Chapter

In Vitro Maturation and Fertilization of Oocytes: From Laboratory Bench to Clinical Practice

Adrian Ellenbogen, Einat Shalom Paz, Medeia Michaeli, Anna Smirnova and Yona Barak

Abstract

Retrieval of immature oocytes from non-stimulated ovaries, followed by in vitro maturation (IVM), was initially proposed in order to avoid side effects of gonadotropin administration. The goal is to eradicate or significantly decrease the risk of ovarian hyperstimulation syndrome (OHSS) in patients with polycystic ovary syndrome (PCOS) and to reduce drug cost and burden of patients. This technology was also proposed for treatment of normal ovulatory women, fertility preservation, or infrequent conditions as failure of oocyte to mature or repeated development of poor-quality embryos. There is no downregulation, and only a small amount of hormones are injected if at all. In vitro maturation of the oocyte procedure obtained up to 35% clinical pregnancy rate in young women, compared with in vitro fertilization (IVF) in many programs. The obstetric and perinatal outcomes of IVM cycles are comparable with IVF/ICSI cycles; therefore it may gradually substitute IVF in certain cases, as the technique continues to develop and pregnancy rates continue to increase. IVM holds great promises as an alternative to assisted reproductive technologies and may be the procedure of choice not only for infertile patients but also for obtaining oocytes for donation or fertility preservation.

Keywords: in vitro fertilization, in vitro oocyte maturation, oocytes, fertilized ovum, pregnancy outcome, pregnancy rates, reproductive techniques

1. Introduction

The concept of in vitro maturation of oocytes (IVM) was firstly mentioned in literature by Pincus and Enzmann, initially in 1935 [1]. They conducted experiments in which ova, taken from tubes at various intervals after fertile matting, were cultured in vitro. Thirty-four years later, Eppig and Schroeder [2] designed the possibility to use IVM rather than protocols of hormonal stimulation currently in use. These authors mentioned "it may be possible to recover immature oocytes from several antral follicles, excluding the dominant preovulatory follicle, and mature them in proper culture." At that time it was impossible to accomplish that assignment due to technical reasons. However, 2 years later, Cha et al. [3] mentioned, "this is an engineering problem with the ultrasound equipment that will be resolved in

the future." Recently, Cha et al. were the first to succeed with IVM in human using immature oocytes retrieved from antral follicles. Trounson et al. [4] were the first who put IVM firmly in the clinical realm, obtaining a live birth from oocytes, recovered from untreated polycystic ovarian patient who underwent in vitro maturation.

In vitro maturation of oocytes have potential advantages over conventional IVF: a simple protocol with decreased or no hormonal stimulation before oocyte retrieval, lower cost of the treatment cycle, and reduced psychological impact. Moreover, and not of less of importance, the risk of OHSS is entirely avoided. Despite these benefits, however, there are still many debatable problems surrounding this treatment. Until one can say that IVM could be an alternative to conventional IVF treatment, these advantages have to be weighed against the pregnancy and delivery rates, children outcome, and possible risks.

There are several basic differences between routine in vitro fertilization (IVF) and IVM. These differences might be related to the size of aspirated follicles, the nuclear and cytoplasmic maturity of the oocytes, and the laboratory procedures. In order to achieve proper fertilization and embryo development, Gougeon and Testart in [5] pointed out that the follicles at the time of the collection for IVM procedures should be in the early antral to antral stage (0.2–14 mm) vs. preovulatory stage (0.16–20 mm) for IVF. After retrieval, majority of the oocytes are immature (MI, GV). However, only few will proceed in vitro to metaphase II (MII) in culture media, some after 6 h, but most of them after 24–48 h and only then will be able to undergo successful fertilization after ICSI. This is in comparison with conventional IVF, where most of the retrieved oocytes are mature, and fertilization might occur immediately after ICSI.

There are many controversial areas of debate, especially regarding the process of oocyte maturation in vitro: (a) nuclear maturation—the process that reverses meiotic arrest at prophase I (GV), driving the progression to MII. This process is followed by the expansion of cumulus granulosa cells and loss of intercellular communications between the cumulus cells and also between cumulus and the surface of the oocyte (a visible laboratory course); (b) cytoplasmic maturation—metabolic and structural modifications within the oocyte that prepares the ovum for activation, fertilization, and embryonic development (an invisible laboratory process) [2, 4, 5]; it should, therefore, be kept in mind that IVM may not be free of possible future complications and counseling of the patients thoroughly before commencing the treatment is essential.

2. Guidelines for IVM treatment

2.1 Ultrasound monitoring

Follicle monitoring follow-up is mainly performed using ultrasound scans of the ovaries and endometrium. However, in contrast to IVF, in IVM cycles, there is no need for serum hormonal level (estradiol, progesterone) follow-up.

First ultrasound should be performed on days 2–3 of the menstrual (or induced) cycle in order to record the number and size of all follicles along with endometrial thickness and a second scan on days 6–8, to determine the presence and size of the largest follicle in each ovary. A third scan should be completed on day of hCG trigger, to measure the endometrial thickness (**Figure 1**).

2.2 FSH priming

Fadini et al. [6] reported that 77.4% of retrieved immature oocytes underwent maturation in vitro followed by 29.8% pregnancy rate vs. 48.4 and 15.2% in primed and non-primed IVM cycles, respectively. Mikkelsen et al. [7] pointed that priming

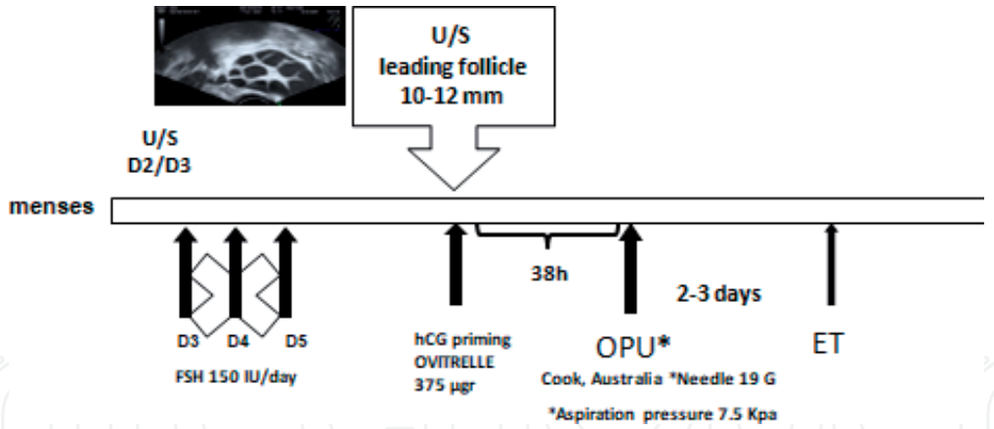

Figure 1.
In vitro maturation oocytes: clinical protocol.

with r-FSH for 2–3 days before the harvesting of immature oocytes from patients with polycystic ovarian syndrome (PCOS) may improve the maturational potential of the oocytes and the implantation rate. Similar results were described by other investigators in PCOS patients [8–11].

2.3 Human chorionic gonadotropin (hCG)/GnRh-agonist priming and retrieval interval

The beneficial effect of hCG priming in IVM cycles and extension of the period of time from 35 to 36 h (routinely administrated in IVF cycles) to 38 h from hCG administration was demonstrated by Son et al. [12]. This hCG priming promotes GV oocytes to reach MI stage and increases the maturation rate of immature oocytes in vitro. Gonadotropin-releasing hormone agonist (GnRH-a) has been used recently in triggering oocyte maturation. In this approach, small follicles were stimulated with gonadotropins for 3–5 days. GnRH-a administration was performed, to trigger ovulation, when the largest follicles were 10–12 mm in diameter. Many immature oocytes, which underwent maturation in vitro, were then harvested, fertilized, and subsequently developed into blastocysts that resulted in live births [13]. This interesting observation might have a great importance in follicular cytoplasmic maturation due to the FSH surge obtained after GnRH triggering. The FSH surge might promote formation of LH receptors on the granulosa cells enhancing LH activity, induce plasminogen activator activity causing dissociation of oocytes from somatic cells of the follicle (therefore more immature oocytes could be obtained), and maintain the opening of gap junction between cumulus cells and oocyte which contributes to oocyte maturation [14–17].

2.4 Timing of collection: at what follicle size?

Son et al. [18] conducted a study in which patients were triggered when the leading follicle was <10 mm, 10–14 mm, or >14 mm. In the group with a leading follicle >14 mm, only one pregnancy was obtained. The authors of the current paper administer, therefore, hCG trigger once a leading follicle of 10–12 mm is developed.

2.5 Endometrial preparation and luteal support

In IVM, estradiol levels are physiological, and oocyte collection is done before the endometrium is fully estrogenized. After aspiration, there is an insufficient support from the corpus luteum for endometrial receptivity. It was demonstrated

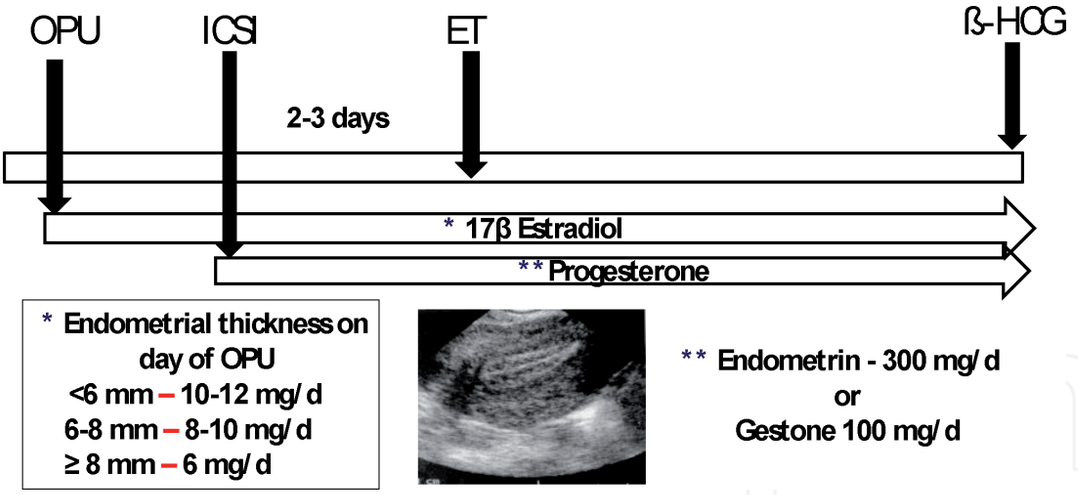

Figure 2.
Endometrial support.

that endometrial thickness is an important predictor of IVM outcome [19]. Thus, endometrial preparation is an important factor for IVM success rates. The protocol for endometrial preparation, which the authors currently use, is similar to that originally described by Trounson et al. [19] and modified by Elizur et al. [20], depending upon the endometrial thickness at day of hCG administration: when endometrial thickness is less than 6 mm, 6–8 mm, or higher than 8 mm, supplementation with 10–12 mg/day, 8–10 mg/day, and 6 mg/day 17-beta estradiol is given, respectively, starting at the day of oocyte retrieval. This approach mimics the natural estrogen rise from the dominant follicle in a natural cycle. In terms of progesterone, supplementation usually begins on the day of oocyte aspiration (as this is the progesterone rise in a natural cycle) using vaginal micronized progesterone (Endometrin, Florish Ltd. Industrial Park Misgav, Israel, Ferring Pharmaceuticals Ltd.) 300 mg daily until pregnancy test is performed (**Figure 2**).

2.6 Oocyte retrieval by transvaginal ultrasound

An high-resolution ultrasound device is obligatory. The oocyte retrieval is done with a single-channel needle 19 G (Swemed, Reduced Single Lumen, Vitrolife, Göteborg, Sweden AB), using a reduced aspiration pressure of 7.5 kPa. This is essential to minimize damage to the immature oocytes.

Usually general anesthesia/sedation is provided. However, local anesthesia with lidocaine 1%, 5 cc injected into the lateral fornixes, could be sufficient.

Oocytes from each ovary are aspirated in separate flask containing 15 ml of flushing medium (Origio, Denmark) and placed on a heated block. Collected cumulus oocyte complexes (OCCs) were classified immediately after OPU (Day 0).

Oocyte cumulus complexes could be classified in one of five groups: (1) expanded cumulus, slack and fluffy multilayer of granulosa cells; (2) full cumulus, multilayer of strictly compact and cubical granulosa cells; (3) full corona, thin layer of strictly compact and cubical granulosa cells; (4) partial cumulus, oocytes surrounded partially with cumulus cells; (5) nude, oocytes without cumulus cells (**Figure 3**). This classification may serve as a prognostic indication of the future maturation and fertilization rate of the immature collected oocytes.

Each complex was then separately cultured in IVM medium (Sage, Cooper Surgical Company, Trumbull, CT, USA) supplemented with FSH + LH (Menogon, Ferring GmbH, Kiel, Germany) with a final concentration of 75 mIU/ml (maturation medium). Oocyte maturation was assessed after 6 (a) and 24–30 h (b),

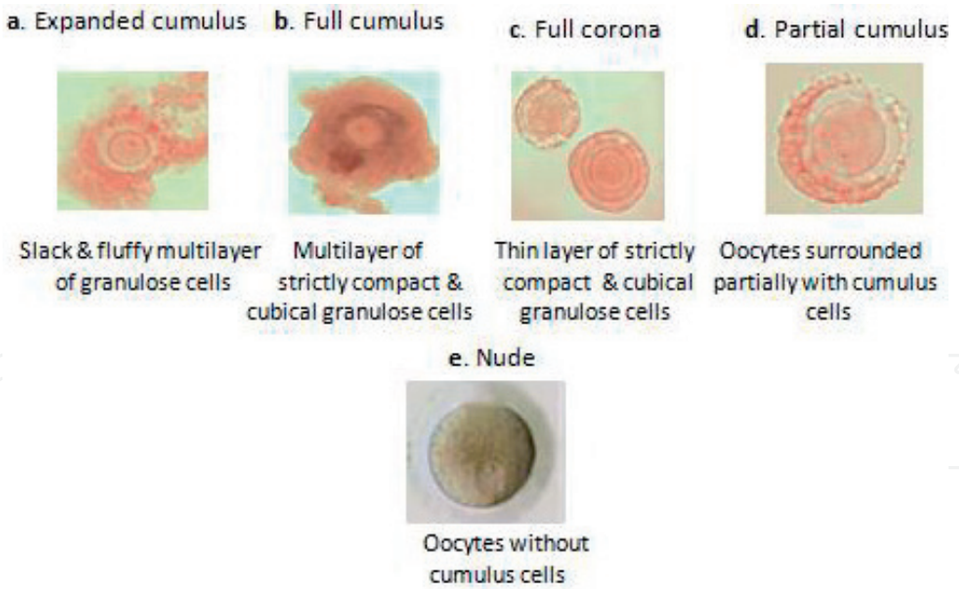

Figure 3.
Classification of collected cumulus complexes.

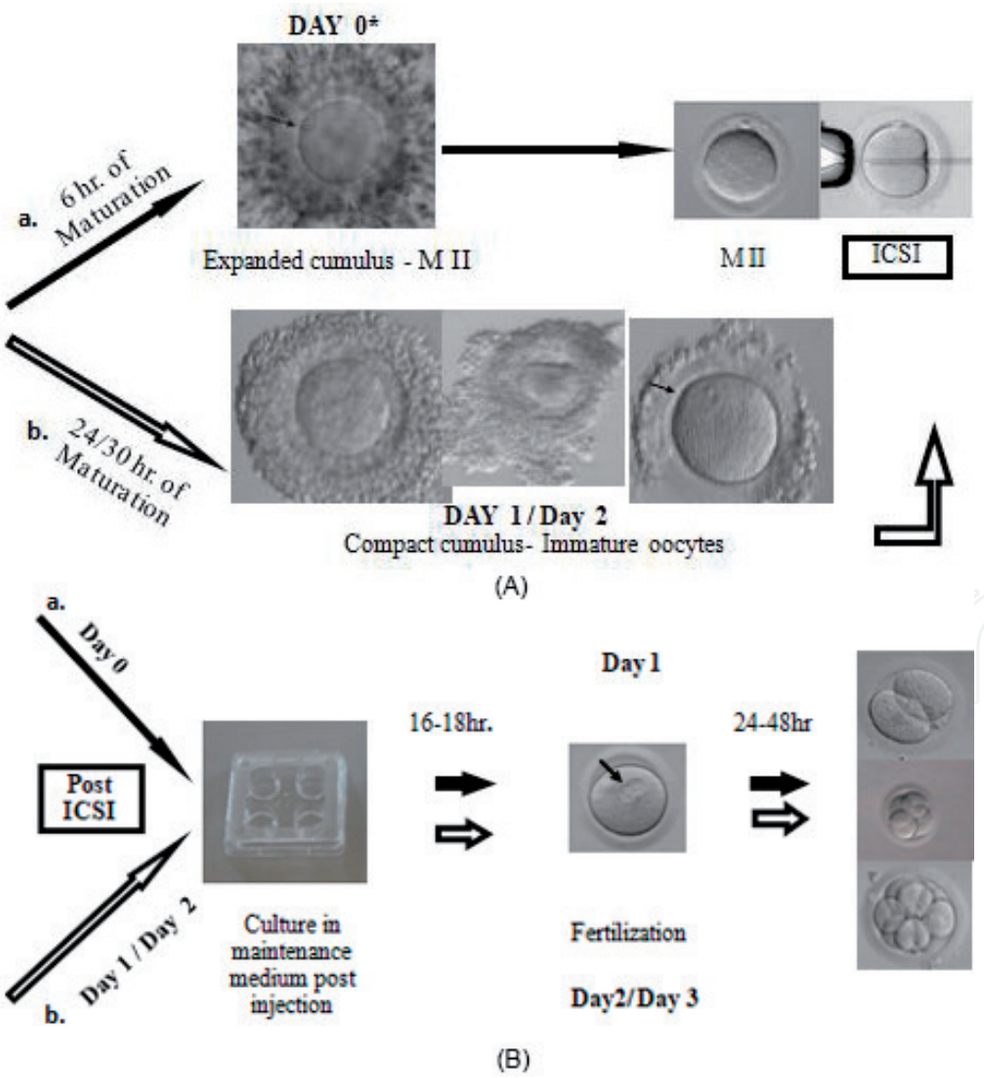

Figure 4.
*(A) In vitro maturation procedure. *Day 0 = day of OPU; [a]MII oocytes detected in maturation medium 6 h after OPU (solid fill arrows); [b]MII oocytes detected in maturation medium 24–30 h after OPU (open arrows) and (B) in vitro maturation procedure. *Day 0 = day of OPU; [a]MII oocytes detected in maturation medium 6 h after OPU (solid fill arrows); [b]MII oocytes detected in maturation medium 24–48 h after OPU (open arrows).*

according to oocyte cumulus complex classification. This was followed by oocyte intracytoplasmic sperm injection (ICSI) for the injection of a single sperm cell into the MII oocytes on the day of OPU or 1–2 days afterward, according to oocyte maturation (**Figure 4A** (a and b) and **B** (a and b)). Two to three embryos were transferred 48–78 h post ICSI. Supplementary embryos were vitrified.

3. Definitions of IVM in human

The biological definition of oocyte IVM is to aspirate GV oocytes from antral follicles and culture them for in vitro maturation to a MII stage. Apart from routine IVF, the clinical definition of an IVM cycle should include an understanding that it involves the retrieval of oocytes from small and intermediate-sized follicles in an ovary before the largest follicle has surpassed 13 mm in mean diameter [21]. However, since modifications of the IVM technique are commonly employed, it is suggested, though, to designating the cycle when an oocyte trigger is given. When oocyte triggering is not performed, the cycle should be designated as IVM without triggering. When the addition of gonadotropin stimulation is given for few days in the early follicular phase, the cycle should be designated as IVM with short gonadotropin stimulation or modified natural cycle IVF with early triggering combined with IVM (if hCG or GnRh-agonist triggering was delivered) [21].

4. Indications for IVM

The indications for IVM include normal ovulatory women (mechanical factor or male infertility), PCOS patients or susceptible to develop ovarian hyperstimulation (OHSS), contraindication to hormonal administration, patients with estrogen-sensitive cancers, or those who require rapid fertility preservation before undergoing potentially gonadotoxic treatments. Other occasional indications may include fertilization failure; poor ovarian response [22]; rescue of oocytes, which have failed to mature in stimulated cycles [23]; or unexplained primarily poor-quality embryos [24].

4.1 IVM in women with normal ovulatory cycles

In early studies, Mikkelsen et al. [7, 25, 26] reported a 17–18% pregnancy rates resulting from in vitro matured oocytes. These pregnancy rates were disappointingly low in comparison with regular IVF results obtained in that time. A constant improvement in pregnancy rate up to 30% was achieved during the last decade, mainly due to application of FSH/hCG priming in IVM protocol [6] and proper patient selection [27]. It seems that in normal ovulatory patients IVM may be an intriguing alternative to conventional IVF techniques resulting in comparable pregnancy rates. It removes the side effects of pituitary suppression and gonadotropin stimulation, especially OHSS; reduces the costs of the entire procedure, both in terms of time consumption and patient/society costs for drugs; and reduces psychological impact.

4.2 PCOS patients

PCOS patients are likely to develop OHSS with conventional IVF treatments. Substituting IVM in PCOS patients eliminates the risk of OHSS and lowers the cost of the treatment. From the early 2000s until nowadays, studies have demonstrated a reassuring pregnancy and delivery rate in PCOS patients undergoing

IVM treatments of 21.9–29.9% [28–31]. Recent studies reported up to 32–44% pregnancy and 22–29% delivery rates [32, 33], compared with IVF pregnancy rate results of 38–45% [33–35]. Junk and Yeap transferred a single blastocyst embryo obtained after IVM in patients with PCOS. A live birth rate of 42.4% per oocyte collection and 45.2% per embryo transfer were obtained [34]. Vitek et al. [35] have recently described implantation, pregnancy, and delivery rates of 17.5, 40, and 40%, respectively, in 20 estrogens suppressed in vitro maturation cycles. In a latest retrospective study comparing results of 61 IVM vs. 53 antagonist protocol cycles in young patients with PCOS, a comparable pregnancy and delivery rates of 30% vs. 40% and 21.3% vs. 28.8%, respectively, was obtained [36]. Those recent reports are encouraging, as in Europe the pregnancy and delivery rates in this group of patients undergoing ICSI was 35.5% and 24.3%, respectively [37]. de Ziegler et al. [38] opposed the need of IVM in the gonadotropin-releasing hormone (GnRH) antagonist era. However, his conclusion is based on outdated publication [28, 39, 40], with poor results in terms of pregnancy and delivery rates in IVM. However, to update, it is ascertained that such data has already improved. GnRH-agonist (GnRH-a) used as a trigger to control the risk of OHSS may cause higher pregnancy losses due to luteal phase defects [41]. In order to overcome this complication in antagonist protocol/agonist trigger, the dual-trigger approach (GnRH-a + low hCG) was proposed; 2.9% of OHSS complications developed [42]. GnRH trigger combined with intensive luteal support in OHSS high-risk patients can facilitate fresh embryo transfer; however, the occurrence of late OHSS was not totally eliminated [43]. Applying the policy of ovarian stimulation with a dual-trigger approach and freezing all of the oocytes or embryos for future use [44] do not, necessarily, eliminate totally OHSS. In a few patients after dual trigger and freeze all, severe OHSS was reported [45]. It seems that in PCOS patient, IVM is a simple, less stressful, and economical protocol of treatment. The puncture is simple and safe, and it may improve the disrupted endocrine environment and induce a spontaneous recovery of ovulation in women with PCOS [46]. It can also avoid short-term complications, such as OHSS, and elude massive hormonal stimulation and long-term complications, such as hormone-dependent neoplasms including breast and ovarian cancer.

4.3 Fertilization failure

Repeated IVF failure is a highly upsetting condition for patients who have apparently normal ovarian stimulation and follicular development, which underwent numerous unsuccessful IVF cycles with no embryos for transfer. Often, these patients are referred to surrogacy or egg donation program, which is also a psychological and economic burden for the couples [47]. Failures following IVF treatment might occur due to many reasons, such as formation of low-quality embryos, maturation arrest of oocytes [48], uncertain diagnosis of oocyte factor, or empty follicle syndrome. Thus, IVM was also proposed for treating patients with poor ovarian response; moreover it might serve the last choice to achieve pregnancy in IVF [22]. Other indications can be applying IVM in rare conditions, such as to rescue oocytes which have failed to mature in stimulated cycles [49] or cases with unexplained primarily poor-quality embryos. Hourvitz et al. [23] examined efficacy of IVM in seven patients with three or more conventional IVF failures due to abnormal oocyte development due to empty follicle syndrome, oocyte maturation arrest, or failure of fertilization. Four women received minimal ovarian stimulation with FSH. Oocytes were obtained in all patients: mean maturation rate was 39.6%, and mean fertilization rate is 45.8%. Embryo transfer was performed in four women; two patients with previous empty follicle syndrome conceived and delivered.

4.4 Fertility preservation

The emerging technology of IVM has recently become another option for fertility preservation. This process can be done without hormonal stimulation [50]. In the cases of cancer patients, who must be started on immediate chemotherapy, IVM might be the only option to preserve fertility by collecting oocytes during the follicular phase, within up to 13 days from cancer diagnosis, and cryopreservation [51, 52]. To shorten the period of time until cancer treatment, studies by Maman et al. [53] reported on luteal phase minimal ovarian stimulation with a reasonable number of harvested oocytes. Therefore, in the cases of cancer patients, especially in whom hormonal treatment is contraindicated and in those who must start chemotherapy without postponement, IVM might be the only choice to preserve fertility [53]. Recently, one successful pregnancy resulting from cryopreserved embryos obtained from IVM oocytes after oophorectomy in an ovarian cancer patient was reported [54]. Other studies have raised the possibility to preserve fertility even in pediatric patients.

Preserving in vitro matured oocytes from antral follicles found in harvested ovarian tissue is an experimental technique that offers a possible advantage over ovarian tissue cryopreservation. Using a mature, frozen, and later thawed oocyte for fertilization might serve as a safer option for fertility preservation than reimplantation of ovarian cortex tissue, due to the risk of malignant cell reseeding [55]. Caravani et al. followed a total of 84 chemotherapy-naïve patients ages < 1–18 years old, who were referred for fertility preservation. Thirty-three children were premenarche and 51 postmenarche. IVM was performed in the pre- and postmenarche groups and in subgroups of very young (up to age 5 years) and older (5–10 years) premenarche patients. The study concluded that IVM is feasible in the prepubertal age group. However, the success of in vitro maturation of those oocytes was correlated with the patient's age (more oocytes were obtained from the post pubertal vs. prepubertal); no mature oocytes are cryopreserved for girls under the age of five [55]. Additionally, it was found that fertilization potential of oocytes was negatively affected after vitrification of IVM oocytes [56]. This implies that vitrification/warming itself could also induce some detrimental effects on IVM oocytes. Actually, present vitrification methods have been adapted to use good-quality in vivo matured oocytes from young women. Therefore, studies to improve survival and further embryological developmental competence of the oocytes retrieved from IVM program are urgently required in order to successfully apply them to IVM fertility preservation program for cancer patients [57].

5. Pregnancy results

Two thousand healthy infants have been born following immature oocyte retrieval and IVM [58].

5.1 Obstetric and fetal complications

Soderstrom-Anttila et al. presented comparable complications and malformations for babies born after IVM and IVF [31]. Buckett et al. commented on a normal pregnancy course for IVM patients compared to routine IVF [59]. Fadini et al. performed a retrospective cohort study involving 196 babies born from IVM cycles compared with 194 children born from conventional ICSI cycles, which were performed during the same period of time. In single births, gestational age at delivery was comparable, but birth weight was significantly higher ($P = 0.009$) in children from IVM cycles (3091 ± 669 vs. 3269 ± 619 g). In a separate analysis of the IVM group, comparing

singleton births derived with certainty from oocytes matured in vitro (n = 71) or in vivo (n = 74), no statistically significant differences were observed in terms of birth weight (3311 ± 637 vs. 3194 ± 574 g, respectively) and gestational age (38.9 ± 2.4 vs. 38.4 ± 2.1 weeks, respectively). In twin births, gestational age was lower in IVM cycles, while weight at birth was comparable (ICSI, 2432 ± 540 g; IVM, 2311 ± 577 g). In single births, major and minor abnormalities were 2 (1.4%) and 6 (4.1%) in the ICSI group and 0 (0.0%) and 8 (5.2%) in the IVM category, respectively. In twin children, major and minor abnormalities were 1 (2.2%) and 2 (4.3%) in ICSI babies and 0 (0.0%) and 2 (4.6%) in IVM cycles, respectively [60].

Obstetric outcome and congenital anomalies of 1421 babies (960 singletons, 442 twins, 15 triplets, and 4 quadruplets) born from 1187 IVM pregnancies were recently summarized by Chian et al. Reassuring results were obtained. The incidence of congenital malformation was 2% in singletons and 1% in twins [61].

6. Future concerns

In vitro maturation as a part of assisted reproductive technologies, may not, yet, be free of possible unidentified future problems. Epigenetic modifications necessary for normal development are established during oocyte growth. In vitro maturation, therefore, may modify the normal maturation of the oocytes [62]. Moreover, the capability of reprogramming the male chromatin after fertilization is dependent upon the maturity of the oocyte. It is questionable, whether this process might be affected by IVM [63]. It was postulated that IVM oocytes were more likely to have abnormal chromosomal configurations and disorganized meiotic spindle microtubules [64]. This finding may be a probable explanation for the reduced developmental potential of oocytes matured in vitro compared to those matured in vivo. However, despite the great achievements obtained in treating infertile couples by standard IVF during the last 34 years, it has become apparent in recent years that ovarian stimulation may itself have disadvantageous effects on oogenesis, with production of aneuploidy [65], reduced embryo quality, and lower endometrial receptivity and might even contribute to perinatal effects [66]. Moreover, human and animal data have demonstrated the potential changes in the implantation process that may occur following superovulation [67]:

1. Changing endometrial gene expression.

2. Causing immunologic changes to the endometrium.

3. Affecting endometrial-embryo interaction causing impairment on fetal development and growth.

4. Increasing the risk of abnormal placentation, leading to increased rates of low birth weight.

7. Improving IVM outcome

There is no doubt that efforts should be made to improve IVM outcome. An adequate learning curve taking into consideration clinical decisions, retrieval procedure, laboratory knowledge, and experience is required [33, 68].

Improving culture condition to optimum must be determined, for instance, adding epidermal growth factor family molecules, such as amphiregulin and epiregulin

to the culture media which augmented oocyte maturation [69], or brain-derived neurotrophic factor (BDNF) and glial-cell-derived neurotrophic factor (GDNF), which recently were reported to improve maturation rates in human oocytes [70], or addition of dibutyryl cyclic adenosine 3′,5′-monophosphate (cAMP) to mouse oocytes in vitro to arrest germinal vesicle break down, in order to combine it with the cytoplasmic maturation [71].

8. Conclusions

The results of the process of IVM may be comparable and may have advantages over standard IVF. It is a simple procedure without pituitary downregulation. For stimulation of IVM cycle, a very small amount of hormones are administered if at all. Treatment time is short with low side effects (no OHSS), resulting in a reduced psychosocial impact.

The method of IVM holds great promise as an alternative to assisted reproductive technologies and may be the procedure of choice not only for infertile patients but also to obtain oocytes for donation or fertility preservation. Improving embryonic-endometrial synchrony through pharmaceutical or other manipulation of endometrial/uterine receptivity will hopefully improve IVM success rates. Appropriate counseling of the patients about the benefits and difficulties of the process should be done routinely [72].

9. Summary points

In vitro maturation of oocytes is a simple and less stressful process of treatment.

Main indication: PCOS patients, selected cases with fertilization failure, and fertility preservation.

Pregnancy rates are comparable.

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References

[1] Pincus G, Enzmann EV. The comparative behavior of mammalian eggs in vivo and in vitro: I. The activation of ovarian eggs. The Journal of Experimental Medicine. 1935;**62**:665-675

[2] Eppig JJ, Schroeder AC. Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation, and fertilization in vitro. Biology of Reproduction. 1989;**41**:268-276

[3] 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. Fertility and Sterility. 1991;**55**:109-113

[4] Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertility and Sterility. 1994;**62**:353-362

[5] Gougeon A, Testart J. Germinal vesicle breakdown in oocytes of human atretic follicles during the menstrual cycle. Journal of Reproduction and Fertility. 1986;**78**:389-401

[6] Fadini R, Dal Canto MB, Mignini Renzini M, Brambillasca F, Comi R, Fumagalli D, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: A prospective randomized study. Reproductive Biomedicine Online. 2009;**19**:343-351

[7] Mikkelsen AL, Lindenberg S. Benefit of FSH priming of women with PCOS to the in vitro maturation procedure and the outcome: A randomized prospective study. Reproduction. 2001;**122**:587-592

[8] Son WY, Tan SL. Laboratory and embryological aspects of hCG-primed in vitro maturation cycles for patients with polycystic ovaries. Human Reproduction Update. 2010;**16**:675-689. DOI: 10.1093/humupd/dmq014 [Epub: 26 May 2010]

[9] Chian RC, Buckett WM, Tulandi T, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. Human Reproduction. 2000;**15**:165-170

[10] Chian RC, Cao YX. In vitro maturation of immature human oocytes for clinical application. Methods in Molecular Biology. 2014;**1154**:271-288. DOI: 10.1007/978-1-4939-0659-8_12

[11] Choi MH, Lee SH, Kim HO, Cha SH, Kim JY, Yang KM, et al. Comparison of assisted reproductive technology outcomes in infertile women with polycystic ovary syndrome: In vitro maturation, GnRH agonist, and GnRH antagonist cycles. Clinical and Experimental Reproductive Medicine. 2012;**39**:166-171. DOI: 10.1007/978-1-4939-0659-8_12

[12] Son WY, Chung JT, Chian RC, Herrero B, Demirtas E, Elizur S, et al. A 38 h interval between hCG priming and oocyte retrieval increases in vivo and in vitro oocyte maturation rate in programmed IVM cycles. Human Reproduction. 2008;**23**:2010-2016

[13] Dahan MH, Zhang L, Chen HY, Tan SL. Early short stimulation modified natural cycle IVF with GnRH agonist trigger and in vitro maturation in a woman with polycystic ovary syndrome: A case report. Journal of Obstetrics and Gynaecology Canada. 2016;**38**(5):465-469. DOI: 10.1016/j.jogc.2016.02.002 [Epub: 14 April 2016]

[14] Galway AB, Lapolt PS, Tsafriri A, Dargan CM, Boime I, Hsueh AJ. Recombinant follicle-stimulating hormone induces ovulation and tissue plasminogen activator expression in hypophysectomized rats. Endocrinology. 1990;**127**(6):3023-3028

[15] Atef A, François P, Christian V, Marc-André S. The potential role of gap junction communication between cumulus cells and bovine oocytes during in vitro maturation. Molecular Reproduction and Development. 2005;**71**(3):358-367

[16] Christenson LK, Stouffer RL. Follicle-stimulating hormone and luteinizing hormone/chorionic gonadotropin stimulation of vascular endothelial growth factor production by macaque granulosa cells from pre- and periovulatory follicles. The Journal of Clinical Endocrinology and Metabolism. 1997;**82**(7):2135-2142

[17] Reich R, Miskin R, Tsafriri A. Follicular plasminogen activator: Involvement in ovulation. Endocrinology. 1985;**116**(2):516-521

[18] Son WY, Chung JT, Herrero B, Dean N, Demirtas E, Holzer H, et al. Selection of the optimal day for oocyte retrieval based on the diameter of the dominant follicle in hCG-primed in vitro maturation cycles. Human Reproduction. 2008;**23**:2680-2685

[19] Reinblatt SL, Son WY, Shalom-Paz E, Holzer H. Controversies in IVM. Journal of Assisted Reproduction and Genetics. 2011;**28**:525-530. DOI: 10.1007/s10815-011-9575-z [Epub: 10 May 2011]

[20] Elizur SE, Son WY, Yap R, Gidoni Y, Levin D, Demirtas E, et al. Comparison of low-dose human menopausal gonadotropin and micronized 17beta-estradiol supplementation in in vitro maturation cycles with thin endometrial lining. Fertility and Sterility. 2009;**92**:907-912. DOI: 10.1016/j.fertnstert.2008.07.1750 [Epub: 30 October 2008]

[21] Dahan MH, Tan SL, Chung J, Son WY. Clinical definition paper on in vitro maturation of human oocytes. Human Reproduction. 2016;**31**(7):1383-1386. DOI: 10.1093/humrep/dew109 [Epub: 09 May 2016]

[22] Liu J, Lu G, Qian Y, Mao Y, Ding W. Pregnancies and births achieved from in vitro matured oocytes retrieved from poor responders undergoing stimulation in in vitro fertilization cycles. Fertility and Sterility. 2003;**80**:447-449

[23] Hourvitz A, Maman E, Brengauz M, Machtinger R, Dor J. In vitro maturation for patients with repeated in vitro fertilization failure due to "oocyte maturation abnormalities". Fertility and Sterility. 2010;**94**:496-501. DOI: 10.1016/j.fertnstert.2009.03.040 [Epub: 08 July 2009]

[24] Tan SL. In vitro maturation: Clinical and biological aspects. Journal de Gynecologie, Obstetrique et Biologie de la Reproduction. 2003;**32**:S52-S56

[25] Mikkelsen AL, Smith SD, Lindenberg S. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Human Reproduction. 1999;**14**:1847-1851

[26] Mikkelsen AL, Smith S, Lindenberg S. Possible factors affecting the development of oocytes in in-vitro maturation. Human Reproduction. 2000;**15**(Suppl 5):11-17

[27] Fadini R, Mignini Renzini M, Dal Canto M, Epis A, Crippa M, Caliari I, et al. Oocyte in vitro maturation in normo-ovulatory women. Fertility and Sterility. 2013;**99**:1162-1169. DOI:

10.1016/j.fertnstert.2013.01.138 [Epub: 20 February 2013]

[28] Child TJ, Phillips SJ, Abdul-Jalil AK, Gulekli B, Tan SL. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. Obstetrics and Gynecology. 2002;**100**:665-670

[29] Child TJ, Sylvestre C, Pirwany I, Tan SL. Basal serum levels of FSH and estradiol in ovulatory and anovulatory women undergoing treatment by in-vitro maturation of immature oocytes. Human Reproduction. 2002;**17**:1997-2002

[30] Ellenbogen A, Atamny R, Fainaru O, Meidan E, Rotfarb N, Michaeli M. In vitro maturation of oocytes: A novel method of treatment of patients with polycystic ovarian syndrome undergoing in vitro fertilization. Harefuah. 2011;**150**:833-836, 876

[31] Soderstrom-Anttila V, Makinen S, TuuriT,SuikkariAM.Favourablepregnancy results with insemination of in vitro matured oocytes from unstimulated patients. Human Reproduction. 2005;**20**:1534-1540

[32] Shalom-Paz E, Almog B, Wiser A, Levin I, Reinblatt S, Das M, et al. Priming in vitro maturation cycles with gonadotropins: Salvage treatment for nonresponding patients. Fertility and Sterility. 2011;**96**:340-343. DOI: 10.1016/j.fertnstert.2011.06.003 [Epub: 30 Junuary 2011]

[33] Shalom-Paz E, Holzer H, Son W, Levin I, Tan SL, Almog B. PCOS patients can benefit from in vitro maturation (IVM) of oocytes. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2012;**165**:53-56. DOI: 10.1016/j.ejogrb.2012.07.001 [Epub: 21 July 2012]

[34] Junk SM, Yeap D. Improved implantation and ongoing pregnancy rates after single-embryo transfer with an optimized protocol for in vitro oocyte maturation in women with polycystic ovaries and polycystic ovary syndrome. Fertility and Sterility. 2012;**98**:888-892. DOI: 10.1016/j.fertnstert.2012.06.055 [Epub: 24 July 2012]

[35] Vitek WS, Witmyer J, Carson SA, Robins JC. Estrogen-suppressed in vitro maturation: A novel approach to in vitro maturation. Fertility and Sterility. 2013;**99**:1886-1890. DOI: 10.1016/j.fertnstert.2013.01.148 [Epub: 18 March 2013]

[36] Shavit T, Ellenbogen A, Michaeli M, Kartchovsky E, Ruzov O, Shalom-Paz E. In-vitro maturation of oocytes vs in-vitro fertilization with a gonadotropin-releasing hormone antagonist for women with polycystic ovarian syndrome: Can superiority be defined? European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2014;**179**:46-50. DOI: 10.1016/j.ejogrb.2014.05.013 [Epub: 02 June 2014]

[37] Ferraretti AP, Goossens V, Kupka M, Bhattacharya S, de Mouzon J, Castilla JA, et al. Assisted reproductive technology in Europe, 2009: European IVF-Monitoring (EIM) Consortium for the European Society of Human Reproduction and Embryology (ESHRE). Human Reproduction. 2013;**28**:2318-2331. DOI: 10.1093/humrep/det278 [Epub: 09 July 2013]

[38] de Ziegler D, Streuli I, Gayet V, Frydman N, Bajouh O, Chapron C. Retrieving oocytes from small non-stimulated follicles in polycystic ovary syndrome (PCOS): In vitro maturation (IVM) is not indicated in the new GnRH antagonist era. Fertility and Sterility. 2012;**98**:290-293. DOI: 10.1016/j.fertnstert.2012.06.043

[39] Chian RC, Gulekli B, Buckett WM, Tan SL. Pregnancy and delivery after

cryopreservation of zygotes produced by in-vitro matured oocytes retrieved from a woman with polycystic ovarian syndrome. Human Reproduction. 2001;**16**:1700-1702

[40] Le Du A, Kadoch IJ, Bourcigaux N, Doumerc S, Bourrier MC, Chevalier N, et al. In vitro oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: The French experience. Human Reproduction. 2005;**20**:420-424

[41] Humaidan P, Bredkjaer HE, Bungum L, Bungum M, Grondahl ML, Westergaard L, et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: A prospective randomized study. Human Reproduction. 2005;**20**:1213-1220

[42] Griffin D, Benadiva C, Kummer N, Budinetz T, Nulsen J, Engmann L. Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist and low-dose human chorionic gonadotropin to optimize live birth rates in high responders. Fertility and Sterility. 2012;**97**:1316-1320. DOI: 10.1016/j.fertnstert.2014.04.028 [Epub: 17 May 2014]

[43] Iliodromiti S, Lan VT, Tuong HM, Tuan PH, Humaidan P, Nelson SM. Impact of GnRH agonist triggering and intensive luteal steroid support on live-birth rates and ovarian hyperstimulation syndrome: A retrospective cohort study. Journal of Ovarian Research. 2013;**6**:93. DOI: 10.1186/1757-2215-6-93

[44] Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. Human Reproduction. 2011;**26**:2593-2597. DOI: 10.1093/humrep/der251 [Epub: 09 August 2011]

[45] Fatemi HM, Popovic-Todorovic B, Humaidan P, Kol S, Banker M, Devroey P, et al. Severe ovarian hyperstimulation syndrome after gonadotropin-releasing hormone (GnRH) agonist trigger and "freeze-all" approach in GnRH antagonist protocol. Fertility and Sterility. 2014;**101**:1008-1011. DOI: 10.1016/j.fertnstert.2014.01.019 [Epub: 15 February 2014]

[46] Ortega-Hrepich C, Polyzos NP, Anckaert E, Guzman L, Tournaye H, Smitz J, et al. The effect of ovarian puncture on the endocrine profile of PCOS patients who undergo IVM. Reproductive Biology and Endocrinology. 2014;**12**:18. DOI: 10.1186/1477-7827-12-18

[47] Smirnova A, Anshina M, Sergeev S, Ellenbogen A. Live birth after *in vitro* maturation of oocytes in a patient with repeated fertilization failure in IVF: A case report. Obstetrics & Gynecology. 2016;**6**(6):1-3. DOI: 10.4172/2161-0932.1000390

[48] Levran D, Farhi J, Nahum H, Glezerman M, Weissman A. Maturation arrest of human oocytes as a cause of infertility: Case report. Human Reproduction. 2002;**17**:1604-1609

[49] Tan SL, Child TJ. In-vitro maturation of oocytes from unstimulated polycystic ovaries. Reproductive Biomedicine Online. 2002;**4**(Suppl 1):18-23

[50] Sonigo C, Grynberg M. In vitro oocyte maturation for female fertility preservation. Gynécologie, Obstétrique & Fertilité. 2014;**42**:657-660. DOI: 10.1016/j.gyobfe.2014.07.009 [Epub: 19 August 2014]

[51] 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. American Journal of Surgery. 2010;**200**:177-183. DOI: 10.1016/j.amjsurg.2009.04.004

[52] Shalom-Paz E, Almog B, Shehata F, Huang J, Holzer H, Chian RC, et al. Fertility preservation for breast-cancer patients using IVM followed by oocyte or embryo vitrification. Reproductive Biomedicine Online. 2010;**21**:566-571. DOI: 10.1016/j.rbmo.2010.05.003 [Epub: 13 May 2010]

[53] Maman E, Meirow D, Brengauz M, Raanani H, Dor J, Hourvitz A. Luteal phase oocyte retrieval and in vitro maturation is an optional procedure for urgent fertility preservation. Fertility and Sterility. 2011;**95**:64-67. DOI: 10.1016/j.fertnstert.2010.06.005 [Epub: 18 July 2010]

[54] Prasath EB, Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, et al. First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient. Human Reproduction. 2014;**29**:276-278. DOI: 10.1093/humrep/det420 [Epub: 09 December 2013]

[55] Karavani G, Schachter-Safrai N, Revel A, Mordechai-Daniel T, Bauman D, Imbar T. In vitro maturation rates in young premenarche patients. Fertility and Sterility. 2019;**112**(2): 315-322. DOI: 10.1016/j.fertnstert.2019.03.026 [Epub: 02 May 2019]

[56] Cohen Y, St-Onge-St-Hilaire A, Tannus S, Younes G, Dahan MH, Buckett W, et al. Decreased pregnancy and live birth rates after vitrification of *in vitro* matured oocytes. Journal of Assisted Reproduction and Genetics. 2018;**35**:1683-1689. DOI: 10.1007/s10815-018-1216-3

[57] Son WY, Henderson S, Cohen Y, Dahan M, Buckett W. Immature oocyte for fertility preservation. Frontiers in Endocrinology. 2019;**10**:464. DOI: 10.3389/fendo.2019.00464

[58] Chian RC. In vitro maturation of immature oocytes for clinical application: Past, today and tomorrow. In: Poncelet C, Sifer C, editors. Physiologie, pathologie, et therapie de la reproduction chez l'human. XXIII ed. Paris, France: Springer-Verlag; 2011. pp. 461-471

[59] Buckett WM, Chian RC, Holzer H, Dean N, Usher R, Tan SL. Obstetric outcomes and congenital abnormalities after in vitro maturation, in vitro fertilization, and intracytoplasmic sperm injection. Obstetrics and Gynecology. 2007;**110**:885-891

[60] Fadini R, Mignini Renzini M, Guarnieri T, Dal Canto M, De Ponti E, Sutcliffe A, et al. Comparison of the obstetric and perinatal outcomes of children conceived from in vitro or in vivo matured oocytes in in vitro maturation treatments with births from conventional ICSI cycles. Human Reproduction. 2012;**27**:3601-3608. DOI: 10.1093/humrep/des359 [Epub: 04 October 2012]

[61] Chian RC, Xu CL, Huang JY, Ata B. Obstetric outcomes and congenital abnormalities in infants conceived with oocytes matured in vitro. Facts Views & Vision in Obgyn. 2014;**6**:15-18

[62] Bao S, Obata Y, Carroll J, Domeki I, Kono T. Epigenetic modifications necessary for normal development are established during oocyte growth in mice. Biology of Reproduction. 2000;**62**:616-621

[63] Gioia L, Barboni B, Turriani M, Capacchietti G, Pistilli MG, Berardinelli P, et al. The capability of reprogramming the male chromatin after fertilization is dependent on the quality of oocyte maturation. Reproduction. 2005;**130**:29-39

[64] Li Y, Feng HL, Cao YJ, Zheng GJ, Yang Y, Mullen S, et al. Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured in vitro. Fertility and Sterility. 2006;**85**:827-832

[65] Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, et al. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: A randomized controlled trial. Human Reproduction. 2007;**22**:980-988

[66] Santos MA, Teklenburg G, Macklon NS, Van Opstal D, Schuring-Blom GH, Krijtenburg PJ, et al. The fate of the mosaic embryo: Chromosomal constitution and development of Day 4, 5 and 8 human embryos. Human Reproduction. 2010;**25**:1916-1926. DOI: 10.1093/humrep/deq139 [Epub: 02 June 2010]

[67] Weinerman R, Mainigi M. Why we should transfer frozen instead of fresh embryos: The translational rationale. Fertility and Sterility. 2014;**102**:10-18. DOI: 10.1016/j.fertnstert.2014.05.019 [Epub: 02 June 2014]

[68] Faundez R, Sikorska O, Rokicki T, Karwacka A, Dworniak T, Barak Y. In vitro maturation in women with PCOS. Fertility and Sterility Supplement. 2008;**90**:S214. DOI: 10.1016/j.fertnstert.2008.07.51269

[69] Ben-Ami I, Komsky A, Bern O, Kasterstein E, Komarovsky D, Ron-El R. In vitro maturation of human germinal vesicle-stage oocytes: Role of epidermal growth factor-like growth factors in the culture medium. Human Reproduction. 2011;**26**:76-81. DOI: 10.1093/humrep/deq290 [Epub: 20 October 2010]

[70] Zhao P, Qiao J, Huang S, Zhang Y, Liu S, Yan LY, et al. Gonadotrophin-induced paracrine regulation of human oocyte maturation by BDNF and GDNF secreted by granulosa cells. Human Reproduction. 2011;**26**:695-702. DOI: 10.1093/humrep/deq390 [Epub: 12 January 2011]

[71] Chen J, Hudson E, Chi MM, Chang AS, Moley KH, Hardie DG, et al. AMPK regulation of mouse oocyte meiotic resumption in vitro. Developmental Biology. 2006;**291**:227-238

[72] Ellenbogen A, Shavit T, Shalom-Paz E. IVM results are comparable and may have advantages over standard IVF. Facts Views & Vision in Obgyn. 2014;**6**:77-80