrieval. Reprod Biomed Online 2004;8:448-53.
108. Radford JA, Lieberman BA, Brison DR, Smith AR, Critchlow JD, Russell SA, *et al.* Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. Lancet 2001;357:1172-5.
109. Demeestere I, Simon P, Buxant F, Robin V, Fernandez SA, Centner J, *et al*. Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: case report. Hum Reprod 2006;21:2010-4.
110. Mhatre P, Mhatre J, Magotra R. Ovarian transplant: a new frontier. Transplant Proc 2005;37:1396-8.
111. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, *et al.* Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005;353:318-21.
112. Oktay K. Spontaneous conceptions and live birth after heterotopic ovarian transplantation: Is there a germline stem cell connection? Hum Reprod 2006;21:1345-8.
113. Hilders CG, Baranski AG, Peters L, Ramkhelawan A, Trimbos JB. Successful human ovarian autotransplantation to the upper arm. Cancer 2004;101:2771-8.
114. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, *et al*. Restoration of ovarian function after orthotopic (intraovarian and periovarian) transplantation of cryopreserved ovarian tissue in a woman treated by bone marrow transplantation for sickle cell anaemia: case report. Hum Reprod 2006;21:183-8.
115. Donnez J, Squifflet J, Dolmans MM, Martinez-Madrid B, Jadoul P, Van Langendonckt A. Orthotopic transplantation of fresh ovarian cortex: a report of two cases. Fertil Steril 2005;84:1018.
116. Sánchez M, Alamá P, Gadea B, Soares SR, Simón C, Pellicer A. Fresh human orthotopic ovarian cortex transplantation: long-term results. Hum Reprod 2007;22:786-91.
117. Callejo J, Salvador C, Miralles A, Vilaseca S, Lailla JM, Balasch J. Long-term ovarian function evaluation after autografting by implantation with fresh and frozen-thawed human ovarian tissue. J Clin Endocrinol Metab 2001;86:4489-94.
118. Porcu E, Bazzocchi A, Notarangelo L, Paradisi R, Landolfo C, Venturoli S. Human oocyte cryopreservation in infertility and oncology. Curr Opin Endocrinol Diabetes Obes 2008;15:529-35.
119. Pennings G. Ethical aspects of social freezing. Gynecol Obstet Fertil 2013;41:521-3.

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