State of the art in in-vitro oocyte maturation

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Purpose of review

The recovery of immature oocytes followed by in-vitro maturation (IVM) and in-vitro fertilization is an attractive alternative to conventional in-vitro fertilization treatment in which controlled ovarian stimulation with gonadotropins is used to increase the number of available oocytes and embryos. Significant progress has been made to improve pregnancy and implantation rates from in-vitro matured oocytes. This review summarizes current knowledge and achievements in human oocyte in-vitro maturation for clinical application, and will highlight recent advances reported in in-vitro maturation treatment.

Recent findings

It has been demonstrated that priming of ovarian immature oocytes with follicle-stimulating hormone or human chorionic gonadotropin prior to immature oocyte retrieval improves oocyte maturation rates and embryo quality as well as pregnancy rates in infertile women with polycystic ovaries or polycystic ovary syndrome. The size of follicles may be important for the subsequent embryonic development, but the developmental competence of oocytes derived from the small antral follicles is not adversely affected by the presence of a dominant follicle. However oocyte maturation *in vitro* is profoundly affected by culture conditions. Currently more than 300 healthy infants have been born following immature oocyte retrieval and in-vitro maturation. In general, the clinical pregnancy and implantation rates have reached 30–35% and 10–15% respectively in infertile women with polycystic ovaries or polycystic ovary syndrome.

Summary

In-vitro maturation treatment can now be offered as a successful option to infertile women with polycystic ovaries or polycystic ovary syndrome. It is possible to combine natural cycle in-vitro fertilization with immature oocyte retrieval followed by in-vitro maturation, and thus offer women with various causes of infertility reasonable pregnancy and implantation rates without recourse to ovarian stimulation. Further research remains to be done to address the mechanism of oocyte maturation in order to refine culture conditions and improve the implantation rate of oocytes matured *in vitro*.

Keywords

immature oocytes, IVM, IVF, PCO, pregnancy

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Abbreviations

FSH	follicle-stimulating hormone
hCG	human chorionic gonadotropin
IVF	in-vitro fertilization
IVM	in-vitro maturation
OHSS	ovarian hyperstimulation syndrome
PCOS	polycystic ovary syndrome

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Introduction

Since the first successful live birth from human in-vitro fertilization was reported [1], assisted reproductive technology has helped many thousands of women overcome infertility problems. Although the initial live births following human in-vitro fertilization (IVF) were produced from a natural cycle IVF, this was slowly replaced by ovarian stimulation IVF, because it is believed that the number of oocytes retrieved relates to the embryos available for transfer, and that this directly affects the probability of successful pregnancy [2–4]. At the beginning, the relatively inexpensive clomiphene citrate was used to stimulate ovaries for producing multiple follicles. However, currently ovarian stimulation protocols use the much more expensive gonadotropin-releasing hormone agonist or antagonist in combination with gonadotropins to generate multi-follicles in the ovaries. Some women are extremely sensitive to stimulation with exogenous gonadotropins and are at increased risk of developing ovarian hyperstimulation syndrome (OHSS), a life-threatening condition [5]. This syndrome may be related to a mutation in the follicle-stimulating hormone (FSH) receptor [6]. In addition, there is anxiety that the long-term side effects of repeated ovarian stimulation may increase the risk of ovarian, endometrial and breast cancers [7,8].

Recovery of immature oocytes followed by in-vitro maturation (IVM) of these oocytes is a potentially useful treatment for women with infertility. In comparison with conventional IVF, the major advantages of IVM treatment include avoidance of the risk of OHSS, reduced cost, and simplified treatment. Recently, it has been demonstrated that immature oocyte retrieval followed by IVM is a successful treatment for women with infertility related to polycystic ovaries or polycystic ovary syndrome (PCOS) because there are numerous antral follicles within the ovaries in this group of patients [9]. Women with polycystic ovaries or PCOS have a significantly higher risk of OHSS when they are

stimulated with exogenous gonadotropins than women with normal ovaries [10]. Although immature oocyte retrieval followed by IVM might be useful in 20–37% of women undergoing IVF treatment who have polycystic ovaries seen on ultrasound scan [11,12], it is important to expand IVM technology for women with various causes of infertility. It seems that there is a beneficial effect with FSH priming on oocyte maturation and subsequent pregnancy, but the results of FSH priming are still controversial. The aim of this review is primarily to focus on the endocrinology of oocyte maturation *in vitro* and on recent clinical outcomes with IVM technology.

Endocrinology of oocyte maturation *in vitro*

At birth, there are approximately one million primordial follicles in the ovaries [13]. Each primordial follicle consists of an oocyte surrounded by a few flattened pregranulosa cells enclosed by a basement membrane. Although large numbers of follicles can leave the primordial pool and begin to grow, there will be very few selected to mature and to ovulate for fertilization. Only after the onset of puberty, the follicles respond to rising levels of gonadotropins to grow and to mature fully and then to ovulate. Therefore, only approximately 400 mature oocytes will be released for potential fertilization from the ovaries during a woman's reproductive life. The process of follicular development within the ovary is directly influenced by gonadotropins, FSH and luteinizing hormone. From the growing cohort of antral follicles, only a proportion of follicles are able to respond to the rising levels of FSH and consequently a large number of follicles die at the early antral stage of development. During the early antral stage, the follicle has multilaminar granulosa cell layers and acquires vascularized and distinct layers of thecal cells that are separated from the granulosa cells by the basement membrane. It seems that approximately 20 antral follicles are selected and continued through to the preovulatory stages of development during each menstrual cycle [14]. In the later antral stage of follicular development, granulosa cells rapidly proliferate and differentiate into two populations: the mural granulosa cells that are adjacent to the basement membrane and the cumulus cells that surround the oocyte. Gonadotropins (FSH and luteinizing hormone) are necessary for follicular development *in vivo*, and both these hormones use the cyclic AMP pathway system as the intracellular second messenger. In addition, there are many other growth factors and cytokines that modulate the actions of gonadotropins; however, the details will be not covered in this review. Although most knowledge of the regulation of oocyte maturation *in vitro* comes from animal experiments, the reader should keep in mind that this may not be relevant to human oocytes, especially the results from the mouse model.

The follicle-enclosed oocyte is arrested at the prophase of the first meiotic division. The resumption of this arrest occurs in preovulatory follicles following the preovulatory luteinizing hormone surge to complete the first meiotic division, which is characterized by the extrusion of the first polar body and then formation of the secondary metaphase plate (metaphase II). Therefore, oocyte maturation is defined morphologically as the reinitiation and completion of the first meiotic division from the germinal vesicle stage to the metaphase II stage. Our knowledge of meiosis at the molecular level has accumulated rapidly in the last two decades. In particular, the discovery of a non-specific factor, the maturation-promoting factor, which is responsible for G2 to M phase transition of the cell cycle, can be considered to be a major breakthrough [15]. Molecular characterization of maturation-promoting factor has shown that the active form is a protein dimer composed of catalytic $p34^{cdc}$ serine/threonine kinase and regulatory cyclin B subunits. The mechanisms involved in maturation-promoting factor driving oocyte maturation are out of this review scope.

Although it is clear that the luteinizing hormone surge triggers the resumption of meiosis *in vivo*, cumulus–oocyte complexes can be spontaneously induced to resume meiosis when they are released from follicles into culture *in vitro*. Therefore, the action of endocrine factors that affect the oocyte maturation *in vitro* may be quite different from in-vivo conditions. Immature oocytes with or without surrounding cumulus cells can be matured to metaphase II. However, the capacity of early embryonic development from the denuded oocytes is questionable. Beneficial effects of cumulus cells on early embryonic development have been reported in many species including human [15]. The actions of endocrine, paracrine and autocrine factors that control oocyte maturation *in vitro*, either directly or indirectly, are mediated by the cumulus cells.

Although FSH and luteinizing hormone play an important role in the development and maturation of preantral, antral and preovulatory follicles *in vivo*, these gonadotropins may not play the same role in promoting oocyte maturation *in vitro*. Currently most IVM protocols supplement FSH or luteinizing hormone into a culture medium for oocyte maturation. However, the effects of FSH or luteinizing hormone on oocyte maturation and subsequent fertilization as well as early embryonic development are still controversial. The idea of supplementing these hormones into a culture medium is based on their physiological role in oocyte maturation *in vivo*. The contradictory reports that FSH or luteinizing hormone are major hormones involved in IVM may be related to cross contamination of FSH with luteinizing hormone, or luteinizing hormone with FSH, since each preparation derives from urinary extracts [16]. While it

has been reported that using the combination of recombinant FSH with recombinant luteinizing hormone in IVM of immature oocytes resulted in significantly higher developmental competence, evident by increased development to the blastocyst stage compared with recombinant FSH alone or no gonadotropins [17], conclusive results require further study. In addition, recently Hreinsson *et al.* [18] showed that using recombinant human chorionic gonadotropin (hCG) or recombinant luteinizing hormone are equally effective in promoting oocyte maturation *in vitro*, although there was no proper control group in their study to confirm this conclusion.

In in-vitro conditions, it was initially considered that FSH and luteinizing hormone probably act to induce oocyte maturation by an indirect action mediated by cumulus cells, because it is believed that there are no FSH or luteinizing hormone receptors on the oocytes [19]. However, recent reports [20,21•] indicate that messenger RNA for FSH and luteinizing hormone receptors is present in mouse and human oocytes, zygotes and preimplantation embryos, indicating a potential role for gonadotropins in the modulation of meiotic resumption and the completion of oocyte maturation. In addition, it has been known that the culture medium supplemented with a physiological concentration of FSH or luteinizing hormone stimulates steroid (estradiol and progesterone) secretions from the cultured granulosa and cumulus cells [22]. Therefore, it is likely that one of the actions of gonadotropins is mediated by either estradiol or progesterone, which may control oocyte maturation *in vitro*. A recent report indicated that luteinizing hormone receptor formation in the cumulus cells that surround porcine oocytes had an important role in oocyte cytoplasmic maturation [23]. However, its importance in oocyte maturation *in vitro* and how this action was linked to other signal transduction (pathways) are still largely unknown.

Estradiol and progesterone are mediators of normal mammalian ovarian function. Inhibition of steroid synthesis in whole cultured follicles impairs subsequent fertilization and developmental capacity of the oocytes in sheep [24]. The presence of estradiol in the culture medium of in-vitro matured human oocytes had no effect on the progression of meiosis but improved the fertilization and cleavage rates [25]. However, it may not be necessary to add estradiol to the oocyte maturation medium when the oocytes are cultured with cumulus cells because the culture medium supplemented with gonadotropins stimulates estradiol secretion from the granulosa and cumulus cells during culture *in vitro* [22]. There is little information about the effect of progesterone contained in the culture medium on oocyte maturation. However, we have found a negative effect of progesterone on bovine oocyte maturation *in vitro* (Chian *et al.*, unpublished data).

It is well known that there are many growth factors contained in follicular fluid. These growth factors must be secreted from the granulosa and cumulus cells that respond to gonadotropins, and subsequently act on the oocyte via paracrine and autocrine pathways. Although a growing number of studies have indicated that there are beneficial effects of growth factors on oocyte maturation, it seems that only denuded oocytes require the supplementation of growth factors in the culture medium for proper oocyte maturation [26]. This suggests that the granulosa and cumulus cells can secrete some growth factors during culture and play some functional roles during oocyte maturation *in vitro*. In practice the culture medium is also supplemented with the patient's own serum or human serum albumin as a protein source. Both serum and human serum albumin are a rich source of growth factors. Therefore, it is not necessary to add growth factors into the IVM medium for oocyte maturation *in vitro*, especially when the IVM medium contains serum or human serum albumin.

Immature oocytes from cesarean section

The first successful IVM birth was from immature oocytes collected at cesarean section for oocyte donation [27]. Therefore, the important question is why the oocytes from the patient at cesarean section still have maturational and developmental potential. In addition, it has been reported that immature human oocytes can be retrieved from the ovaries during either follicular or luteal phases of ovulatory cycles [28], indicating that age, pathology, day of the menstrual cycle, and cyclic versus non-cyclic ovaries influence the number of immature oocytes collected. Although the data are insufficient, it seems that the number of immature human oocytes collected from an ovary decreases as the patient's age increases, but there is no statistical difference in the oocyte maturation and cleavage rates among donors in the different age groups. Interestingly, it has been indicated that immature oocytes from the luteal phase had a significantly higher maturation rate than those obtained from the follicular phase [29]. Later, a pregnancy obtained from immature oocytes derived from cesarean section was further confirmed by Hwang *et al.* [30], indicating that the oocyte maturation rate was relatively low when those oocytes were cultured *in vitro* [31]. However, a recent paper [32] reported that the immature oocytes from small follicles at cesarean section followed by IVM/IVF could develop to the blastocyst stage when the embryos were co-cultured with human ampullary epithelial cells.

The main problem with an oocyte donation program is the limited source of donors, because the discomfort and

increased risks associated with ovarian stimulation as well as oocyte retrieval limit availability. Therefore, immature oocytes derived from cesarean sections followed by IVM may be a source of donors who do not have to go through laborious ovarian stimulation. However, more research is required to clarify the developmental potential of immature oocytes derived from cesarean sections.

Immature oocytes from women with polycystic ovaries or polycystic ovary syndrome

Infertile women with polycystic ovaries or PCOS show numerous antral follicles on pelvic ultrasound examination. As mentioned, this group of patients has an increased risk of OHSS from gonadotropins compared with women with normal ovaries. The first pregnancy in a woman with anovulatory infertility following IVM of immature oocytes and IVF was reported by Trounson *et al.* [33]. Another pregnancy was reported in a group of patients with PCOS treated with IVM combined with intracytoplasmic sperm injection and assisted hatching [34]. Subsequent studies indicated that although immature oocytes recovered from untreated patients with polycystic ovaries or PCOS can be matured, fertilized and developed *in vitro*, the implantation rate of these cleaved embryos is disappointingly low [35,36,37]. However, recent data indicate that with an alternative IVM treatment in these patients, using priming with FSH or hCG before immature oocyte retrieval, clinical pregnancy and implantation rates of 30–35% and 10–15% respectively can be attained [9]. Currently the prime candidates for IVM treatment are women with polycystic ovaries or PCOS regardless of whether they are ovulatory or anovulatory [38,39]. It has been reported that the pregnancy rate after IVM treatment correlates with the number of immature oocytes retrieved, indicating that the best predictor for successful IVM treatment is the number of antral follicles measured by ultrasonography [40]. In addition, Gulekli *et al.* [41] reported that pregnancy has been established using immature oocytes from donors followed by IVM, suggesting that IVM technology may increase the number of women who have polycystic ovaries and are prepared to be oocyte donors.

Priming with follicle-stimulating hormone before immature oocyte retrieval

As an alternative approach, a truncated course of ovarian stimulation with FSH before immature oocyte retrieval has been applied, indicating that FSH pre-treatment promotes efficient recovery of immature oocytes and maturation *in vitro* [42]. It has been reported that the immature oocytes from stimulated cycles from normal cycling woman without hCG can be matured and fertilized *in vitro* and subsequently pregnancy and live birth can be obtained [43]. However, another report [44] indicated that FSH priming with a fixed dose (150 IU/day) for 3 days from day 3 of the menstrual cycle does not increase the number of oocytes obtained per aspiration and does not improve oocyte maturation, cleavage rates or embryo development when women with normal cycling ovaries are treated. Furthermore, Suikkari *et al.* [45] reported that although using low-dose FSH priming started from the previous luteal phase improved the efficiency of immature oocyte recovery, maturation and fertilization rates, the average number of immature oocytes collected, the rates of oocyte maturation and fertilization were not different between women with regular menstrual cycles and those with irregular cycles and polycystic ovaries. Nevertheless, it has been reported that priming with recombinant FSH during the follicular phase before harvesting of immature oocytes from patients with PCOS improves the maturational potential of the oocytes and the implantation rate of the cleaved embryos. This indicated that significantly higher pregnancy (29%, seven out of 24) and implantation (21%, eight out of 37) rates were obtained when priming with FSH before immature oocyte retrieval [46]. More recently, Mikkelsen *et al.* [47] also reported that there were no differences in the rates of oocyte maturation, fertilization, cleavage or implantation when 2 and 3 days was the time (coasting) interval between FSH priming and aspiration of immature human oocytes for IVM. In this study normal cycling women were primed with 150 IU FSH/day for 3 days starting on day 3. It is evident that optimizing IVM treatment for different groups of patients is important and that after individualized IVM treatment the pregnancy and implantation rates rate per embryo transfer can reach 23 and 14% respectively [18].

Interestingly, it has been reported that FSH priming with 75 IU/day for 6 days in combination with hCG priming 36 h before immature oocyte retrieval has no additional benefit in women with PCOS [48••]. Although these results are conflicting on the benefits of using FSH priming in women with regular menstrual ovaries or irregular menstrual cycles associated with polycystic ovaries, theoretically the use of FSH priming at the beginning of follicular or luteal phases may enhance more follicular development and the maturational competence of immature oocytes *in vivo*. However, further research is required to confirm these beneficial results of priming with FSH before immature oocyte retrieval from women with regular menstrual cycles and normal ovaries compared with irregular menstrual cycles and polycystic ovaries.

Priming with human chorionic gonadotropin before immature oocyte retrieval

A few germinal vesicle stage oocytes may be retrieved from the standard stimulated cycles even 36 h after hCG

administration. These immature oocytes are capable of undergoing IVM and then normal fertilization and development. Although successful pregnancies have been established using such in-vitro matured oocytes [49–52], the pregnancy rate was unacceptably low. It has been observed that morphological and molecular differences exist between the immature oocytes collected from stimulated cycles and those collected during cesarean sections [53]. In addition, it has been found that the time course of germinal vesicle breakdown and oocyte maturation is different between these oocytes, although the final rates of oocyte maturation are not different in the groups [15]. It appears that the oocytes retrieved from follicles in women undergoing ovarian stimulation respond to hCG that may promote the initiation of oocyte maturation *in vivo*. It has been demonstrated that the time course of oocyte maturation *in vitro* is hastened and the rate of oocyte maturation is increased by priming with 10 000 IU hCG 36 h before retrieval of immature oocytes from women with polycystic ovaries or PCOS [54,55]. Therefore, it is possible that pregnancy rates may potentially be improved by priming with hCG prior to immature oocyte retrieval [56]. This hypothesis was confirmed by several reports [48••,57–59].

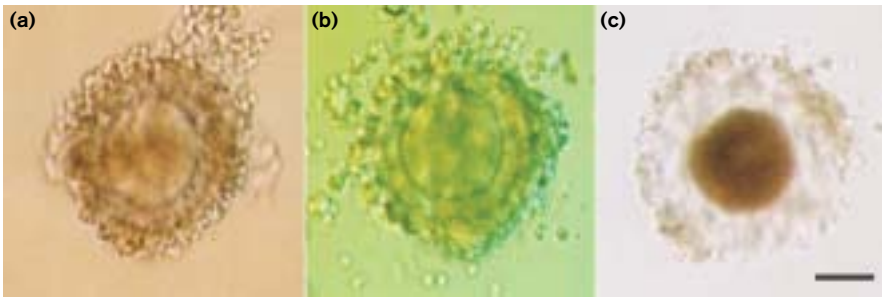
Lin *et al.* [48••] reported that a 36.4% clinical pregnancy rate was obtained from 33 cycles of IVM treatment when priming with hCG was used before immature oocyte retrieval from women with PCOS. This study indicated the beneficial effect of hCG priming on IVM treatment. Based on more than 1000 IVM cycles in a multicenter study with hCG priming before immature oocyte retrieval from women with polycystic ovaries or PCOS, the pregnancy and implantation rates reached 30–35% and 10–15% respectively (Chian *et al.*, unpublished data). Interestingly, recent findings indicated that in women with polycystic ovaries or PCOS, the time course and maturation rates are different when germinal vesicle stage oocytes were divided into different groups based on the morphology of cumulus cells after hCG priming [60]. Therefore, it seems that hCG priming both promotes some oocytes to undergo maturation to the metaphase-I stage, when derived from the relatively larger follicles (>10 mm in diameter), and also enhances some germinal vesicle stage oocytes from small follicles to acquire maturational and developmental competence *in vivo*. However, the exact mechanism of hCG action on small follicles is still largely unclear. Further, we also need to understand the functional role of hCG priming before immature oocyte retrieval on small size follicles in women with polycystic ovaries or PCOS (Fig. 1).

Immature oocytes from poor responders or over-responders

Currently most infertility treatments employ ovulation stimulation protocols to increase the number of oocytes available for fertilization. However, some of these stimulated cycles have to be cancelled either due to the risk of OHSS or to a delayed response to gonadotrpin stimulation. Immature oocyte retrieval followed by IVM and IVF may provide an important alternative to cancellation of these cycles. Initially, one live birth was reported from immature oocytes that were collected from a patient at potential risk of developing OHSS [61]. Subsequently the possibility of immature oocyte retrieval followed by IVM, IVF and embryo transfer was further confirmed although a pregnancy was not obtained [62]. Recently, it was reported that 47.1% (eight out of 17) clinical pregnancies were achieved from patients with potential risk of developing OHSS followed by immature oocyte retrieval and IVM [63]. To prevent the likelihood of OHSS in susceptible patients, gonadotropin stimulation or hCG administration are withdrawn to cancel the cycle. In order to carry out IVM for this group of patients, 10 000 IU hCG was given 36 h before oocyte collection when the leading follicle reached a mean diameter of 12–14 mm. Although it is often a concern that the presence of hCG is likely to be associated with OHSS [64], there was no OHSS reported when the leading follicle reached 12–14 mm in diameter in patients at risk of developing the syndrome [63]. So far, there have been more than 30 healthy live births obtained from this group of patients following

Figure 1. Human oocytes from women primed with or without human chorionic gonadotropin 36 h before retrieval

(a) Immature oocyte retrieved without human chorionic gonadotropin (hCG) priming, showing compact cumulus cell layers surrounding the oocyte. (b) Immature oocyte retrieved with hCG priming, showing some dispersed cumulus cells surrounding the oocyte. (c) Dead (or atretic) oocyte collected after hCG priming, showing dark-yellow cytoplasm; normally, approximately 5–10% of oocytes retrieved from women with polycystic ovary syndrome look like this oocyte. Scale bar = 50 μm.



oocyte retrieval and IVM treatment at the Maria Infertility Hospital, Korea. In order to avoid OHSS these patients were given 10 000 IU hCG in preparation for immature oocyte retrieval 36 h later, administered at an early stage of ovarian stimulation, when patients appeared to develop a potential risk of OHSS. It is important to mention here that about 11.6% of oocytes have already reached the metaphase II stage at collection when the leading follicles attain a 12–14 mm diameter, 36 h after hCG priming. Therefore, the patients who are at risk of developing OHSS can be provided an extra chance by hCG administration followed by oocyte retrieval and IVM treatment rather than cancelling the cycle.

Poor response to ovarian stimulation by gonadotropins is defined as a plasma estradiol level less than 1000 pg/ml on the day of hCG administration, and usually less than four mature oocytes can be retrieved [65]. Poor response to gonadotropin stimulation occurs more often in older women but may also be present in young women with an abnormal or normal endocrinological profile [66]. Some poor responders appear to respond to stimulation but have a low estrogen level or few or slow growing follicles. Normally these groups of patients require a prolonged stimulation time and higher doses of gonadotropins. Following gonadotropin stimulation, the number of follicles may be normal, but their size may be smaller than in the usual treatment cycles. These patients experienced a higher cancellation rate because of the smaller number or size of follicles. However, Check *et al.* [52] reported that two pregnancies were established following IVM when germinal vesicle or metaphase I stage oocytes were retrieved after hCG administration from such poor responders. Recently, Liu *et al.* [67••] reported that a 37.5% (three out of eight) pregnancy rate was obtained following immature oocyte retrieval and IVM without hCG administration before oocyte collection, suggesting that IVM may be a viable alternative to cancellation in poor responders to conventional stimulated IVF cycles. It should be noted here that, in our view, giving 10 000 IU hCG 36 h before oocyte retrieval followed by IVM would optimize the successful pregnancy rate in such poor responders because at least some in-vivo matured oocytes can be collected after hCG administration. Indeed, these mature oocytes pooled together with IVM of immature oocytes will maximize the successful IVF treatment without cycle cancellation.

Combination of natural cycle in-vitro fertilization with in-vitro maturation

There is an increasing interest in natural cycle IVF among patients, primarily because it is more comfortable, there are less side effects and the only disadvantage is failed oocyte collection with no embryo available for transfer as a consequence [68]. Literature reports for pregnancy rates per embryo transfer in the natural cycles vary between 0 and 30% [69–71]. However, the implantation and live birth rates per started cycle seem almost similar to one ovarian stimulated cycle, when compared with a natural cycle with intracytoplasmic sperm injection [72]. In addition, life table analysis to calculate cumulative success rates after successive cycles of treatment has indicated that the cumulative probability of pregnancy is 46% with an associated live birth rate of 32% after four cycles of treatment [73]. Therefore, it is important to ask what is the best option for the patients undergoing two or three natural cycle treatments compared with one ovarian stimulated cycle in the same time span with similar pregnancy and live birth rates. The most important advantage of natural cycle IVF is to simplify the treatment and to avoid side effects, particularly the still unknown long-term effects of repeated ovarian stimulation with gonadotropin-releasing hormone and gonadotropins.

In women, although only a single follicle usually grows to the preovulatory stage and releases its oocytes for potential fertilization, there are many small follicles that also develop during the same follicular phase of the menstrual cycle. It is believed that approximately 20 antral follicles are selected and continue to the preovulatory stages of development during each cycle [14]. Recently it has been documented that there are two or three waves of ovarian follicular development in women during each menstrual cycle based on daily transvaginal ultrasonography, challenging the traditional theory of a single cohort of antral follicles that grow only during the follicular phase of the menstrual cycle [74•,75]. Interestingly, it seems that atresia does not occur in the non-dominant follicles even after the dominant follicle is selected during folliculogenesis, because immature oocytes retrieved from non-dominant follicles have been successfully matured *in vitro*, fertilized, and resulted in several pregnancies and healthy live births [76,77]. Animal model studies also support the finding that oocyte quality and early embryonic developmental competence of immature oocytes following maturation *in vitro* are not detrimentally affected by the presence of the dominant follicle in the ovaries [78,79]. Therefore, one very attractive possibility for enhancement of the success of natural cycle IVF treatment is its combination with immature oocyte retrieval and IVM. If the mature oocyte from the dominant follicle together with immature oocytes from small follicles were collected as well, the chances of a pregnancy would be greatly increased when we manage to mature these immature oocytes and produce several viable embryos. However, it is very important to prevent ovulation from the dominant follicle due to a natural luteinizing hormone surge when patients are treated

with natural cycle IVF combined with IVM. Our experiences indicate that 10 000 IU hCG can be administered 36 h before oocyte retrieval when the size of the dominant follicle reaches 14 mm in diameter. Most oocytes collected from the dominant follicles were at metaphase II stage. A pilot study has indicated that the clinical pregnancy rate attained was 45–50% per embryo transfer following natural cycle IVF combined with IVM (Chian *et al.*, in preparation; Lim *et al.*, unpublished data).

Conclusion

It has been demonstrated that priming with FSH or hCG prior to immature oocyte retrieval improved oocyte maturation and pregnancy rates when immature oocytes were retrieved from women with polycystic ovaries or PCOS. The size of follicles may be an important feature for the subsequent embryonic development, but the developmental competence of oocytes derived from small antral follicles seems not to be adversely affected by the presence of a dominant follicle. Approximately 300 healthy infants have been born following immature oocyte retrieval and IVM. In general, the clinical pregnancy and implantation rates have reached 30–35% and 10–15% respectively in women with polycystic ovaries or PCOS. Therefore, as an option, IVM treatment can be offered to women whose infertility is due to polycystic ovaries or PCOS. The combination of natural cycle IVF with immature oocyte retrieval followed by IVM is an attractive treatment for women with all types of infertility without recourse to the usual ovarian stimulation. Further research remains to be done to address the mechanism of oocyte maturation in order to refine culture conditions and to improve the implantation rate of in-vitro matured oocytes.

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