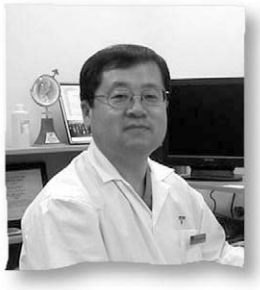
Outlook

In-vitro maturation of immature oocytes for infertile women with PCOS



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Abstract

Immature oocyte retrieval followed by in-vitro maturation (IVM) is a promising potential treatment option, especially for women who are infertile through polycystic ovarian syndrome (PCOS). Although the pregnancy and implantation rates of IVM treatment are not as high as conventional IVF treatment, IVM treatment has many advantages for infertile women with PCOS, because this group of patients is extremely sensitive to stimulation with exogenous gonadotrophins and is at increased risk of developing ovarian hyperstimulation syndrome (OHSS). Different protocols have been used before immature oocyte retrieval, indicating that there are beneficial effects with FSH or LH priming on oocyte maturation. To date, the clinical pregnancy and implantation rates obtained from IVM treatment in infertile women with PCOS are approximately 30–35% and 10–15% respectively. Therefore, as an option, IVM treatment can be offered to women with PCOS instead of conventional IVF treatment with ovarian stimulation.

Keywords: *IVM, OHSS, oocyte, PCOS*

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common reproductive disorders in women of childbearing age. It has a heterogeneous presentation that is clinically characterized by anovulation and hyperandrogenism, and on pelvic ultrasound examination shows numerous antral follicles within the ovaries (Adams *et al*., 1986). Women with PCOS often present with anovulatory infertility, and a significant proportion are resistant to induction of ovulation by clomiphene citrate. Ovulation can be induced successfully in 75% of non-responders with human menopausal gonadotrophin (HMG) or recombinant FSH (rFSH), but gonadotrophin use requires intensive monitoring and involves a distinct risk of ovarian hyperstimulation syndrome (OHSS) (Healy *et al*., 1980; Wang and Gemzell, 1980), because there is a significant higher risk of OHSS in this group of patients compared with women with normal ovaries (MacDougall *et al*., 1993).

Recovery of immature oocytes followed by in-vitro maturation (IVM) of these immature oocytes is a potentially useful treatment for women with PCOS related infertility. In comparison with conventional IVF, the major benefits of IVM treatment include avoidance of the risk of OHSS, reduced cost, and less complicated treatment. The first pregnancy in a woman with anovulatory infertility following IVM of immature oocytes and IVF was reported by Trounson *et al*. (1994). Meanwhile, another pregnancy was achieved in a group of patients with PCOS treated with IVM combined with intracytoplasmic sperm injection (ICSI) and assisted hatching (Barnes *et al*., 1995). In addition, previous studies indicated that although immature oocytes recovered from unstimulated PCOS can be matured, fertilized and developed *in vitro*, the implantation rate of these cleaved embryos is disappointingly low (Barnes *et al*., 1996; Trounson *et al*., 1998; Cha *et al*., 2000). However, recent data indicate that IVM treatment with FSH or human chorionic gonadotrophin (HCG) priming before immature oocyte retrieval can improve the clinical pregnancy and implantation rates in infertile women with PCOS (Chian *et al*., 2004).

Pregnancy rate after IVM treatment is related to the number of immature oocytes retrieved (Child *et al*., 2001, 2002), indicating that the best predictor for successful IVM treatment is the antral follicle number as measured by ultrasonography of the patient's ovaries (Tan *et al*., 2002). Although immature oocyte retrieval followed by IVM might be useful in 20–37% of women undergoing IVF treatment who have polycystic ovaries (PCO) seen on ultrasound scan (Frank, 1989; Buckett *et al*., 1999), it is important to extend IVM technology for women with all types of infertility indications. Interestingly, Gulekli *et al*. (2001) reported that a pregnancy has been established with immature oocytes derived from donors who have PCO seen under ultrasonography followed by IVM. Therefore, it seems that IVM technology may lead to an increase in the number of women who are prepared to be oocyte donors. Since this author has written another review paper dealing with IVM treatment for different types of patients, the present study will focus on IVM treatment for women with PCOS.

Principles of oocyte maturation *in vitro*

Gonadotrophins, FSH and LH are necessary for follicular development *in vivo*, and both FSH and LH utilize the cyclic AMP (cAMP) pathway system as the intracellular second messenger. The follicle enclosed oocyte is arrested at the prophase of the first meiotic division. Resumption of this arrest occurs in preovulatory follicles following the preovulatory LH 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. The oocyte and cumulus cells are coupled by gap junctions, suggesting that this communication may play an important role in the resumption of oocyte meiosis. The gap junctions permit regulatory molecules, such as steroids, Ca^{2+}, inositol-1,4,5-trisphosphate (IP_3), cAMP and purines, to pass freely between the cumulus cells and the cytoplasm of the oocyte. The mechanism of oocyte maturation involves LH inducing the loss of communication (gap junction) between the oocyte and cumulus cells (Cha and Chian, 1998). Oocyte maturation is defined as the reinitiation and completion of the first meiotic division from the germinal vesicle (GV) stage to metaphase-II (M-II) stage, with accompanying cytoplasmic maturation necessary for fertilization and early embryonic development.

Although it is clear that the LH surge triggers the resumption of meiosis *in vivo*, cumulus–oocyte complexes (COC) can spontaneously induce the resumption of meiosis when they were released from the follicles during culture *in vitro*. However, the capacity of early embryonic development from these in-vitro matured oocytes is questionable, because animal model studies have indicated that oocyte cytoplasmic maturation might not occur properly when the oocytes matured *in vitro* (Thibault *et al*., 1975; Thibault, 1977). In addition, the action of endocrine factors that affect oocyte maturation *in vitro* may be quite different from the in-vivo condition. However, it has been found that the first morphological change of COC during culture *in vitro* is the release of gap junctions between the oocyte and cumulus cells (Chian *et al*., unpublished data).

FSH and LH play an important role in the development and function of pre-antral, antral and pre-ovulatory follicles *in vivo*. However, these gonadotrophins may not play the same role in oocyte maturation *in vitro*. Currently, most IVM protocols supplement FSH and/or LH into oocyte culture medium. The rationale of addition of FSH and/or LH into culture medium is based on the physiological role of FSH and LH in oocyte maturation *in vivo*. However, the effects of FSH and LH on oocyte maturation *in vitro* and subsequent fertilization as well as embryonic development are still controversial. It has been indicated that these contradictory reports may result from the use of urinary gonadotrophins and the contamination of FSH or LH preparations, which have been used as a major hormone source in oocyte IVM (Bevers *et al*., 1997). Although the recent use of recombinant FSH and LH has been beneficial to oocyte maturation (Anderiesz *et al*., 2000), the resulting conclusions require further analysis. Furthermore, it has been reported that using recombinant HCG or LH are equally effective in promoting oocyte maturation *in vitro* (Hreinsson *et al*., 2003).

The concentrations of FSH and LH in culture medium should be the same as that contained in the follicular fluid surrounding the fully mature oocyte *in vivo*. It has been reported that exposure of cattle and human oocytes to a 1:10 ratio of FSH:LH resulted in significantly higher developmental competence, as evidenced by increased development to blastocyst stage compared with FSH alone or no gonadotrophins (Anderiesz *et al*., 2000). However, a recent report indicated that the ratio of FSH:LH is not important for oocyte maturation *in vitro* and subsequent embryonic development *in vitro* (Choi *et al*., 2001). Culture medium supplemented with physiological concentrations of FSH and/or LH stimulates steroid (oestradiol and progesterone) secretion from the cultured granulosa and cumulus cells (Chian *et al*., 1999a). Therefore, one of the actions of gonadotrophins must be mediated by either oestradiol or progesterone, to control oocyte maturation *in vitro*. In addition, it has been reported that LH receptor formation in the cumulus cells surrounding porcine oocytes is important for oocyte cytoplasmic maturation, indicating that cAMP pathway links oocyte cytoplasmic maturation (Shimada *et al*., 2003). Therefore, there is little doubt about the beneficial effect of oocyte-surrounded cumulus cells on oocyte cytoplasmic maturation during culture *in vitro*. Animal model studies indicated that the beneficial effects of the cumulus cells not only on early embryonic development but also on the formation of a male pronucleus after fertilization (Chian *et al*., 1994). Moreover, it has been found that the protein synthesis pattern is different in the cytoplasm between denuded oocytes and oocytes surrounded with cumulus cells for IVM, and that FSH modulates the protein synthesis pattern of the cumulus cell-intact oocytes (Chian and Sirard, 1995).

Oestradiol and progesterone are mediators of normal mammalian ovarian function. Inhibition of steroid synthesis in whole cultured follicles impairs subsequent fertilization and early embryonic developmental capacity following IVM in sheep (Moor *et al*., 1980). The presence of oestradiol in the culture medium of in-vitro matured human oocytes had no effect on the progression of meiosis, but its presence improves fertilization and cleavage rates (Tesarik and Mendoza, 1995). It is generally considered that the effect of oestradiol in the culture medium at a concentration of 1.0 μg/ml in the bovine follicular fluid of preovulatory follicles shortly after the LH surge (Dieleman *et al*., 1993) can be adapted to human oocyte IVM (Trounson *et al*., 1994; Barnes *et al*., 1995, 1996). However, it may not be necessary to add oestradiol to the culture medium when the oocytes are cultured with the cumulus cells, because the culture medium supplemented with

gonadotrophins stimulates oestradiol secretion from the cumulus cells during culture *in vitro* (Chian *et al*., 1999a). It seems that there is a negative effect of the presence of progesterone in the culture medium on oocyte maturation and subsequent early embryonic development *in vitro* (Chian *et al*., unpublished data).

It is known that there are many growth factors in follicular fluid. These growth factors must be secreted from the granulosa and the cumulus cells responded to gonadotrophins, acting on oocytes through 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 (Chian and Tan, 2002), suggesting that the granulosa and cumulus cells could secrete some growth factors during culture *in vitro* and play some functional roles in oocyte maturation. Usually the culture medium is supplemented with some volume of the patient's own serum or human serum albumin (HSA) as a protein source. Serum and HSA are rich with growth factors. Therefore, it is not practically necessary to add growth factors into IVM medium for oocyte maturation *in vitro* when the culture medium has been supplemented with serum or HSA.

Complex culture medium, such as tissue culture medium 199 (TCM-199), Ham's-F10, and Chang's medium buffered with bicarbonate or HEPES (N-2-hydroxyethylperazine N-2-ethane sulphonic acid) have been most widely used in research or clinical application of oocyte IVM (Trounson *et al*., 1998). Although major beneficial components seem already present in these media (Bevers *et al*., 1997), it is clear that some components in these media have negative effects on oocyte cytoplasmic maturation *in vitro* (Chian and Tan, 2002). Recently, a new IVM medium has been developed that is more optimal for immature human oocytes culture *in vitro*, and its use has attained clinical pregnancy and implantation rates of 37 and 18% respectively, based on approximately 200 IVM treatment cycles in infertile women with PCO or PCOS following using an HCG priming protocol (Chian *et al*., unpublished data).

More attention is necessary in determining specific metabolic needs and optimal culture conditions required by maturing oocytes for appropriate gene expression and regulation. It is only after activation of the embryonic genomes that the embryo begins to control its own unique genomes. In the human embryo, this is thought to occur between the 4-cell and 8-cell stage (Braude *et al*., 1988). The extended culture of embryos *in vitro* in the presence of serum may affect the expression of the embryonic genomes abnormally evidenced by the production of large offspring in ruminant species, so-called 'large calf/lamb syndrome' (see reviews by Leese *et al*., 1998; McEvoy *et al*., 2003). It is important to mention that most embryo transfers are performed at the blastocyst stage in these ruminant species, suggesting that there is a potential risk for the embryos cultured *in vitro* to blastocyst stage because the activation of the embryonic genomes occurs after 8-cell stage of embryos in these ruminant species. It has been reported that some genetic and structural defects in oocyte maturation are induced environmentally (Eichenlaub-Ritter *et al*., 2002) and are related to the age of the woman (Keefe *et al*., 2003). Therefore, some theoretical concern regarding the safety of IVM has been raised, especially the potential impact in the expression of imprinting genes (Wolffe and Matzke, 1999; John and Surani, 2000; Fauser *et al*., 2002; Albertini *et al*., 2003; Reik *et al*., 2003). However, based on approximately 300 live births from IVM so far, it is difficult to conclude that IVM increases the risk of imprinting gene damage over that of conventional IVF (i.e. hormone stimulated IVF cycles).

Priming with FSH or HMG before immature oocyte retrieval

As an alternative approach, mild ovarian stimulation with FSH or HMG before immature oocyte retrieval has been applied, indicating that FSH or HMG pretreatment promotes efficient recovery of immature oocytes and maturation rate of the oocytes from women either with or without PCOS (Wynn *et al*., 1998). However, it has been reported 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 on oocyte maturation, cleavage rates or embryonic development in women with normal cycling ovaries (Mikkelsen *et al*., 1999). Nevertheless, the same authors reported that oocyte maturation and implantation rates are significantly improved by rFSH priming before harvesting of immature oocytes from patients with PCOS (Mikkelsen and Lindenberg, 2001). More recently, they also reported that the rates of oocyte maturation, fertilization, cleavage or implantation were not different between 'coasting' for 2 days and 3 days before immature oocyte retrieval when the normal menstrual cycling women were given 150 IU FSH/day for 3 days, starting from day 3 (Mikkelsen *et al*., 2003). Interestingly, Suikkari *et al*. (2000) reported that using low-dose FSH priming starting at the luteal phase improves the efficiency of immature oocyte recovery, maturation and fertilization rates, but the average number of immature oocytes collected, the rates of oocyte maturation and fertilization were not different between women with regular menstrual cycles and women with irregular cycles realted to PCO. In addition, 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 (Lin *et al*., 2003). Although these results are contrary to the benefits of using FSH priming in women with regular menstrual ovaries or irregular menstrual cycles of PCOS, theoretically the use of FSH priming before immature oocyte retrieval may enhance follicular development. Further research is required to confirm the beneficial results of FSH priming before immature oocyte retrieval from women with normal ovaries or PCOS.

Priming with HCG before immature oocyte retrieval

It has been noted that morphological and molecular differences exist between immature oocytes collected from stimulated cycles and from Caesarean section (Chian *et al*., 1997). It has also been found that the time course of germinal vesicle breakdown (GVBD) and oocyte maturation was different between these two groups, although the final rates of oocyte maturation were not different in these two groups (Cha and Chian, 1998). It appears that the oocytes retrieved from follicles in women undergoing ovarian stimulation respond to HCG, which may promote the initiation of oocyte maturation *in vivo*. It has been demonstrated that the time course of oocyte maturation *in vitro* is shortened 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 PCO or PCOS (Chian *et al*., 1999b, 2000). Therefore, it is possible that pregnancy rate may potentially be improved by priming with HCG (Chian *et al*., 1999c). Furthermore, it seems that HCG priming 36 h prior to oocyte collection may increase the number of oocytes retrieved (Chian *et al*., 2001) (**Figure 1**). This hypothesis was confirmed by other reports (Hwang *et al*., 2002; Nagele *et al*., 2002; Son *et al*., 2002; Lin *et al*., 2003).

Lin *et al*. (2003) reported that 36.4% clinical pregnancy rate was obtained based on 33 cycles of IVM treatment primed with HCG before immature oocyte retrieval from infertile women with PCOS, indicating that the beneficial effect of HCG priming on IVM treatment and the combination of FSH and HCG priming has no additional beneficial effect on IVM. Based on approximately 1000 IVM cycles in a multi-centre study with HCG priming before immature oocyte retrieval from women with PCO or PCOS, the pregnancy and implantation rates achieved were approximately 30–35% and 10–15% respectively (Chian *et al*., unpublished data). Interestingly, it has been reported that the time course and maturation rate of the GV stage oocytes were different when COC were collected with different morphologies after HCG priming from infertile women with PCOS (Yang *et al*., 2001). HCG priming seems to promote and initiate oocyte maturation process from GV to metaphase I stage in the relatively larger size of follicles (>10 mm in diameter). At the same time, HCG priming may enhance some GV stage oocytes from the small size of follicles to acquire maturational and developmental competence *in vivo*. However, the exact mechanism of action of HCG on these small follicles is still unclear. Further research is necessary to confirm the functional role of HCG priming on the small size of follicles before immature oocyte retrieval in infertile women with PCO or PCOS is attempted (**Figure 2**).

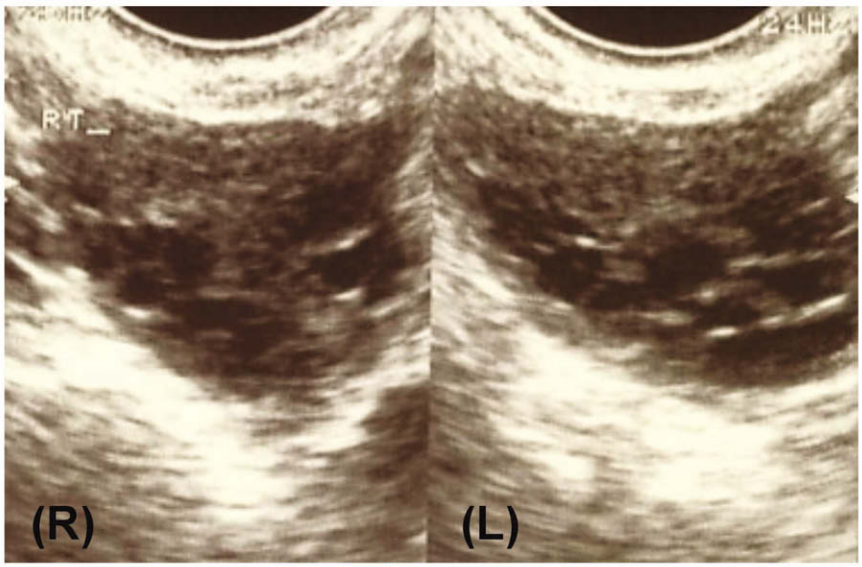

Figure 1. Transvaginal ultrasound view of polycystic ovaries in patient with polycystic ovarian syndrome. In total, 63 immature oocytes were retrieved from this patient at oocyte collection 36 h after HCG priming. The patient became pregnant and delivered a healthy male infant (see Chian *et al*., 2001). (R): right-side ovary; (L): left-side ovary.

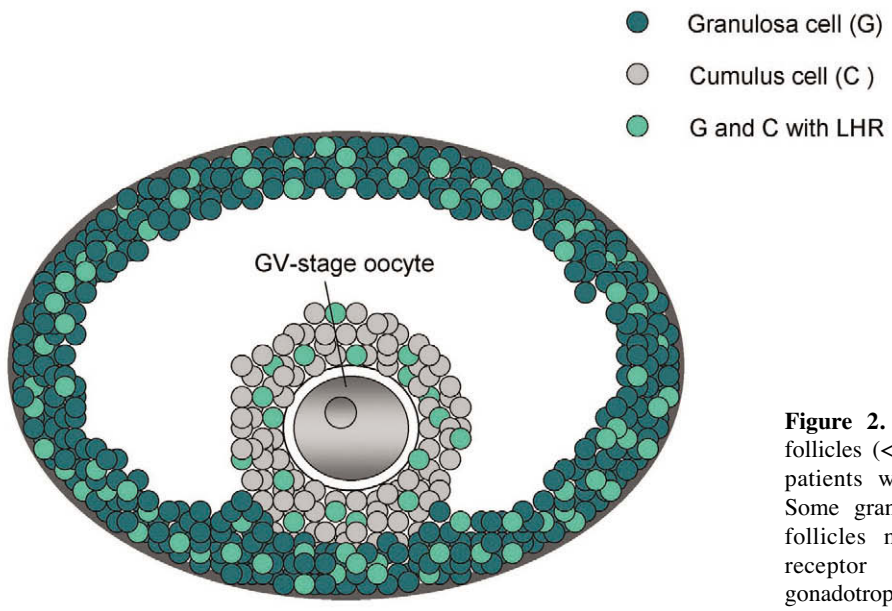

Figure 2. Hypothetical model for the small follicles (<8 mm in diameter) in ovaries from patients with polycystic ovarian syndrome. Some granulosa and cumulus cells in small follicles may possess luteinizing hormone receptor (LHR) or human chorionic gonadotrophin (HCG) receptor.

Conclusions

Priming with FSH or HCG prior to immature oocyte retrieval improves oocyte maturation and embryo quality as well as pregnancy rates, when these immature oocytes are retrieved from infertile women with PCO or PCOS. Approximately 300 healthy infants have been born following immature oocyte retrieval and IVM after either FSH or HCG priming protocols. In general, clinical pregnancy and implantation rates following IVM treatment are approximately 30–35% and 10–15% respectively in infertile women with PCOS. Therefore, IVM treatment is a viable treatment option and an alternative to conventional IVF for infertile women with PCO or PCOS.

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