Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

In Vitro Maturation (IVM) of Oocytes from Ovarian Tissue: Based on In Vitro Oogenesis

Sherman Silber
Infertility Center of St. Louis
Sierra Goldsmith
sierra.goldsmith@yahoo.com

Infertility Center of St. Louis
Yifei Lu
Department of Obstetrics and Gynecology, Penn State College of Medicine, Division of Reproductive Endocrinology and Infertility
Nestor Rodriguez
Infertility Center of St. Louis
Alexis Jones
Infertility Center of St. Louis
Katsuhiko Hayashi
Graduate School of Medicine Osaka University

Research Article

Keywords:

Posted Date: November 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11681376/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

TITLE

In Vitro Maturation (IVM) of Oocytes from Ovarian Tissue: Based on In Vitro Oogenesis

ABSTRACT

Purpose: Using in-vitro gametogenesis results in mice, can we perform simple, easy to perform, but robust in-vitro maturation (IVM) of oocytes from human ovarian tissue?

Methods: This is a prospective non-randomized feasibility pilot study to develop a simplified methodology for IVM based upon our results with in vitro oogenesis in mice. 197 women have undergone ovary tissue cryopreservation since 1979 at our center. 41 consecutive post-pubertal patients who received no ovarian stimulation underwent in-vitro maturation of oocytes (IVM) derived from dissected ovarian tissue prior to their ovary tissue cryopreservation. We tested whether prior natural in vivo exposure to gonadotropin would have already prepared GV's in vivo for in vitro maturation using ordinary IVF media containing FSH and HCG.

Results: Harvested germinal vesicle oocytes in all post-pubertal patients who had not yet received chemotherapy developed into mature metaphase II oocytes without specialized "IVM media", without co-culture, and with HCG concentrations varying widely from 10mIU/l to 1000 mIU/l. The actual number of CC's in the spent media decreased with age, but the IVM rate per CC did not significantly decrease with age, varying widely up to 76%. Prior hormone replacement therapy reduced the IVM rate.

Conclusions: Simple IVM from ovarian tissue could provide additional fertility preservation for patients undergoing ovary tissue cryopreservation, and could also eventually lead to IVF without the need for ovarian stimulation. Patients on hormonal contraception and prepubertal patients had variable IVM rates, and will be the subject of a future feasibility study.

INTRODUCTION

The promptest way to preserve fertility in cancer patients is oophorectomy and cryopreservation of ovarian cortical tissue [1-10]. This avoids months of delay and the high cost required for several cycles of ovarian stimulation and ultrasound guided oocyte retrieval for oocyte cryopreservation. It also allows for natural conception and hormone function when the tissue is thawed and replaced years later. However, this procedure, like all procedures, does not always result in pregnancy. It would be useful also to be able to culture eggs (GV's) obtained from the ovary cortical medullary dissection and mature them into MII oocytes in vitro (IVM) with a simple methodology [8,10]. That might provide two sources for future fertility, ovarian tissue transplantation of course, but also IVF.

It makes sense to utilize in our clinical patients, knowledge gained from in vitro oogenesis in animals to better understand the actual mechanisms of oocyte maturation [10]. The transcripts which transform fibroblasts into stem cells are well studied and universal in most species [7, 11]. The transcripts that serially transform stem cells into fetal epiblast cells and then into primordial germ cells (PGC's) are also well established. However, transforming primordial germ cells into

competent oocytes requires transcripts induced by fetal (not adult) granulosa cells that are species specific, and more difficult to ascertain [12]. In the mouse it is now possible to use these mouse specific transcripts to make fetal granulosa cells in separate culture. This allows us to make competent oocytes from primordial germ cells without having to retrieve fetal granulosa cells from other mice. This principle could eventually lead to making human oocytes from somatic cells. [13-17].

We have discovered there are three basic phases of oocyte development that in vitro oogenesis studies have elucidated. We call these phases “IVD”, “IVG”, and “IVM”. “IVD” refers to differentiation of PGC’s into competent oocytes that are sensitive to gonadotropin [13-17]. IVD is a term that includes genetic changes in the early germ cells that are not gonadotropin sensitive, and that non-hormonally prepare the oocyte for gonadotropin sensitivity. For clinical purposes in human adults, we know from countless ovarian tissue transplants that recruited primordial follicles must go through a 4 month phase of IVD to be prepared for gonadotropin sensitivity (Figure 1a-b) [7,8].

After IVD, it is known from in vitro oogenesis experiments that 5-12 days of in vitro exposure to FSH (and a minute amount of LH) is necessary for oocyte maturation, which is consistent generally with in vivo clinical IVF experience [13]. Thereafter, exposure to HCG or LH results in fertile MII oocytes. Less than 5 days of exposure to gonadotropin will yield a lower maturation rate. Greater than 12 days of in vitro exposure to FSH will may result in poorer quality oocytes or degeneration.

We postulated from these results with in vitro oogenesis that in the ovary of any woman on any given day there will be germinal vesical (GV) oocytes that have completed IVD in vivo, and have been exposed to gonadotropin in vivo for a varying number of days after first becoming sensitive to gonadotropin [7,8]. On any given day there will be a variety of GV oocytes in different stages of exposure to gonadotropin. If these GV oocytes have been exposed to less than 5 days of gonadotropin since emerging from IVD phase, we postulated many are not fully ready to mature to MII under the influence of HCG or LH. In most patients, on any given day about 35% to 70% of oocytes in the ovary at the time of cortical medullary dissection for cryopreservation will have been exposed to gonadotropin for greater than 5 days and less than 13 days and thus without any pretreatment they will be ready for robust and simple IVM with HCG or LH (Figure 1b). Being on birth control pills of varying formulations will reduce the in vivo gonadotropin levels to a varying degree, and represent thus a major confounder.

Thus from in vitro gametogenesis experiments, oocytes appear to require 5 to 12 days of exposure to gonadotropin after IVD to become capable of maturation to metaphase II. That would predict that without any prior treatment or specialized media, possibly 35% to 70% of all oocytes retrieved from ovary tissue dissection have already been prepared in vivo for simple IVM. With patient selection and follicle selection, the percentage of IVM competent oocytes with no special added treatment might be much higher. We decided to test this hypothesis in patients undergoing oophorectomy for ovarian cortical tissue cryopreservation, in an attempt to introduce a simpler technology for IVM.

MATERIALS AND METHODS

A total of 41 consecutive post-pubertal women who had not yet received chemotherapy underwent ovary cortical tissue cryopreservation along with an attempt to obtain mature oocytes from the tissue before freezing. GV oocytes were collected from spent media from the dissected ovarian tissue. These 41 consecutive patients had no prior chemotherapy, and were post-pubertal. They were the most recent from a total series of 197 cases of ovary tissue freeze for fertility preservation that began in 1997.

The cortex was dissected away from the medulla using the original Gosden technique [18]. A sharp scalpel first was used to bivalve the whole ovary, and then each half was dissected in room air. The residual medullary tissue was scraped off the internal surface of the remaining cortex. Dissection was “minimally disruptive” in early cases (Figure 2). With deeper dissection smaller follicles (4-6mm) were obtained. Our goal was simply to assess feasibility of simple IVM from ovarian tissue and not follicle selection. There was continual irrigation with hepes buffered IVF culture media and the dissection was performed under optical magnification from 2.5x to 10x. Ovarian cortex was vitrified in the manner already described [19,20].

After ovarian cortex dissection, the spent media was examined for cumulous complexes (CC’s), which contained immature oocytes which were placed in a variety of different media with widely

varying concentrations of FSH and HCG (Table 1) (Figures 3a-d). Most commonly ordinary IVF media was used, but in some cases "IVM media" was used. There was no prior hormone administration. In vitro cultures were checked periodically until 48 hours. Thereafter no maturation was noted. Ovarian tissue in all cases was cryopreserved with vitrification. All oocytes which matured to MII were also vitrified. We thus tested whether prior in vivo exposure to gonadotropin would prepare newly formed GV's for in vitro maturation with simple IVF media supplemented with HCG in any concentration. The purpose of this study was not to make comparisons. It was purely a proof of a concept feasibility project in an attempt to see if IVM could be a simple and easy methodology, based on what we have learned from our in vitro oogenesis technology. Ethical approval was obtained from the appropriate institutional review board. Informed consent was obtained from all participants prior to study procedures, including consent for publication of the data. Informed consent was obtained prior to performing any procedures, including permission for publication on all data.

STATISTICAL EVALUATION

Patients were stratified by age at the time of oophorectomy into two groups: ≤30 years (n = 28) and >30 years (n = 13). The primary outcomes evaluated included the number of germinal vesicle (GV) oocytes in CC's recovered per patient, the number of metaphase II (MII) oocytes obtained following IVM per patient, and IVM maturation efficiency, defined as the proportion of recovered GV oocytes that successfully matured to the MII stage.

GV oocyte recovery and MII oocyte yield were summarized as mean ± standard deviation (SD). Differences between age groups were evaluated using two-sided Mann–Whitney U tests. IVM maturation efficiency was calculated for each age group by dividing the total number of MII oocytes obtained by the total number of GV oocytes recovered, expressed as a percentage. Differences in pooled maturation efficiency between age groups were assessed using a two-sided patient-label permutation test, preserving patient-level clustering of oocyte outcomes. Ninety-five percent confidence intervals (95% CIs) for pooled maturation rates were estimated using patient-cluster bootstrap resampling to account for within-patient correlation. All statistical tests were two-sided, and p-values <0.05 were considered statistically significant.

RESULTS

Table 1 gives details of the 41 cases. Harvested germinal vesicle (GV) oocytes developed into mature metaphase II oocytes in all cases with simple IVF culture media apparently as well as with “IVM media”, and with HCG concentrations varying widely from 10 mIU/ml to 1,000 mIU/ml (Table 1). These results should therefore be easily reproducible in any IVF lab even if it is not specialized in IVM.

Younger patients demonstrated a greater number of CC’s but no greater IVM maturation rate per CC compared with patients older than 30 years, demonstrating a clear age related decline in oocyte quantity but not in maturation rate (Figure 4). The age related decline in IVM is caused

by a decrease in oocyte quantity much more than a decline in in vitro maturation competence [21].

Of a total of 732 GV oocytes, 251 became mature MII's (34%) varying from 12% to 76%. The only single case with low maturation rate occurred when the cumulous was stripped immediately instead of at 15-24 hours. Patients aged ≤30 years yielded 602 CCs (mean 21.5 CCs per patient) and 217 mature MII oocytes (mean 7.8 per patient). Patients aged >30 years yielded 130 CCs (mean 10 per patient) and 34 mature MII oocytes (mean 2.6 per patient) (Table 2). These differences in age groups were highly significant for both CC yield and mature MII oocyte number per patient (both $p < 0.006$) (Table 2). However the IVM decline in maturation rates per CC was not statistically significant (Figure 4). The IVM rate per patient decreased with age because of the decrease in number of CC's (Table 3a-b). The IVM decline per patient was thus mostly caused by a decline in number of CC's. The IVM rate per GV was also somewhat lower for older patients, but this was not statically significant (Table 2).

It is very important to note that there were a group of cases that had consistently higher maturation rates not related to age (Table 4). These patients had not been using any hormonal contraception or HRT, which can have variable impact on gonadotropin concentration. Also these GV's came from smaller follicles 4-6mm that were obtained with deeper dissection. There are a variety of GV's in every ovary that are either developing or degenerating. The IVM rates for patients who were on hormonal birth control were unpredictable, but those not on any birth control were consistently high in ordinary IVF culture media despite no ovarian stimulation.

DISCUSSION

The most remarkable aspect of these findings is the potentially great simplicity of IVM methodology. In fact the point of this study was simply the feasibility for widespread adoption of IVM with no special technology. On any given day approximately 35% to 70% of the oocytes which have emerged from IVD and appear in the spent media from the cortical-medullary dissection have already been prepared for IVM in vivo by greater than 5 days and less than 13 days of exposure to gonadotropin (Figure 1b). This is just a gross average with wide variation dependent on the depth of dissection, prior hormonal contraception, and possibly age. There appears to be no impact on results from widely different concentrations of HCG in the media, or in whether specific IVM media or standard IVF media was used. There was no pre treatment ovarian stimulation of the patient, and specialized media was not needed. But approximately 35% to 70% or more of the oocytes in the spent media had already received naturally in vivo an appropriate exposure to gonadotropin to prepare them for maturation to MII in culture over 24-48 hours with just HCG and FSH in the media. However, the consistency of IVM rate per GV was altered by the prior use of hormonal contraception, possibly because of the variable suppression of gonadotropin by different birth control pill formulations. Although it is well established that oocyte quality declines with age it is interesting that the capacity to mature does not appear to decline significantly with age [21].

The basis of our postulate that IVM can be simplified arose from our studies on in vitro oogenesis in the mouse [13-17]. Fully potent, mature oocytes have been generated in culture from embryonic stem cells or induced pluripotent stem cells (IPS cells) derived from mouse tail fibroblasts. This was accomplished by first exposing the fibroblasts to 4 genes (Oct4, Sox2, c-Myc, and Klf4) for 3-4 weeks to make IPS cells (the near equivalent of embryonic stem cells). These stem cells were then transformed into epiblast like cells (recreating the normal steps of early embryonic development) by incubating for 2 days in two genes (Activin A and bFGF). Germ cell specification begins in the epiblast of the early embryo. These epiblast like cells are then incubated for 6 days in four genes (BMP4, SCF, LIF, and EGF) to make primordial germ cells (PGC's), again recreating early embryo development of germ cells (which could then become either oocytes or spermatogonia). These PGC's, or PGC like cells are then incubated for 3 weeks with fetal granulosa cells to make competent gonadotropin sensitive oocytes. PGC's will not become gonadotropin sensitive oocytes if incubated with adult granulosa cells. Only fetal granulosa cells can do this. This phase of transformation of PGC's into gonadotropin sensitive oocytes has been termed "IVD". This process has now been partly reproduced in the human (from menopausal patients as old as 55 years) up to the PGC stage [14]. Primordial resting follicles in the human ovarian cortex do not become gonadotropin sensitive until they are recruited and then go through 4 months of IVD (Figure 1b) [7,8].

Our study did not try to mature resting primordial follicles or early developing follicles. Primordial follicles which were arrested in the fetus in early meiotic prophase are all located in the dense fibrous tissue of the cortex. They enter meiosis as soon as they reach the dorsal ridge at 3 weeks because they encounter retinoic acid from the adrenal gland. Retinoic acid upregulates

the gene Stra8, which initiates meiosis [22-26]. This is unlike in the male, where the Sertoli cells of the testis block the action of retinoic acid, allowing the primordial germ cells to become spermatogenic stem cells. If the meiosis of the female germ cells were not arrested, girls would rapidly run out of eggs before birth. What arrests the early prophase of meiosis in the female fetus is simply the mechanism of tissue pressure in the dense fibrous tissue of the ovarian cortex, which causes early oocyte dormancy [15,27]. They remain insensitive to gonadotropin until they are recruited from the resting dormant phase and undergo 4 months of IVD [8]. The oocytes that have emerged from IVD are all that we can mature with simple IVM, because they are then gonadotropin sensitive. Our studies with ovary transplantation have demonstrated that it is tissue density and pressure gradient in the ovary cortex that arrests these fetal oocytes in early meiosis, and which controls the entry of primordial follicles into the IVD phase (Figure 5) [19-20].

In the mouse, normal competent oocytes have now been made without the need to harvest fetal granulosa cells. Hayashi's group has done the painstaking work of using reverse transcriptase to reveal the genes that generate the fetal granulosa cells. Thus they can transform PGC's into competent (of course not yet mature) oocytes by using two different culture systems, one which produces PGC's from IPS cells and one which produces fetal granulosa cells from IPS cells. They have even learned how to make functional oocytes from male (not female) stem cells by replacing the Y chromosome with a second x chromosome [28]. In humans we will most likely not be able to harvest fetal granulosa cells from abort uses. We will have to generate them from IPS cells. This phenomenon is species specific and so it has to be human fetal granulosa cells that are generated [12]. Thus until the genes which in the human can transform PGC's into fetal

granulosa cells are known, there may be no further progress in human in vitro oogenesis. However we can nonetheless use this knowledge now today for IVM.

These results support the evidence that recruited follicles go through 4 months of non-hormonally sensitive development before emerging from IVD to finally become sensitive to gonadotropin (Figure 5) [19,20]. Every day follicles that had been recruited four months earlier become gonadotropin sensitive. The IVM rate of up to 76% from follicles retrieved on any given day indicates that ≥5 days of exposure to gonadotropin, and less than 13 days of exposure is the ideal window that prepares them for HCG or LH induced conversion to metaphase 2. Less than 5 days exposure will hinder meiotic competence and maturation. More than 12 days of exposure will result in a poorer quality oocyte or frank degeneration. However there was a wide variability in IVM rate, with many patients having over 50% and some less (Table 4). We postulate that this for two likely reasons. We obtained all different stages of GV oocytes both developing and degenerating. With focus on the smaller follicles (4-6mm) the IVM rate might be consistently over 50%. However another explanation for the wide variation is that some women in this series on hormonal birth control of a variety of formulations or low dose HRT had unpredictable IVM rates. Those not on hormonal replacement (HRT) or birth control had consistently greater than 50% maturation rate regardless of age.

The ovaries of pre-pubertal girls will not easily undergo IVM, and similarly combined estrogen and progestin hormonal contraception can interfere unpredictably with IVM, because these eggs will not have been exposed adequately to gonadotropin. Nonetheless progestin only birth control

pills and low dose pills may not always hinder IVM because gonadotropin levels may not have been drastically reduced. Prior toxic chemotherapy will prevent IVM because developing follicles will be destroyed, although many primordial follicles will survive, making ovary transplantation still a viable option even when IVM is not [4,10]. Our simplistic approach to IVM is based on the fact that prior in vivo exposure to gonadotropin has prepared the oocytes for in vitro maturation, with robust success.

The question remains whether these normal appearing MII oocytes can lead to healthy children. There have already been prior successful reports on IVM from ovary tissue, the first report being in 2014, of IVM and normal pregnancy after oophorectomy for ovarian cancer, and another case report of a healthy pregnancy in 2015 [29,30]. An ongoing pregnancy with live birth from De Vos was reported in 2020 [31,32]. These are scattered case reports.

However, the Vietnamese have had astonishing success with IVM, and many healthy births. However instead of maturing oocytes from the spent media of whole intact ovaries, they aspirate small follicles from unstimulated IVF patients with very narrow gauge needles under transvaginal ultrasound. Also none of their patients were on birth control or HRT. They demonstrated unmistakably that these IVM oocytes with no hormonal stimulation result in acceptable pregnancy and delivery rates [33-35]. In fact they found no difference in pregnancy and baby rates, between IVM retrieved oocytes and routine stimulated cases. Despite the wide variety of stages of development after IVD, there are always at least 35% to 70% of recoverable

oocytes in every ovary at any time that have completed IVD after recruitment, and that have had adequate in vivo exposure to gonadotropin for maturation in vitro.

That maturation rate may even become higher using the CAPA system, which is very simple and easy to apply. Or it may just be follicle selection or patient selection. Possibly none of this will matter for patients not on hormonal contraception or HRT. The Vietnamese group differ from our series not only using CAPA, but also in needle retrieval rather than retrieval from dissected ovary tissue. They put the aspirated GV's in non-hormonal culture for 24 hours after retrieval and then add HCG [36]. They also showed that better IVM rates can be achieved with GV's from smaller follicles, because larger follicles may be in the process of degeneration because of too many days in vivo exposure to gonadotropin. With ovarian tissue dissection, there will be many GV's that came from larger follicles that could explain their higher IVM rate per GV. Furthermore, prior hormonal birth control or HRT in some of our population of patients may have variably suppressed gonadotropin secretion, and thereby affected IVM rate. However, the Vietnamese approach, like ours, reveals the potential simplicity and possible robustness of IVM. This experience may lead the way to a future of IVF without the need for ovarian stimulation, particularly in younger patients with more oocytes.

Another value of this pilot feasibility series, aside from verifying the potential simplicity and robustness of IVM and adding another dimension to fertility preservation for cancer patients, is in clarifying in the human certain aspects of oocyte development that have been learned from in vitro gametogenesis. Resting primordial follicles are released daily from meiotic arrest by a

tissue pressure gradient from the densest fibrous tissue in the outer cortex to the least dense in the inner cortex and medulla [9,10,15]. They then require 4 months after recruitment to become gonadotropin sensitive Thereafter, competent GV's emerging from non-hormonally sensitive IVD require exposure to gonadotropin to become capable of maturation to MII under the influence of HCG or LH. This correlates remarkably with results of ovarian stimulation well known in all IVF programs.

There are several important limitations of this pilot feasibility report. It is not large yet and will require over 100 cases, carefully controlled, to determine if the significance or difference or lack of difference according to age or HRT treatment will hold up statistically. There needs in the future to be a careful evaluation of success related to specified different media, although with ovarian tissue there will always be a heterogenous stage of follicle development, which can be obtained with the Vietnamese approach of transvaginal ultrasound guided needle aspiration. Nonetheless it makes sense that in older women there will be less CC's and therefore a parallel lower success with IVM. Also there are confounders like possible PCOS that could not be evaluated prior to cancer treatment or oophorectomy. Furthermore, our minimally disruptive dissection might minimize retrieval of smaller follicles. A huge problem also is patient recruitment. In the United States where insurance does not cover fertility preservation for cancer patients, recruitment of patients has to be basically a free service, which severely limits the volume of patient recruitment. Furthermore, at least half of patients undergoing oophorectomy for cryopreservation have already undergone early chemotherapy, hormone replacement, or are pre-pubertal. Those patients will be the subject of a future study.

Nonetheless, even with these limitations, several truths are evident from this simple methodology report. Firstly, IVM is a simple and robust procedure to enhance fertility preservation in many patients undergoing ovarian cryopreservation. Early chemotherapy will not preclude success with ovarian cryopreservation and subsequent transplantation, because primordial follicles are not actively developing and are somewhat resistant to chemotherapy compared to developing follicles. IVM will not work in this group, but ovary tissue transplant in them can be successful nonetheless. Most importantly, if the IVM maturation rate could be increased in all women with CAPA or better selection methods, or simply avoiding hormonal contraception or HRT, IVF could eventually possibly be performed without ovarian hyperstimulation in properly selected patients.

CONCLUSIONS

The most intriguing aspects of this study are the simple robustness of IVM from ovarian tissue, the irrelevance of specific culture media or co-culture (based on principles of in vitro gametogenesis), the unimportance of specific HCG concentration in the media, and the ease of obtaining mature oocytes without the need for ovarian hyperstimulation. This provides two sources for fertility preservation with one procedure: ovary tissue cryopreservation, and IVM of oocytes derived from the ovarian tissue.

REFERENCES

1. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B, van Langendonckt A. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004 Oct 16-22;364(9443):1405-10.

2. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Sanchez Serrano M, Schmidt KT, Ernst E, Luyckx V, Andersen CY. Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. Fertil Steril. 2013 May;99(6):1503-13.

3. Donnez J, Dolmans MM. Fertility Preservation in Women. N Engl J Med. 2017 Oct 26;377(17):1657-1665.

4. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, Schiff E, Dor J. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med. 2005 Jul 21;353(3):318-21.

5. Cadenas J, Pors SE, Kumar A, Kalra B, Kristensen SG, Andersen CY, Mamsen LS. Concentrations of oocyte secreted GDF9 and BMP15 decrease with MII transition during human IVM. Reprod Biol Endocrinol. 2022 Aug 19;20(1):126.

6. Andersen CY, Rosendahl M, Byskov AG, Loft A, Ottosen C, Dueholm M, Schmidt KL, Andersen AN, Ernst E. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. Hum Reprod. 2008 Oct;23(10):2266-72.

7. Silber SJ, Goldsmith S, Castleman L, Hayashi K. In Vitro Maturation, In Vitro Oogenesis, and Ovarian Longevity. Reprod Sci. 2024 May;31(5):1234-1245.

8. Silber SJ, Goldsmith S, Castleman L, Hurlbut K, Fan Y, Melnick J, Hayashi K. In-vitro maturation and transplantation of cryopreserved ovary tissue: understanding ovarian longevity. Reprod Biomed Online. 2022 Mar;44(3):504-514.

9. Silber SJ, DeRosa M, Goldsmith S, Fan Y, Castleman L, Melnick J. Cryopreservation and transplantation of ovarian tissue: results from one center in the USA. J Assist Reprod Genet. 2018;35(12):2205-2213.

10. Silber S. Ovarian tissue cryopreservation and transplantation: scientific implications. J Assist Reprod Genet. 2016;33(12):1595-1603.

11. Yamanaka S. Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. Cell Prolif. 2008 Feb;41 Suppl 1(Suppl 1):51-6.

12. Yamashiro C, Sasaki K, Yabuta Y, Kojima Y, Nakamura T, Okamoto I, Yokobayashi S, Murase Y, Ishikura Y, Shirane K, Sasaki H, Yamamoto T, Saitou M. Generation of human oogonia from induced pluripotent stem cells in vitro. Science. 2018 Oct 19;362(6412):356-360.

13. Hikabe O, Hamazaki N, Nagamatsu G, Obata Y, Hirao Y, Hamada N, Shimamoto S, Imamura T, Nakashima K, Saitou M, Hayashi K. Reconstitution in vitro of the entire cycle of the mouse female germ line. Nature. 2016;539:299–303.

14. Pandolfi EC, Hsu FM, Duhon M, Zheng Y, Goldsmith S, Fu J, Silber SJ, Clark AT. In vitro germ cell induction from fertile and infertile monozygotic twin research participants. Cell Rep Med. 2022 Oct 18;3(10):100782.

15. Nagamatsu, G., Shimamoto, S., Hamazaki, N., Nishimura, Y., & Hayashi, K. 2019. Mechanical stress accompanied with nuclear rotation is involved in the dormant state of mouse oocytes. Science Advances, 5(6), [eaav9960].

16. Hamazaki N, Kyogoku H, Araki H, Miura F, Horikawa C, Hamada N, Shimamoto S, Hikabe O, Nakashima K, Kitajima TS, Ito T, Leitch HG, Hayashi K. Reconstitution of the oocyte transcriptional network with transcription factors. Nature. 2021 Jan;589(7841):264-269.

17. Yoshino T, Suzuki T, Nagamatsu G, Yabukami H, Ikegaya M, Kishima M, Kita H, Imamura T, Nakashima K, Nishinakamura R, Tachibana M, Inoue M, Shima Y, Morohashi KI, Hayashi K. Generation of ovarian follicles from mouse pluripotent stem cells. Science. 2021 Jul 16;373(6552):eabe0237.

18. Gosden RG, Baird DT, Wade JC, Webb R. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196 degrees C. Hum Reprod. 1994 Apr;9(4):597-603.

19. Silber S, Pineda J, Lenahan K, DeRosa M, Melnick J. Fresh and cryopreserved ovary transplantation and resting follicle recruitment. Reprod Biomed Online. 2015 Jun;30(6):643-50.

20. Silber S. Unifying theory of adult resting follicle recruitment and fetal oocyte arrest. Reprod Biomed Online. 2015 Oct;31(4):472-5.

21. Silber SJ, Kato K, Aoyama N, Yabuuchi A, Skaletsky H, Fan Y, Shinohara K, Yatabe N, Kobayashi T. Intrinsic fertility of human oocytes. Fertil Steril. 2017 May;107(5):1232-1237.

22. Koubova J, Menke DB, Zhou Q, Capel B, Griswold MD, Page DC. Retinoic acid regulates sex-specific timing of meiotic initiation in mice. Proc Natl Acad Sci U S A. 2006 Feb 21;103(8):2474-9.

23. Anderson EL, Baltus AE, Roepers-Gajadien HL, Hassold TJ, de Rooij DG, van Pelt AM, Page DC. Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. Proc Natl Acad Sci U S A. 2008 Sep 30;105(39):14976-80.

24. Koubova J, Hu YC, Bhattacharyya T, Soh YQ, Gill ME, Goodheart ML, Hogarth CA, Griswold MD, Page DC. Retinoic acid activates two pathways required for meiosis in mice. PLoS Genet. 2014 Aug 7;10(8):e1004541.

25. Baltus AE, Menke DB, Hu YC, Goodheart ML, Carpenter AE, de Rooij DG, Page DC. In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. Nat Genet. 2006 Dec;38(12):1430-4.

26. Chassot AA, Le Rolle M, Jolivet G, Stevant I, Guigonis JM, Da Silva F, Nef S, Pailhoux E, Schedl A, Ghyselinck NB, Chaboissier MC. Retinoic acid synthesis by ALDH1A proteins is dispensable for meiosis initiation in the mouse fetal ovary. Sci Adv. 2020 May 22;6(21):eaaz1261.

27. Bendsen E, Byskov AG, Andersen CY, Westergaard LG. Number of germ cells and somatic cells in human fetal ovaries during the first weeks after sex differentiation. Hum Reprod. 2006;21(1):30–35.

28. Murakami K, Hamazaki N, Hamada N, Nagamatsu G, Okamoto I, Ohta H, Nosaka Y, Ishikura Y, Kitajima TS, Semba Y, Kunisaki Y, Arai F, Akashi K, Saitou M, Kato K, Hayashi K. Generation of functional oocytes from male mice in vitro. Nature. 2023 Mar;615(7954):900-906.

29. Prasath EB, Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, Loh SF, Chia YN. First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient. Hum Reprod. 2014;29(2):276-8

30. Uzelac PS, Delaney AA, Christensen GL, Bohler HC, Nakajima ST. Live birth following in vitro maturation of oocytes retrieved from extracorporeal ovarian tissue aspiration and embryo cryopreservation for 5 years. Fertil Steril. 2015;104(5):1258-60.

31. Segers I, Mateizel I, Van Moer E, Smitz J, Tournaye H, Verheyen G, De Vos M. In vitro maturation (IVM) of oocytes recovered from ovariectomy specimens in the laboratory: a promising "ex vivo" method of oocyte cryopreservation resulting in the first report of an ongoing pregnancy in Europe. J Assist Reprod Genet. 2015;32(8):1221-31.

32. Segers I, Bardhi E, Mateizel I, Van Moer E, Schots R, Verheyen G, Tournaye H, De Vos M. Live births following fertility preservation using in-vitro maturation of ovarian tissue oocytes. Hum Reprod. 2020 Sep 1;35(9):2026-2036.

33. Vuong LN, Ho VNA, Ho TM, Dang VQ, Phung TH, Giang NH, Le AH, Pham TD, Wang R, Smitz J, Gilchrist RB, Norman RJ, Mol BW. In-vitro maturation of oocytes versus conventional IVF in women with infertility and a high antral follicle count: a randomized non-inferiority controlled trial. Hum Reprod. 2020 Nov 1;35(11):2537-2547.

34. Vuong LN, Le AH, Ho VNA, Pham TD, Sanchez F, Romero S, De Vos M, Ho TM, Gilchrist RB, Smitz J. Live births after oocyte in vitro maturation with a prematuration step in women with polycystic ovary syndrome. J Assist Reprod Genet. 2020 Feb;37(2):347-357.

35. Vuong LN, Ho VNA, Le AH, Nguyen NT, Pham TD, Nguyen MHN, Le HL, Le TK, Ha AN, Le XTH, Pham HH, Tran CT, Huynh BG, Smitz JEJ, Gilchrist RB, Ho TM. Hormone-free vs. follicle-stimulating hormone-primed infertility treatment of women with polycystic ovary syndrome using biphasic in vitro maturation: a randomized controlled trial. Fertil Steril. 2025 Feb;123(2):253-261.

36. Gilchrist RB, Ho TM, De Vos M, Sanchez F, Romero S, Ledger WL, Anckaert E, Vuong LN, Smitz J. A fresh start for IVM: capacitating the oocyte for development using pre-IVM. Hum Reprod Update. 2024 Jan 3;30(1):3-25.

FIGURES

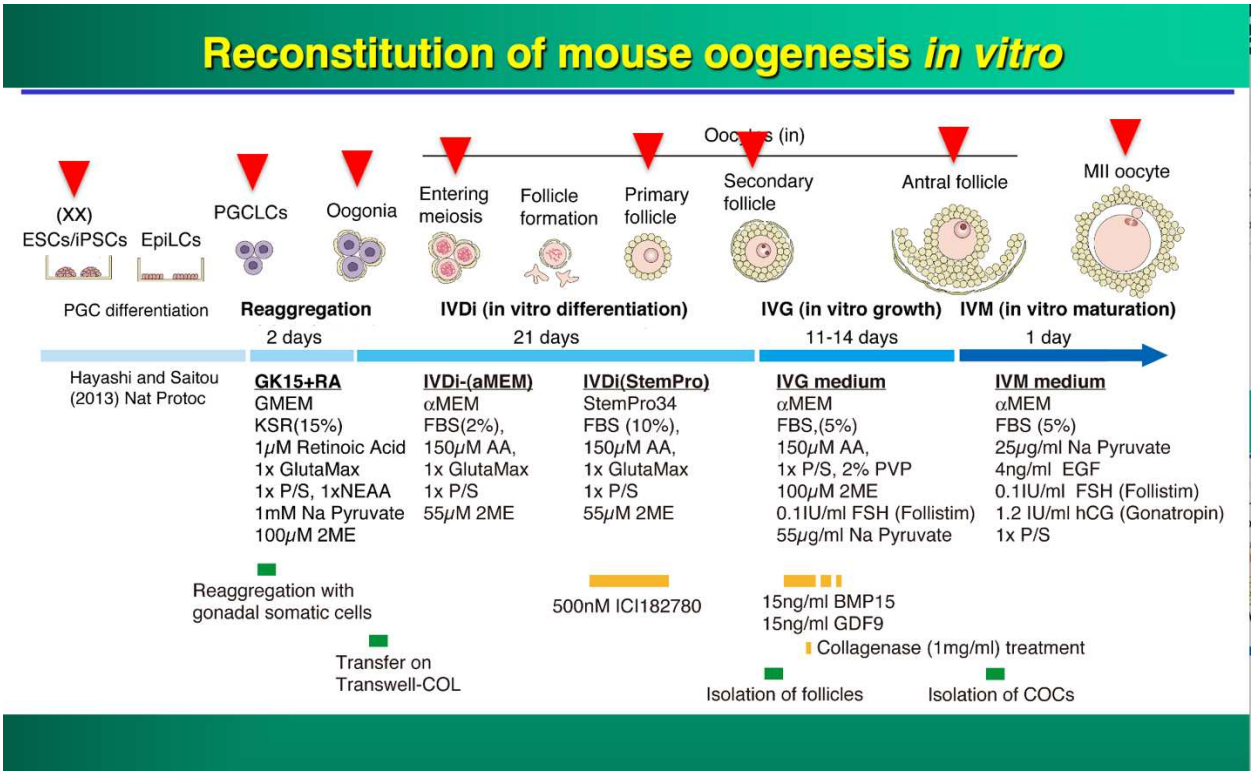

Figure 1a: Diagram explaining in vitro maturation (IVM) of oocytes from ovarian tissue in human, based on mouse model of in vitro oogenesis [13].

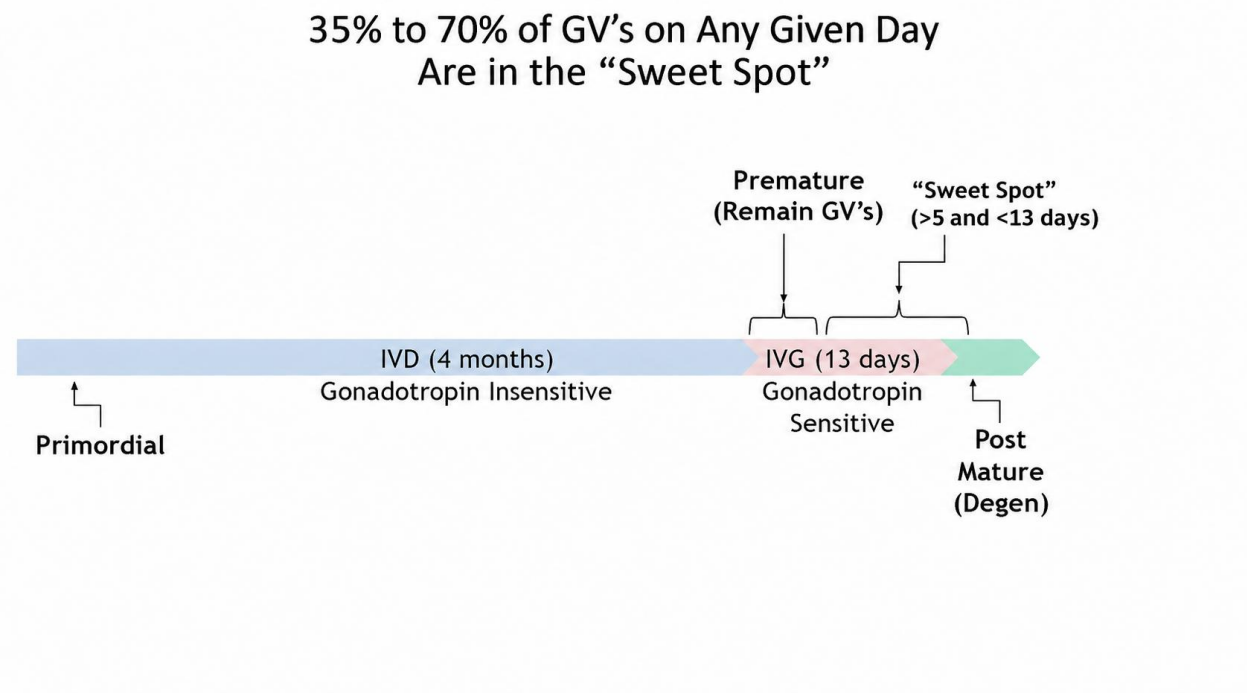

Figure 1b: Diagram explaining in vitro maturation (IVM) of oocytes from ovarian tissue in human, based on mouse model of in vitro oogenesis.

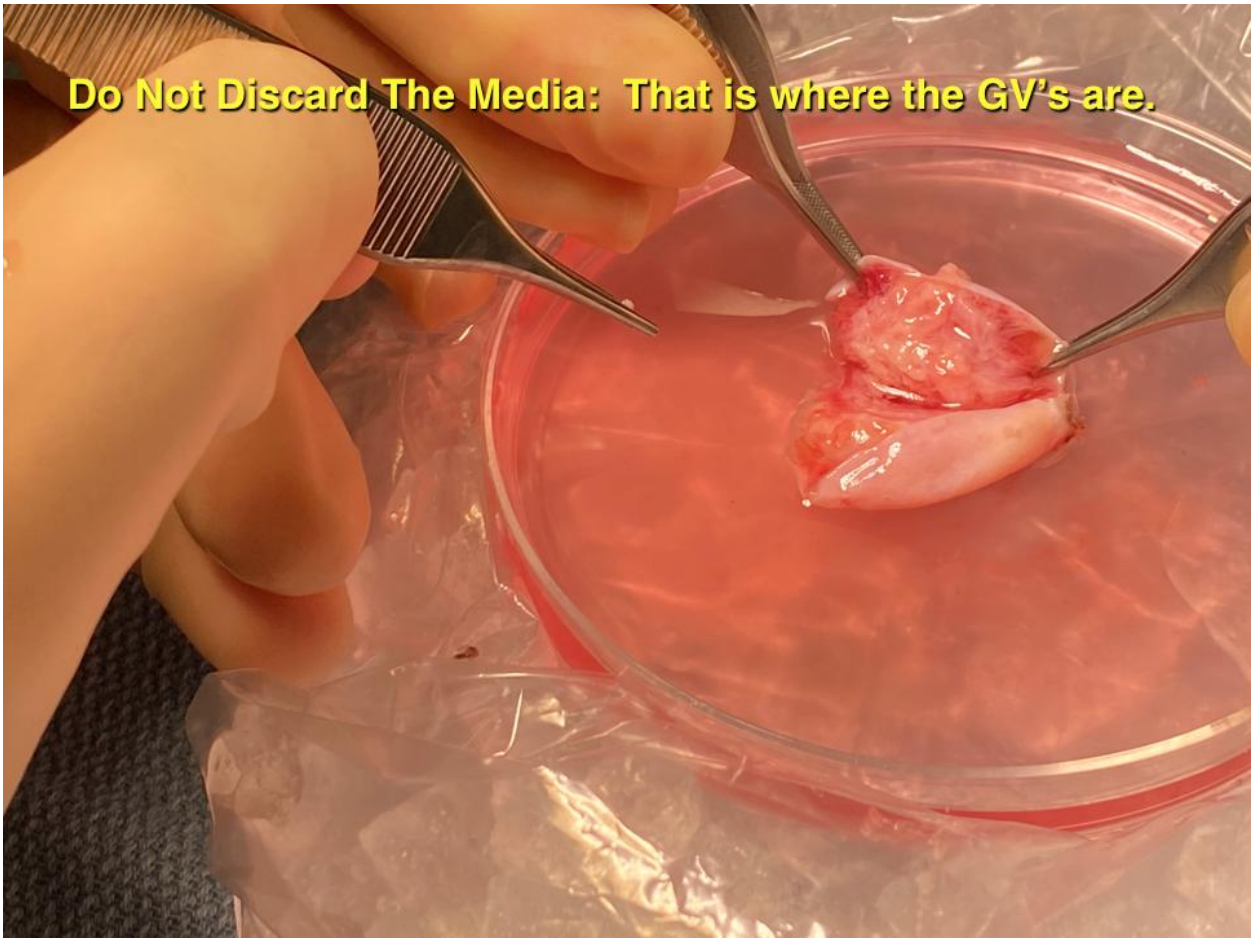

Figure 2: Dissection of ovarian cortex from the medulla.

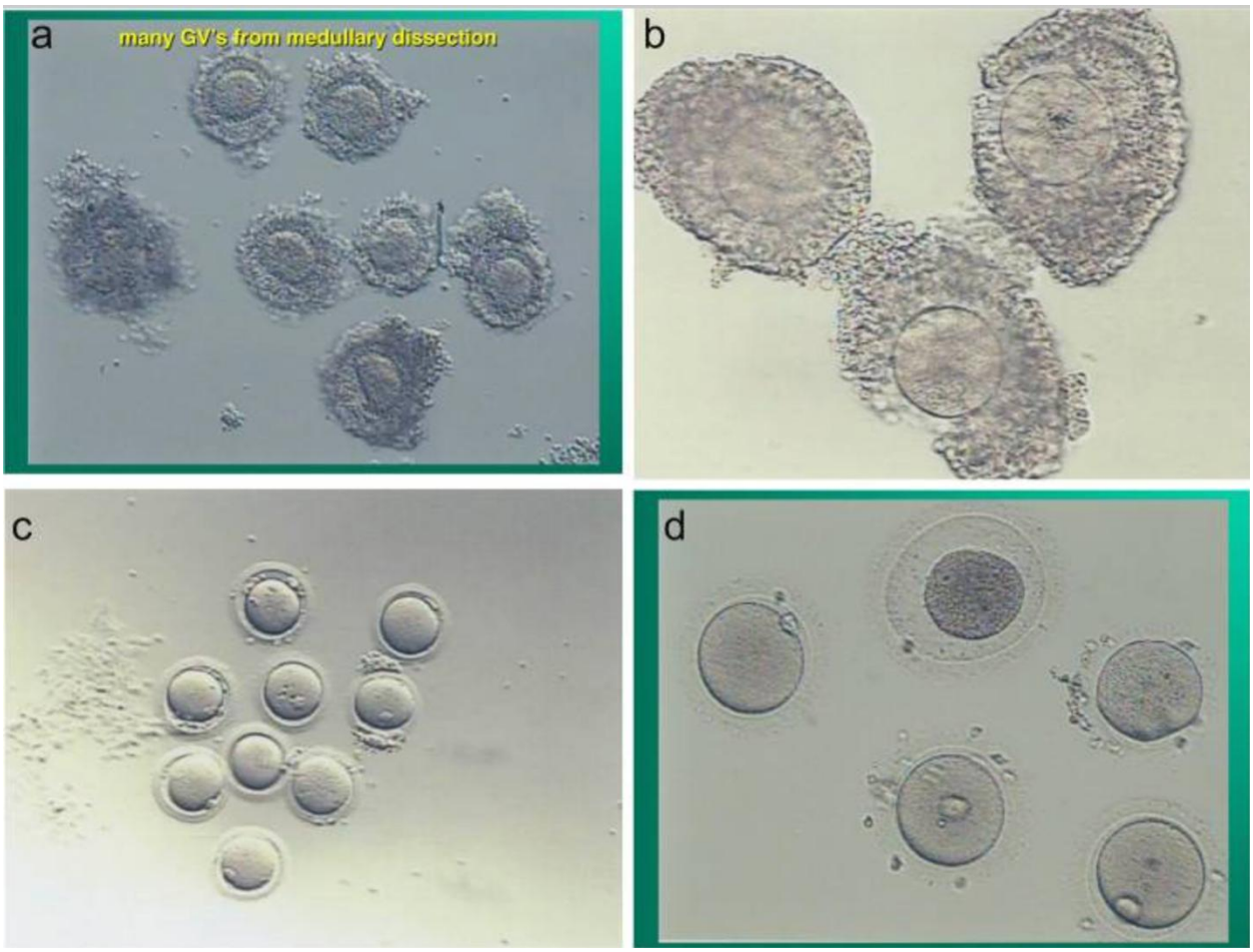

Figure 3a: Day 0 Compact appearance of cumulous complexes (CC's) at time of dissection (Silber et al. 2022).

Figure 3b: Day 1 Spreading of cumulous complexes after 24 hours in culture with HCG or LH (Silber et al. 2022).

Figure 3c: Day 1 Metaphase II oocytes after 24 hours in culture with HCG or LH (Silber et al. 2022).

Figure 3d: Three normal M II oocytes, one M I oocyte, and one degenerated oocyte, resulting from cultured ovarian tissue GV's, on day 2 (Silber et al. 2022).

CCs, MII Oocytes, and IVM Rate by Age Group

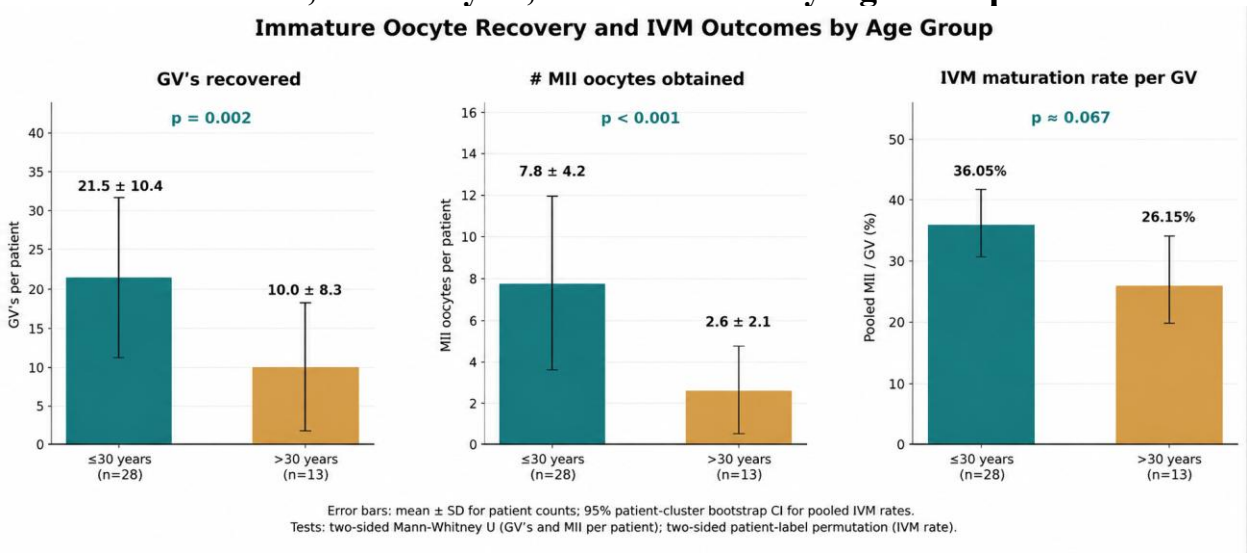

Figure 4: Comparison of cumulus oocyte complexes (CCs), mature metaphase II (MII) oocytes, and in vitro maturation (IVM) rate between patients aged ≤30 years and >30 years. Bars represent mean ± SEM. Patients aged ≤30 years had significantly higher CC yield, MII oocyte yield, and IVM maturation rate compared with patients aged >30 years.

Figure 5: Levels of FSH and anti-Müllerian hormone (AMH) levels for women who underwent cryopreserved ovary transplantation. Women reported laboratory results to the center on a monthly basis (on average). Blood was drawn on day three of the woman's cycle and follow-up

continued throughout the functional life of the ovarian tissue. The best fit curves show a clear increase in AMH levels (mean = 0.43, SD = 0.52) before their return to normal as well as the decrease in FSH levels (mean = 34.49, SD = 40.35) 4-5 months after recruitment. (Silber et al., 2015). This indicates an over-recruitment of primordial follicles at the time of transplant and long term continued ovarian function despite low AMH (because of decrease in follicle recruitment when the ovarian reserve is low).

TABLES

Patient Age	Reason For Cryopreservation	Culture Media Base Used	FSH/HCG	Number of CCs	M II Oocytes Frozen	IVM Rate	Total OTF Pieces
27	Large B Cell Lymphoma of the Cervix	Quinn's Cleavage	75mIU/ml FSH +10mIU/ml HCG	25	12	48%	21
33	Triple Negative Breast Cancer: BRCA Positive	Quinn's Cleavage	75mIU/ml FSH+ 1000mIU/ml HCG	11	(cumulus 1 stripped immediately)	9%	17
39	Fertility Preservation (Social)	Medicult IVM	75mIU/ml FSH +1000mIU/ml HCG	3	2	67%	17
17	Large B Cell Lymphoma	Medicult IVM	75mIU/ml FSH+ 1000mIU/ml HCG	10	2	20%	20
14	Ewing Sacroma	Quinn's Cleavage	150mIU/ml FSH +20mIU/ml HCG	34	10	29%	21
30	Fertility Preservation for Turner's Syndrome Daughter	Quinn's Cleavage	75mIU/ml FSH +10mIU/ml HCG	24	9	38%	10
18	Breast Cancer	Quinn's Cleavage	75mIU/ml FSH +10mIU/ml HCG	8	2	25%	16
33	Breast Cancer	Quinn's Cleavage	75mIU/ml FSH +1000mIU/ml HCG	26	5	19%	10
25	Breast Cancer	Sage IVM	75mIU/ml FSH +1000mIU/ml HCG	14	5	36%	10
14	Non-Hodgkin's Lymphoma	Sage IVM	75mIU/ml FSH +100mIU/ml HCG	37	14	38%	20
19	Hodgkins Lymphoma	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	40	14	35%	22
13	Rhabdomysacroma	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	25	14	56%	16
22	Fertility Preservation for Premature Ovarian Failure Sister Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	34	14	41%	22 (d)
20	Fertility Preservation Premature Ovarian Failure Sister Recipient	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	10	4	40%	22 (r)
23	Breast Cancer	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	40	10	25%	33
33	Breast Cancer	Quinn's Cleavage	75mIU/ml FSH 100mIU/ml HCG	3	1	33%	9
41	Breast Cancer	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	5	2	40%	13
33	Rhabdomyosarcoma	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	18	6	33%	24
36	Premature Ovarian Failure	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	20	7	35%	12
20	Hodgkins Lymphoma	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	15	3	20%	22
27	Breast Cancer BRCA+	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	20	4	20%	26
38	Fertility Preservation for Turner Syndrome Daughter Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	3	1	33%	16
17	Lymphoma	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	18	6	33%	10
33	Breast Cancer	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	1	1	100%	17
26	Fertility Preservation for unrelated Woman with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	22	4	18%	32
16	Malignant neoplasm of an unspecified kidney	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	13	3	23%	12
17	Hodgkins Lymphoma	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	15	5	33%	17
33	Fertility Preservation for niece with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	9	3	33%	18
22	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	24	9	37%	12
25	Fertility Preservation for unrelated Woman in remission from Leukemia Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	15	5	33%	11
25	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	17	13	76%	14
24	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	41	6	14%	12
26	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	15	9	60%	15
31	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	20	3	15%	14
27	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	27	7	25%	14
36	Fertility Preservation for Daughter with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	8	1	12%	10
26	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	20	10	50%	10
Average IVM Rate 33% M II's Vitrified			Average Number Ovary Pieces Vitrified 17				

Table 1: 41 consecutive post-pubertal IVM in patients with no prior chemotherapy.

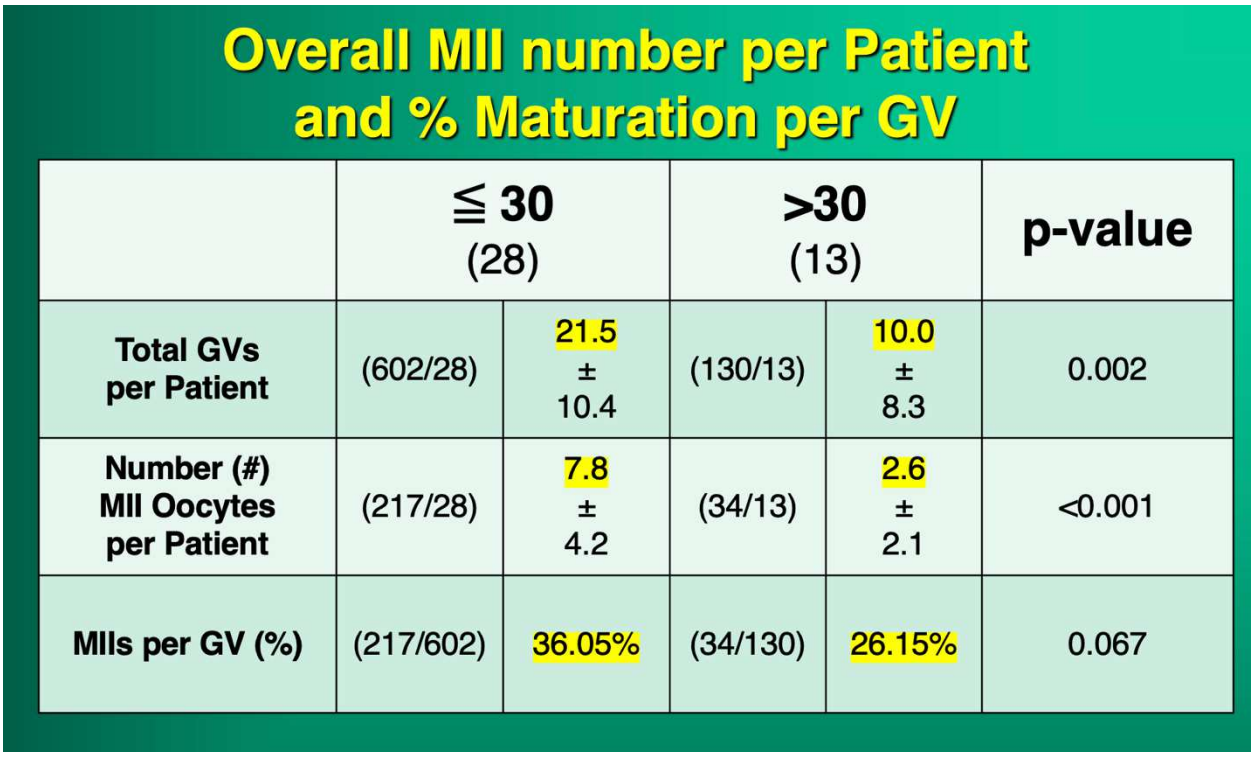

Table 2: Data represent the mean ± standard deviation (SD) of germinal vesicle (GV) oocytes recovered and metaphase II (MII) oocytes obtained per patient, along with pooled in vitro maturation (IVM) rates. Patients were stratified into two age groups: ≤30 years (n = 28) and >30 years (n = 13). Pooled IVM maturation rates were calculated as the total number of MII oocytes obtained divided by the total number of GV oocytes recovered within each age group, expressed as a percentage. Between-group comparisons of GV and MII oocyte yields were performed using two-sided Mann–Whitney U tests, while differences in pooled IVM maturation rates were assessed using a two-sided patient-label permutation test. Statistical significance was defined as $p < 0.05$.

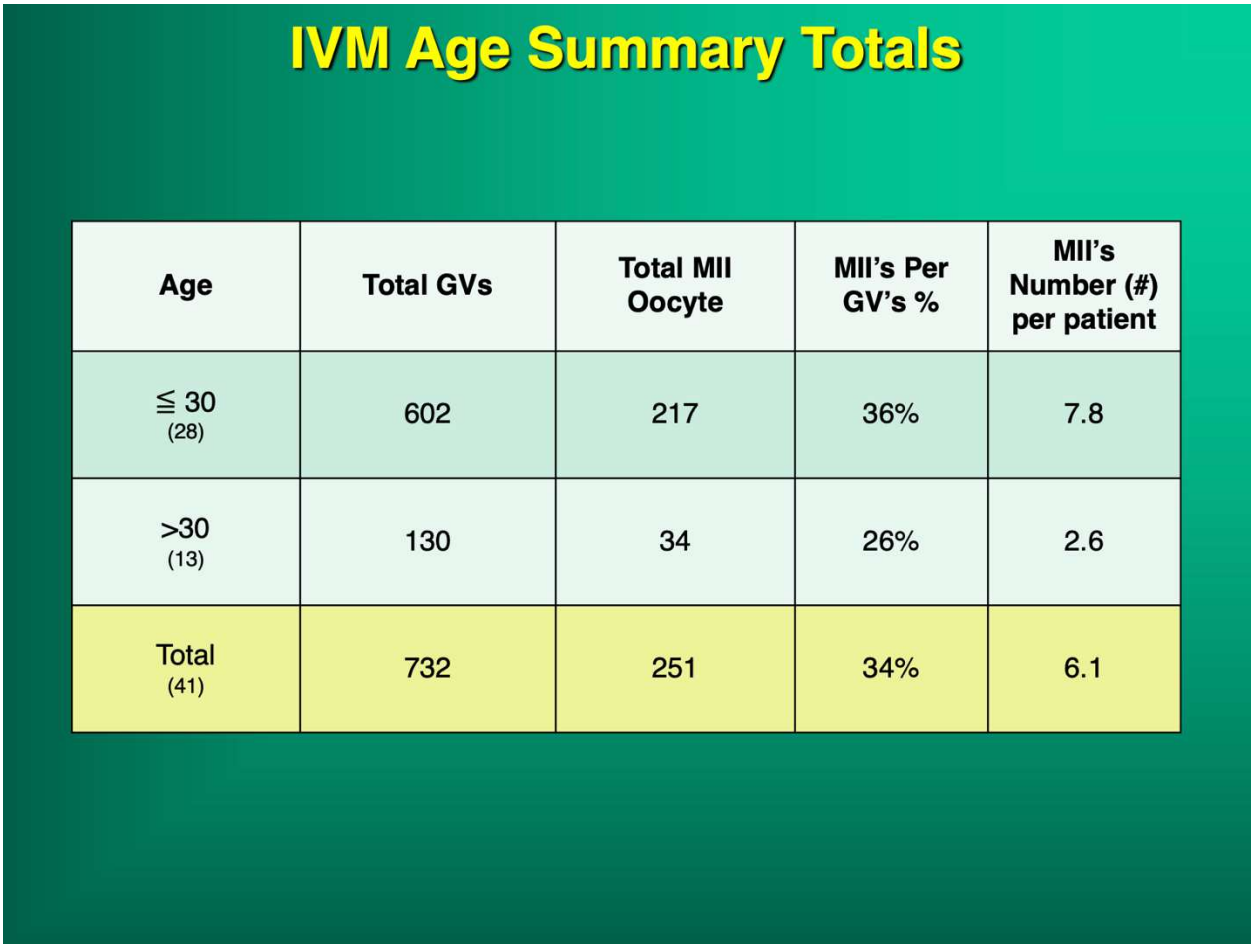

Table 3a: Data represent the total number of germinal vesicle (GV) oocytes recovered, total metaphase II (MII) oocytes obtained following in vitro maturation (IVM), pooled IVM maturation rate (MII/GV × 100), and mean number of MII oocytes obtained per patient. Patients were stratified into two age groups: ≤30 years (n = 28) and >30 years (n = 13). The pooled IVM maturation rate was calculated by dividing the total number of MII oocytes obtained by the total number of GV oocytes recovered within each age group.

IVM Age Summary Totals

Age	Total Number Of GVs	GVs Per Patient	MII Oocyte Per GV	IVM Rate Per GV
7-22 (14)	289	20.6	104	36%
23-26 (9)	192	21.3	67	35%
27-30 (5)	121	24.2	46	38%
>30 (13)	130	10.0	34	26%
Total (41)	732	17.8	251	34%

Table 3b: Data represent the total number of germinal vesicle (GV) oocytes recovered, total metaphase II (MII) oocytes obtained following in vitro maturation (IVM), pooled IVM maturation rate (MII/GV × 100), and mean number of MII oocytes obtained per patient. Patients were stratified into two age groups: ≤30 years (n = 28) and >30 years (n = 13). The pooled IVM maturation rate was calculated by dividing the total number of MII oocytes obtained by the total number of GV oocytes recovered within each age group.

Variable High IVM Rates

Patient Age	Reason For Cryopreservation	Culture Media Base Used	FSH/HCG	Number of CCs	M II Oocytes Frozen	IVM Rate	Total OTF Pieces
27	Large B Cell Lymphoma of the Cervix	Quinn's Cleavage	75mIU/ml FSH +10mIU/ml HCG	25	12	48%	21
39	Fertility Preservation (Social)	Medicult IVM	75mIU/ml FSH +1000mIU/ml HCG	3	2	67%	17
13	Rhabdomysacroma	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	25	14	56%	16
25	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	17	13	76%	14
26	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	15	9	60%	15
26	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	20	10	50%	10
24	Fertility Preservation for Sister with Turner's Syndrome Donor	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	8	5	62%	8
7	Congenital Neutropenia	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	6	4	66%	5
30	Fertility Preservation for Sister with Leukemia	Quinn's Cleavage	75mIU/ml FSH +100mIU/ml HCG	25	14	56%	13

Table 4: Representative cases demonstrating high in vitro maturation (IVM) rates across patients of varying ages and indications for ovarian tissue cryopreservation. Data include patient age, indication for cryopreservation, culture medium, gonadotropin supplementation, number of cumulus–oocyte complexes (CCs) recovered, number of metaphase II (MII) oocytes cryopreserved, IVM maturation rate, and total ovarian tissue fragments (OTF) obtained. IVM maturation rates were calculated as the number of MII oocytes divided by the number of CCs recovered, expressed as a percentage. Despite an overall pooled IVM maturation rate of 34.3% in the full cohort, considerable interpatient variability was observed, with several patients achieving maturation rates exceeding 50%. This variability may reflect differences in the developmental competence of recovered immature oocytes, including the presence of both developing and degenerating oocytes. We hypothesize that selective recovery of immature oocytes from smaller antral follicles (4–6 mm) may improve maturation efficiency, potentially achieving rates exceeding 50%; however, this hypothesis requires further investigation.