Review Article

Potential Use of Immature Oocyte to Improve Fertility Preservation Outcome: A Narrative Review

Batara Sirait1,2,3, Ahmad Aulia Jusuf4, Budi Wiweko1,6,7, Ninings Handayani1,8, Daniel Abidin Aubry1, R. Muharam6,7

Fertility preservation through gamete vitrification has become one of the critical strategies to secure a childbearing potential in patients who are diagnosed with cancer or risks of infertility. Preserving the gametes would prevent the deleterious effects of cancer drugs or radiotherapy exposure on the quality of the gametes. Furthermore, in vitro fertilisation of vitrified mature human oocytes has lately demonstrated promising results that are reflected in the increased survival rate of thawed oocytes and the resultant clinical pregnancy rate. However, limitations in the cryopreservation of mature oocytes of cancer patients persist. Ovarian stimulation protocols which comprise administering gonadotrophin-releasing hormones could aggravate cancer or delay essential cancer therapy. Considering such circumstances, vitrification of immature oocytes would become a rational option. While the vitrification procedure of mature oocytes has been established, the vitrification of immature oocytes remains controversial due to a low post-thaw in vitro maturation and fertilisation rate. Apparent cryoinjuries to the immature oocytes post thawing or warming have been observed in both human and animal model oocytes. An alternative strategy was therefore proposed to improve the effectiveness of utilising immature oocytes for fertility preservation by conducting the in vitro oocyte maturation process first before vitrification. This method has prevailed, especially in oncofertility patients. Although the success rate of the clinical outcomes remains low, this approach, in conjunction with proper counselling, might provide oncofertility patients with an opportunity to preserve their reproductive potential.

Keywords: Cancer-related infertility, fertility preservation, immature oocytes, in vitro fertilisation, vitrification

INTRODUCTION

The first successful births of twin human babies from fertilisation of frozen-thawed pre-ovulatory mature oocyte were reported in Australia in 1986.[1] The strategy of utilising vitrified mature oocytes in an in vitro fertilisation (IVF) programme, however, has endured a slow acceptance due to the low survival rate of the oocytes post thawing. Experimental research on oocyte cryopreservation indicates the difficulties of vitrifying a large single oocyte cell.[2] The urgency to improve the success rate of oocyte cryopreservation has risen in several countries such as Italy, Austria, Germany and Switzerland due to legislation that restricts embryo cryopreservation.[3] A significant improvement in cancer prognosis after treatments in young adolescent patients has also validated the demand for an oocyte cryopreservation programme as means to preserve the reproductive potential of girls or women who are about

Address for correspondence: Dr. Batara Sirait, Department of Obstetrics and Gynaecology, Faculty of Medicine Universitas Kristen Indonesia, Mayjen Sutoyo Street Number 2, RT.9/RW.6, Cawang, Kramat Jati, East Jakarta, 13630, Indonesia. E-mail: batarasirait@gmail.com

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to undergo cancer therapies.

Oocyte freezing as an alternative method to treat infertility was first approved in the UK by the Human Fertilisation and Embryology Authority in 2000[6] followed by the American Society for Reproductive Medicine in 2013.[7]

Vitrification is preferably performed when the oocyte is at a mature stage, namely metaphase II (MII). To promote in vivo maturation of oocytes, ovarian stimulation begins at day 2 or 3 of a menstrual cycle during the follicular phase. Exogenous gonadotropin hormone is administered once daily to support follicular growth until a minimum of two or three follicles has reached 18 mm. Oocyte maturation trigger utilising gonadotropin-releasing hormone agonist or human chorionic gonadotropin is then injected and the ovum pick-up procedure is commenced 36 h later.[8] Nonetheless, the gonadotropin stimulation approach is unsuitable for certain patients with underlying conditions such as cancer. Women with breast cancer, for instance, are particularly sensitive to the elevation of serum oestradiol (oestrogen-sensitive tumour) and are therefore advised to avoid undergoing ovarian stimulation using the gonadotropin hormone.[9]

In addition to the risks of cancer aggravation, the time required for ovarian stimulation procedure could delay crucial cancer treatments. Investigating the potential use of immature oocytes as an alternative option for fertility preservation therefore becomes admissible. Contrary to the collection of in vivo matured oocytes, immature oocytes can be retrieved conveniently at any stage of the ovarian cycle.[10] More importantly, the necessity of the ovarian stimulation protocol could be bypassed. A case report published in 2012 demonstrated the convenient method of retrieving immature oocytes from antral follicles during a conservative surgery for ovarian cancer, indicating the feasibility and development prospects of such strategy.[11]

Several investigations were carried out to establish an optimal fertility preservation strategy using immature oocytes that could assure the chance of bearing a child in a specific group of cancer patients.[12-14] In the earlier decade of oocyte cryopreservation history, a low survival rate of cryopreserved mature oocyte post thawing has led to a postulation of a higher cryoinjury in resistance in germinal vesicle (GV) immature oocyte compared to the MII mature oocyte. At that point, GV was believed to be the ideal stage for vitrification rather than MII because of its lack of a microtubular spindle system. Moreover, chromosomes in GV are enclosed by a nuclear membrane, which was considered to reduce the risk of chromosome injuries and prevent the polyploidy or aneuploidy occurrence possibly induced by the extreme cooling condition during vitrification.[15] However, increasing evidence has suggested that the GV-stage oocytes are as vulnerable to cryoinjuries as the mature-stage oocytes.[16-19] Some studies have subsequently recommended performing an in vitro maturation (IVM) of the immature oocytes before vitrification,[20-24] while others have opposed this idea.[25-27] This literature provides a comprehensive review on the safety and current progress of vitrifying immature human oocytes for possible use of fertility preservation.

**METHODS**

The literature review was conducted using several search engines including Google Scholar and PubMed. Boolean search strategy (AND, OR, NOT) has been applied to identify the relevant articles using terms such as oocyte, egg, vitrification, freezing, slow freezing, cryopreservation, immature and fertility preservation. Keyword phrases included in the search were ‘immature egg freezing’, ‘immature oocyte vitrification’ and ‘immature oocyte freezing’ [Figure 1].

**DISCUSSION**

The clinical indications for women who could benefit from fertility cryopreservation using immature oocytes

Several factors including the cancer type and grade, urgency of the cancer treatment and marital status should be taken into consideration when opting for the fertility preservation programme. The clinical algorithm for female cancer patients aiming to retain their reproductive potential has been well defined.[14,9,28] As previously described, gonadotropin ovarian stimulation may be contraindicated in certain cancer patients with hormonal-sensitive tumours such as desmoplastic tumours and breast cancer (especially in oestrogen receptor-positive type and breast adenocarcinoma).[13,29] Administering exogenous gonadotropin during ovarian stimulation could induce an increased oestradiol level up to 15 times more than the natural cycle.[30] Thus, oncologists may advise the patients to undergo oocyte retrieval without ovarian stimulation.[12,13] Likewise, women who cannot delay their cancer treatments for a 2- to 6-week stimulation protocol could benefit from the retrieval and cryopreservation of immature oocytes.[9]

Clinical evidence on the benefits of preserving fertility using immature oocytes was proven in several studies.[10,12-14,29] A 2010 study demonstrated a novel approach of collecting immature oocytes without ovarian stimulation in 38 oncofertility women diagnosed with breast cancer. The oocyte retrieval was performed under sedation 36 h following a 10,000 IU HCG injection.
GV-stage oocytes were subsequently matured in vitro through a 24-h culture and the resultant mature oocytes were used for further treatment. The yield of the in vitro mature oocytes ranged between 1 and 22 and the median vitrified embryos was 4 with a range of 1–13. Of the 38 women, 18 opted for the IVM process followed by the oocyte vitrification while the remaining agreed to fertilise their mature oocytes and vitrify the resultant embryos. An increased number of in vitro matured oocytes for vitrification was reported in two oncofertility women through a combination of immature oocyte aspiration at both the follicular and the luteal phase. Moreover, evident benefits of retrieving immature oocytes at the luteal phase were demonstrated in a woman aged 21 who was incapable of suspending her cancer treatment and was advised against receiving the gonadotropin therapy. Supporting the previous results, a prospective study on 248 breast cancer women has proven a similar IVM rate of immature oocytes aspirated at either the follicular or the luteal phase. The increasing yield of mature oocytes post IVM has certainly generated an interest in the practice of such strategy as an urgent approach for fertility preservation.

Another plausible method of fertility preservation is through immature oocytes aspiration from excised ovarian tissue combining with an ovarian tissue cryo-banking. This strategy was applied in four women who were diagnosed with Hodgkin lymphoma, breast cancer and rectal cancer. Immature oocytes were collected from the excised ovarian tissue and were subjected to the IVM culture. The mean oocyte maturation rate post IVM was 79%. All of the oncofertility patients managed to acquire at least one mature oocyte for vitrification. Furthermore, a large retrospective cohort study in 2015 comprising 255 cancer patients has validated the safety and advantages of harvesting the ovarian tissue for immature oocyte collection as means to attain an increased total number of in vitro matured oocytes and fertilisation rate.

As the insemination of the in vitro matured oocytes was decided based on the marital status, patient preference or...
age, most studies on fertility preservation using immature oocytes have heretofore reported the yield of the in vitro matured oocytes as the main outcome.[10,12-14,29] Only few studies managed to describe the downstream IVF outcomes including the total number of embryos derived from the in vitro matured oocytes.[13,14] Hourvitz et al. reported a mean number of vitrified embryos between 1.67 ± 0.56 and 3.39 ± 0.73 depending on the immature oocyte collection procedure.[14] Although the results were encouraging, clear benefits of utilising immature oocytes to obtain embryos for further treatment are difficult to define due to the small sample sizes of the available reports. Therefore, detailed information regarding the current success rate of fertility preservation should be informed to the patients who wish to undergo the program.

Immature oocyte collection in IVF could also benefit women with polycystic ovary syndrome who are at risk of an ovarian hyperstimulation syndrome subsequent to ovarian stimulation.[31] The fertility preservation programme would also cater to the increasingly modern trend of postponing childbearing due to social or non-medical reasons. A study in the UK provided several background and clinical characteristics of women who underwent fertility cryopreservation. The mean age of the 27 women involved in the study was 36.7 years. They were highly educated, and half of the participants were professionally employed.[32]

**Competency of in vitro matured germinal vesicle or post germinal vesicle breakdown metaphase I oocytes: A lesson from a stimulated fresh in vitro fertilisation cycles**

Clinical use of immature oocytes obtained during a stimulated fresh IVF cycle is disputable even without the vitrification processing. In vitro matured GV and MI oocytes lack the competency to improve the clinical pregnancy.[33-35] Although a similar fertilisation rate was observed between the in vivo and in vitro matured oocytes, Shu et al. concluded that the clinical pregnancy and live birth rate of transferring embryos derived from the in vitro matured oocytes were unsatisfactory.[33] A 2010 study has also shown the inconspicuous effectiveness of using immature oocytes derived from the stimulated IVF cycles. Two hundred and sixty-three immature oocytes subjected to IVM were compared with their sibling in vivo matured oocytes (n = 234). Although both groups acquired comparable fertilisation rates, the developmental quality of the day 2 cleavage stage in the immature oocyte group was lower in regard to the blastomeric number and symmetry. Moreover, none of the 17 transferred embryos derived from the in vitro matured oocytes were successfully implanted.[34] Another study also observed a low clinical efficacy of the in vitro matured oocytes which did not culminate to a single clinical pregnancy in the five cases of embryo transfer.[35]

Nonetheless, a promising utilisation of in vitro matured non-GV or germinal vesicle breakdown (GVBD) has recently been reported by Olid et al.[36] IVM of GVBD oocytes in G-2™ PLUS media (Vitrolife, Sweden) resulted in a 10% clinical pregnancy rate and a 5.6% live birth rate. Concordantly, an evidence on the clinical benefit of in vitro matured MI-stage oocyte was recorded through one successful live birth of a healthy normal baby.[37]

An IVF using in vitro matured oocytes is undeniably less favourable than the in vivo matured oocytes. However, performing IVM of non-GV immature oocytes is another option, particularly in cycles which yield a low number of mature oocytes. The possible reasons underlying the low developmental competence of in vitro matured non-GV stages were implied by Ferrer-Vaquer et al.[38] This study highlighted the several differences in the cytoplasmic maturation between in vivo and in vitro matured oocytes. Alterations in the mitochondrial membrane potential, cluster number of endoplasmic reticulum and actin cytoskeleton were particularly observed in in vitro matured oocytes.

**Should in vitro maturation be performed before or after vitrification?: A lesson from the use of immature oocytes in in vitro fertilisation to retain reproductive potential**

Following the successful birth of a human female baby derived from a frozen-thawed in vitro matured GV immature oocyte in 1998 Georgia – America, cryopreservation of immature oocytes has been proposed as an alternative strategy to preserve the gametes.[39] Over the past two decades, numerous studies have revealed the challenges of immature oocyte vitrification. Several limitations of the approach are well discussed in an existing review as follows: (i) even though the survival rates of the frozen-thawed mature and immature oocyte are similar, the capacity of the surviving immature oocytes to undergo maturation in vitro is considerably low; (ii) fertilisation capacity of in vitro matured oocytes is also lower compared to the in vivo matured oocytes; and (iii) vitrification has been shown to evoke injuries of the chromosomes and cytoplasmic organelles even in the GV stage, disclaiming the theories that were previously suggested. The irreversible injuries of the organelles could be accountable for the lack of maturation and fertilisation capacity of the vitrified immature oocytes.[16,19] However, several contradictory
studies have proved that the outcomes of vitrifying oocytes at immature stage were not inferior to that of mature oocytes.\cite{25-27}

As presented in Table 1, most studies recommended performing IVM before vitrification to improve the clinical utilisation of the immature oocytes. Nonetheless, three studies suggested otherwise, proving that vitrifying oocytes before IVM or after were equally efficient. An in-depth evaluation is therefore still required to identify the factors which led to the different outcomes and to confidently determine whether vitrification of immature oocytes should be done before or after IVM. The current methodology for IVM is not sufficient enough to induce oocytes with a viability that is on par with the in vivo matured oocytes. Advancements in the IVM process might become an essential key to enhance the quality of in vitro matured oocytes and a stepping stone towards improvement in the fertility preservation programme.\cite{18}

A standard procedure for IVM is to culture the immature oocytes in media supplemented with human serum albumin and highly purified gonadotrophins for up to 30 h until a polar body extrusion is observed.\cite{40} Of note, the collection of immature oocytes from small antral follicles may slightly be more time – and technically – demanding than the collection of mature oocytes. A cell strainer with minute pores might be utilised to search for immature oocytes with minimal cumulus complex.\cite{40} No particular commercialised brand of media has been deemed more effective in promoting IVM.

Advancements for IVM of oocytes could only be achieved with a clear insight into the in vivo maturation process. The mechanism involves a series of complex signalling pathways that are regulated by specific factors expressed through different cells found within the cumulus-oocyte complex. These cells are connected by gap junctions and connexins that need to be maintained in vitro to preserve the intra- and intercellular communications. In the pursuit of mimicking an in vitro condition which complements the in vivo micro-environments, several pre-maturation enhancements of IVM have been attempted. Vanhoutte et al.\cite{41} designed a three-dimensional (3D) culture system utilising an extracellular matrix composed of collagen. Retrieved immature oocytes with intact cumulus complex were cultured on polymerised collagen supplied with IVM media and phosphodiesterase-3 inhibitor (meiotic arrester). Subsequently, the oocytes were recovered from the gel, washed and denuded for fertilisation through conventional IVF. The 3D culture managed to maintain the communication framework between cells found in the cumulus-oocyte complex that in turn brought on more meiotically competent oocytes and improved embryo development compared to the conventional IVM method.

In addition to the pre-maturation culture, several studies have shown an enhanced efficacy of IVM by fine-tuning the media with paracrine oocyte maturation-promoting factors such as FSH and amphiregulin\cite{42} and oocyte-secreted factors such as bone morphogenetic protein 15 and growth differentiation factor 9.\cite{43} Nonetheless, while the promising outcomes of these experiments could bridge the gap in the effectiveness between an IVM-IVF cycle and a conventional IVF cycle, large prospective studies are obligatory before these findings could be translated to clinical practice. Additionally, methods to reduce the cryoinjuries during vitrification should also be pursued to accomplish an altogether viable strategy for fertility preservation.

**The cellular and molecular causes of low fertility potential in immature oocytes after vitrification**

Several studies have revealed the consequences of immature oocyte vitrification in humans\cite{44,45} and animal models\cite{46-49} on a cellular and molecular level. In a 1988 study conducted by Van Blerkom et al., mouse GV vitrification was shown to cause premature chromosome condensation, extrusion of chromatin fragmentation in the cytoplasm, microtubule defects, impaired relocation of mitochondria and protein synthesis alteration.\cite{46} An experimental study that examined the post-vitrification cycle cell status and chromosomal integrity of GV-stage rat oocytes also pointed out similar findings. Following a warming procedure, the GV-stage oocytes immediately entered the M-phase cell cycle but failed to maintain chromosomal integrity due to multiple chromatin fusion. The study also highlighted a remarkably low number of F-actin in both the cytoplasm and the cortical area and an irregular retraction of trans-zona pellucida post vitrification.\cite{47} Several defects of the cytoskeleton, aberrant density of the mitochondrial matrix and microtubule were also observed in other animal studies.\cite{48,49}

Utilising transmission electron microscope, frozen-thawed GV oocytes were shown to prematurely release peripheral cortical granules onto the subzonal space during IVM. Additionally, the number of cortical granules was reduced remarkably causing an increased density of the zona pellucida ultrastructure and subsequent zona pellucida hardening. Aggregates of small mitochondria-smooth endoplasmic reticulum (M-SER) were found to be randomly distributed within the cytoplasm.\cite{44} Furthermore, detrimental effects of cryoprotectant exposure on the chromosomes and microtubules of immature human
Table 1: Summary table of studies evaluated the various outcomes of vitrified immature oocyte before or after in vitro maturation

| Study design       | Year | Main objective                                                                 | Sample size                                                                 | Main outcome                                                                                                                                  | Conclusion                                                                 | References |
|--------------------|------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|------------|
| In vitro experimental study | 2009 | To compare the efficacy of vitrification before and after IVM of immature oocytes | Immature human oocytes (n=472) Group 1 consisted of 219 GV that was vitrified before IVM Group 2 comprised 253 GV that was vitrified after IVM (n=79, while the remaining (n=99) were setting for control group) 184 immature (GV or MI) human oocytes were randomly allocated to Group 1 (vitrified after IVM, n=100) and Group 2 (vitrified before IVM, n=84) | A comparable survival rate post vitrification was observed between Group 1 and 2 (85.4% vs. 86.1%, respectively) However, the number of matured oocytes was significantly higher in Group 2 than in Group 1 A similar outcomes including fertilisation rate, cleavage and blastocyst rate were observed | IVM of GV-stage oocytes before vitrification yields better outcomes in terms of maturation rate | Cao et al.[20] |
| In vitro experimental study | 2012 | Comparing the effectiveness of IVM of immature oocytes before and after vitrification | Immature oocytes were obtained from 120 IVF patients GV-stage oocytes were allocated to freezing prior to IVM (n=109) and after IVM (n=107) | While the survival rates were similar between Group 1 and 2 (86.9% and 84.5%), the maturation rate was significantly higher in Group 1 than Group 2 (46% vs. 23.8%) Fertilisation rate and embryo development potency between the Group 1 and 2 were comparable However, no transferable blastocysts were obtained in both groups | A high number of in vitro matured oocytes survived the vitrification procedure because IVM was conducted before vitrification | Fasano et al.[21] |
| In vitro experimental study | 2012 | To investigate the best stage for vitrification of cumulus-free immature oocytes | Infertile human oocytes obtained from 120 IVF patients were chosen as control group (fIVM) and 50 immature oocytes were vitrified prior to IVM (vIVM) | A comparable survival rate between vitrified-GV before and after IVM was observed (69.7% vs. 70.5%, respectively) However, the low maturation rate (51.3% vs. 75.7%) and high spontaneous cleavage were observed in GV oocytes of vitrified before IVM | Vitrification of in vitro matured GV oocytes acquired more preferable outcomes than GV oocytes vitrified before IVM | Wang et al.[21] |
| Cross-sectional study | 2013 | Evaluating the maturation rate and viability of immature oocytes post warming | Infertile human oocytes A total of 53 fresh immature oocytes were subjected to fresh IVM as a control group (fIVM) and 50 immature oocytes were vitrified prior to IVM (vIVM) | Maturation rate of fIVM was superior over vIVM (88.7% vs. 56%, respectively, P<0.001) | Performing IVM prior to vitrification attained the maturation capacity of immature oocytes and the viability to further support embryonic development | Yazdanpanah et al.[24] |
| Experimental study | 2016 | Observing cryoinjuries on nuclear integrity such as spindle, DNA, as well as embryonic aneuploidy and ZPD after warming | Infertile human oocytes Thirty-one in vivo mature oocytes were used as control (Group A, with or without vitrification) Two hundred-twenty-six immature oocytes were allocated to Group B (IVM group before vitrification, n=113) and Group C (immature group, n=110) | Aneuploidy rate was comparable amongst groups (P<0.05). However, comet cells were significantly lower in Group A than Group B and C (P<0.05) Group C showed a lower occurrence and retardance value of spindle compared to Group A and B cleavage rate was also lower than that of Group A and B (P<0.05) | According to post warming molecular analysis, the most suitable stages for oocyte vitrification are at the in vivo mature and in vitro matured stages | Song et al.[22] |

Contd...
Oocyte vitrification is imperative to deliver the clinical benefits of immature oocyte vitrification for fertility preservation consequently embryos for cryopreservation. Ineffectiveness could increase the availability of mature oocytes and consequently embryos for cryopreservation. Ineffectiveness of immature oocyte vitrification for fertility preservation persists on account of the vitrification injuries observed in frozen-thawed oocytes of both the animal models and humans. Alternatively, performing IVM before vitrification would increase the survival and fertilisation rate of the immature oocytes. Developing methods that could significantly improve IVM and reduce cryoinjuries during vitrification is imperative to deliver the clinical benefits of immature oocyte cryopreservation as means to preserve fertility.

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**Conflicts of interest**
There are no conflicts of interest.

**REFERENCES**

1. Chen C. Pregnancy after human oocyte cryopreservation. Lancet 1986;1:884-6.
2. Argyle CE, Harper JC, Davies MC. Oocyte cryopreservation: Where are we now? Hum Reprod Update 2016;22:440-9.
3. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo and blastocyst cryopreservation in ART: Systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update 2017;23:139-55.
4. Kim SY, Lee JR. Fertility preservation option in young women with ovarian cancer. Future Oncol 2016;12:1695-8.
5. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011:...
The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin 2011;61:212-36.

6. Wise J. UK lifts ban on frozen eggs. BMJ 2000;320:334.

7. American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: A guideline. Fertil Steril 2013;99:37-43.

8. Cobo A, Garcia-Velasco JA, Coello A, Domingo J, Pellicer A, Remohi J. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril 2016;105:755-68.

9. Rodrigo-Wallberg KA, Oktay K. Options for fertility preservation in female cancer patients. Cancer Treat Rev 2012;38:354-61.

10. Grynegberg M, Poulain M, Le Parco S, Sifer C, Fanchin R, Frydman N. Similar in vitro maturation rates of oocytes retrieved during the follicular or luteal phase offer flexible options for urgent fertility preservation in breast cancer patients. Hum Reprod 2016;31:623-9.

11. Fadini R, Dal Canto M, Mignini Renzini M, Milani R, Fruscio R, Cantu MG, et al. Embryo transfer following in vitro maturation and cryopreservation of oocytes recovered from antral follicles during conservative surgery for ovarian cancer. J Assist Reprod Genet 2012;29:779-81.

12. Huang YJ, 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.

13. Huang YJ, Chian RC, Gilbert L, Fleiszer D, Holzer H, Dermits 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.

14. Hourvitz A, Yerushalmi GM, Maman E, Raanani H, Elizur S, Hourvitz O, et al. Oocyte vitrification in the luteal phase to preserve fertility in cancer patients. Reprod Biomed Online 2008;17:520-3.

15. Polim A, Handayani N, Aprilliana T, Silvia R, Sirait B, Boediono A, et al. Association between estradiol levels and clinical outcomes of IVF cycles with single blastocyst embryo transfer. Asian Pac J Reprod 2021;10:49-55.

16. Lim KS, Chae SJ, Choo CW, Yu H, Lee HJ, Hur CY, et al. In vitro maturation: Clinical applications. Clin Exp Reprod Med 2013;40:143-7.

17. Lavery S, Baldwin K, Mitchell H, Culley L, Hudson N. Oocyte cryopreservation for social reasons: Demographic profile and disposal intentions of UK users. Reprod Biomed Online 2015;31:239-45.

18. Shu Y, Gehrhardt J, Watt J, Lyon J, Dasig D, Behr B. Fertilization, embryo development, and clinical outcome of immature oocytes from stimulated intracytoplasmic sperm injection cycles. Fertil Steril 2007;87:1022-7.

19. Reichman DE, Politch J, Ginsburg ES, Racowsky C. Extended in vitro maturation of immature oocytes from stimulated cycles: Analysis of fertilization potential, clinical outcomes and reproductive outcomes. J Assist Reprod Genet 2010;27:347-56.

20. Shin SB, Cho JW, Lee SH, Yang KM, Lim CK, Lee HS. Fertilization and pregnancy potential of immature oocytes from stimulated intracytoplasmic sperm injection cycles. Clin Exp Reprod Med 2013;40:7-11.

21. Martin-Palomo Olid N, Garcia D, Rodriguez A, Vassena R. Could fertility clinics offer a sizable improvement of live birth rates by maturing post-GVBD oocytes in vitro? J Assist Reprod Genet 2019;36:1927-34.

22. Vanhoutte L, De Sutter P, Van der Elst J, Dhont M. Clinical benefit of metaphase I oocytes. Reprod Biol Endocrinol 2005;3:71.

23. Ferrer-Vasquez A, Barragan M, Rodriguez A, Vassena R. Altered cytoplasmatic maturation in rescued in vitro matured oocytes. Hum Reprod 2019;34:1005-105.

24. Tucker MJ, Wright B, Morton PC, Massey JB. Birth after cryopreservation of immature oocytes with subsequent in vitro maturation. Fertil Steril 1998;70:578-9.

25. Vanhoutte L, De Sutter P, Van der Elst J, Dhont M. Clinical benefit of metaphase I oocytes. Reprod Biol Endocrinol 2005;3:71.
of hCG-primed *in vitro* maturation cycles for patients with polycystic ovaries. Hum Reprod Update 2010;16:675-89.

41. Vanhoutte L, Nogueira D, Dumortier F, De Sutter P. Assessment of a new *in vitro* maturation system for mouse and human cumulus-enclosed oocytes: Three-dimensional prematuration culture in the presence of a phosphodiesterase 3-inhibitor. Hum Reprod 2009;24:1946-59.

42. Sánchez F, Lolicato F, Romero S, De Vos M, Van Ranst H, Verheyen G, *et al.* An improved IVM method for cumulus-oocyte complexes from small follicles in polycystic ovarian syndrome patients enhances oocyte competence and embryo yield. Hum Reprod 2017;32:2056-68.

43. Hussein TS, Thompson JG, Gilchrist RB. Oocyte-secreted factors enhance oocyte developmental competence. Dev Biol 2006;296:514-21.

44. 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.

45. 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.

46. Van Blerkom J. Maturation at high frequency of germinal-vesicle-stage mouse oocytes after cryopreservation: Alterations in cytoplasmic, nuclear, nucleolar and chromosomal structure and organization associated with vitrification. Hum Reprod 1989;4:883-98.

47. Kim SS, Olsen R, Kim DD, Albertini DF. The impact of vitrification on immature oocyte cell cycle and cytoskeletal integrity in a rat model. J Assist Reprod Genet 2014;31:739-47.

48. Wu C, Rui R, Dai J, Zhang C, Ju S, Xie B, *et al.* Effects of cryopreservation on the developmental competence, ultrastructure and cytoskeletal structure of porcine oocytes. Mol Reprod Dev 2006;73:1454-62.

49. Turathum B, Saikhun K, Sangsuwan P, Kitiyanant Y. Effects of vitrification on nuclear maturation, ultrastructural changes and gene expression of canine oocytes. Reprod Biol Endocrinol 2010;8:70.