—Review—

Oocyte Maturation in Humans: the Potential Relevance to Reproductive Medicine

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Introduction

Mammalian oogenesis is characterized by alternating periods of active meiotic progression and intermittent, long periods of meiotic arrest. The oocyte undergoes major growth and developmental processes in the period prior to ovulation. At the time of birth, most oocytes are arrested in the dictyate stage of meiosis; they remain quiescent for an indeterminate period until they begin growing in response to as yet undefined local signals [1]. From the beginning of the growth phase until the time of ovulation, oocytes increase in volume by at least two orders of magnitude and during this time exhibit intense metabolic activity. Resumption of meiosis only occurs in a fully grown, meiotically competent oocyte after the luteinizing hormone (LH) surge when oocytes undergo germinal vesicle breakdown (GVBD), complete first meiosis, and mature to metaphase II. In many mammals such as the mouse, pig, cow and man, completion of meiosis is dependent on fertilization that triggers progression to anaphase II, and to the first mitotic interphase with formation of female and male pronuclei. The process of maturation encompasses a complex series of molecular and structural events, culminating in the arrest of the oocyte chromosomes on the metaphase II plate in anticipation of sperm penetration and activation for fertilization. This review will focus on the biology of oocyte maturation and the potential relevance of maturation of human oocytes *in vitro* to reproductive medicine.

Received: February 10, 2003
Accepted: May 18, 2003
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Biology of Oocyte Maturation

Functions of cumulus cells

Intercellular communication between the oocyte and surrounding follicle cells is of vital importance, first to maintain the oocytes arrested at prophase I of meiosis and later to urge the oocyte to resume meiosis at the time of ovulation. Direct transfer of substances between cumulus cells and the oocyte is important for the maintenance of meiotic arrest [1,2]. This transfer is mediated by gap junctions allowing the exchange of ions and small molecules between cells. Several substances are believed to act as meiotic inhibitors, such as purines and cAMP [2–5]. In small follicles, tonic levels of cAMP are continuously transferred to the oocyte to maintain meiotic arrest [2]. In mammals cAMP in granulosa/cumulus cells promotes the generation/activation of a maturation-inhibiting factor which is transferred to the oocyte and is maintained in an active form by a cAMP-dependent process in the oocyte. Cumulus cells are clearly in control of meiosis, since compounds which increase intracellular cAMP levels inhibit or delay meiotic resumption in cumulus-enclosed oocytes, but not in denuded oocytes [2, 4]. The maintenance of an optimal intracellular cAMP concentration in combination with meiotic arrest may be necessary to allow oocytes to complete cytoplasmic maturation [6–8].

Follicular responses to the preovulatory surge of LH lead to production of hyaluronic acid, resulting in the mucification and expansion of the cumulus granulosa cells with the attendant termination of gap junctional contact between the cumulus cells and the oocyte [1, 2, 8]. This loss of intercellular communication may serve as a trigger for the resumption of meiosis in mature oocytes *in vivo* as it will reduce cAMP concentrations

within the oocyte, lead to deactivation of cAMP dependent protein kinase A (PKA), and reduce the inhibitory effects of purines on the maintenance of meiotic arrest in the oocyte. These changes in the somatic cell compartment occur simultaneously with the breakdown of the oocyte nuclear membrane as the cell proceeds to metaphase and complete the first division with expulsion of the first polar body [8].

Factors affecting oocyte maturation

In vivo, LH is a physiological signal that triggers resumption of meiotic maturation and all changes necessary in the follicle to obtain a fertilizable gamete, but in addition to gonadotropins, a number of other factors have been reported to affect oocyte maturation [9]. Granulosa and cumulus cells secrete a wide variety of growth factors that may either amplify or attenuate gonadotropin action in the ovary in a paracrine-autocrine manner. These include insulin-like growth factors (IGFs), growth hormone (GH), epidermal growth factor (EGF), pituitary adenylate cyclase activating peptide (PACAP), and many others.

We have demonstrated that IGF-I stimulates the meiotic maturation of rabbit follicle-enclosed oocytes in the absence of gonadotropin in a dose-dependent manner [10,11]. This finding is consistent with previous data showing that IGF-I enhanced the meiotic maturation of cumulus-enclosed oocytes in humans [12]. Furthermore, IGF-I-stimulated oocyte maturation is significantly blocked by the concomitant addition of αIR-3, a monoclonal antibody to IGF-I receptor, suggesting that the maturation-promoting action of IGF-I may be mediated through the IGF-I receptor [10]. These findings also suggest that the IGF-I receptors in the oocyte are functional. Although most investigations of the biological action of IGF-I have focused on the somatic cells of the ovary, it seems highly likely that IGF-I interacts with oocytes. Expression of IGF ligands and receptor genes has been observed in the growing oocytes of human infant ovaries and in mature oocytes of adult ovaries, suggesting that IGF may be involved in the processes of oocyte maturation as well as oogenesis [13]. The physiological significance of these observations remains to be established in humans, but the potential of IGF-I to act at the oocyte level, as indicated by the presence of receptors in oocytes and IGF-I-induced stimulation of meiotic maturation [10,13], suggests that in the presence of endogenous follicular factors and gonadotropins, IGF-I may regulate the selection and meiotic maturation of oocytes during follicular development *in vivo*.

Growth hormone (GH) induces *in vitro* maturation of follicle-enclosed oocytes in mammals [14, 15]. GH treatment has been shown to increase the ovarian content of immunoreactive IGF-I and its mRNA in rabbits [10, 15]. The blockade of the stimulatory effect of GH observed in the presence of antibodies to IGF-I [14] indicates that GH requires the intermediary of endogenously generated granulose cell-derived IGF-I to accomplish its effect on oocyte maturation. In rabbit ovaries perfused *in vitro*, hCG also stimulates both the ovarian expression of IGF-I mRNA and oocyte maturation. The hCG-induced oocyte maturation can be inhibited by IGFBP-3, but not IGFBP-1 [10]. In addition, IGFBP-3 inhibits IGF-I-induced oocyte maturation in a dose-dependent manner, suggesting that IGFBP-3 may exert an inhibitory signal for IGF-I-induced oocyte maturation. In humans, the concentrations of IGFBP-3 in follicular fluids decrease as oocytes progress to mature [16]. These findings suggest that IGFBP-3 may play a significant role in oocyte maturation in the gonadotropin-induced ovulatory process. In contrast, the concentrations of IGFBP-1 in follicular fluids with mature oocytes are significantly higher than those in follicular fluids with intermediate or immature oocytes [16]. Significantly positive correlations are found between the concentrations of IGFBP-1 and E2 and P in follicular fluids. It is suggested that the follicular fluid IGFBP-1 level may be a valuable marker of oocyte maturation.

Most growth factors directly affect the oocyte or exert their action via cumulus cells. EGF stimulates cumulus expansion and increases the proportion of denuded and cumulus-enclosed oocytes attaining metaphase II [17, 18]. Goud *et al.* have demonstrated that EGF improves denuded human oocyte maturation but the overall cleavage rates after fertilization in cumulus-intact oocytes were higher than in denuded oocytes cultured with EGF [19]. Even though EGF can affect isolated oocytes, the presence of cumulus cells appears to be beneficial for oocyte maturation and early development. Therefore, supplementation of the maturation medium with EGF and maintenance of the cumulus during culture improve the nuclear and cytoplasmic maturation of human oocytes.

A new peptide of the vasoactive intestinal peptide/glucagone/GRF family, PACAP, has been found to affect the meiotic maturation of oocytes [20]. The peptide had the opposite effect on cumulus-enclosed and denuded oocytes: the effect is stimulatory in the presence of cumulus cells and inhibitory in the absence of them. PACAP acts directly on denuded oocytes,

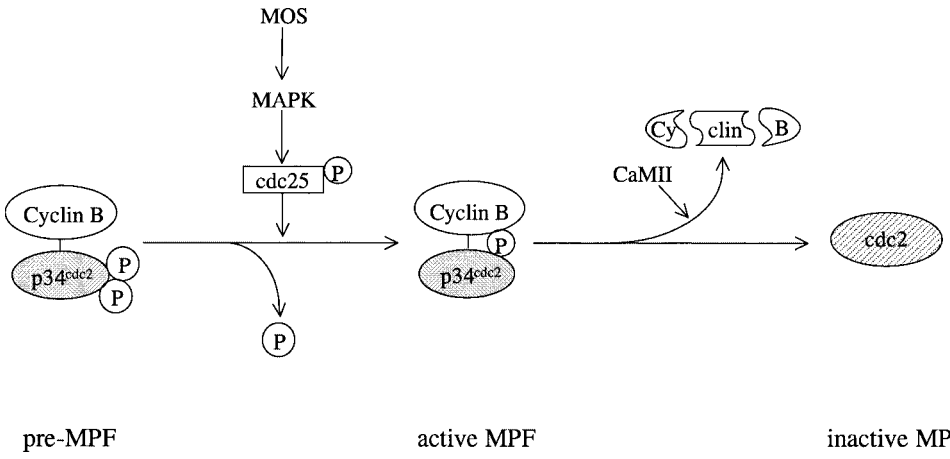

Fig. 1. Molecular mechanism of activation of maturation promoting factor (MPF). MPF is shown as a heterodimer composed of $p34^{cdc2}$ and cyclin B. In the pre-MPF stage, the MPF heterodimer is phosphorylated on tyrosine 15 and threomine 14. MOS activates mitogen activated protein kinase (MAPK) and cdc25. The phosphorylated form of cdc25 is responsible for dephosphorylating the cdc2 protein on tyrosine 15 and threonin 14, thus activating MPF. The MPF activity increases until metphase I and decreases during the anaphase to telophase transition. The activity increases again until the oocyte reaches metaphase II and is maintained at a high level until fertilization. Calmodulin (CaM) kinase II induces cyclin degration, resulting in the inactivation of MPF.

delaying meiotic maturation by modifying the intracellular cAMP levels in oocytes.

Molecular control of oocyte maturation

The maintenance of meiotic arrest in growing oocytes and the reinitiation of meiosis after the preovulatory gonadotropin surge can be explained in terms of activation and inactivation of cell cycle proteins [21, 22]. Regardless of the signaling system which starts GVBD, nuclear maturation in mammalian species appears to be mediated by the production of active maturation promoting factor (MPF) in the ooplasm (Fig. 1). MPF is a serine-threonine kinase protein heterodimer composed of a catalytic subunit, $p34^{cdc2}$, and a regulatory subunit, cyclin B [23]. During the cell cycle, MPF activity is regulated by the phosphorylation-dephosphorylation of $p34^{cdc2}$ and its association with cyclin B [24].

Cyclic MPF activity is a characteristic feature of oocytes and is attributable to the periodic activation and inactivation of the MPF heterodimer [22]. MPF activity is consistently low in GV stage oocytes, and is detected just before, or concomitantly with, GVBD. MPF activity increases until metaphase I and decreases during the anaphase to telophase transition. The activity increases again until the oocyte reaches metaphase II, and is maintained at a high level by the interaction of cytostatic factor (CSF) and the viral oncogene *c-mos* until fertilization. Inactivation of MPF results in exit from the metaphase, and during oocyte maturation MPF inactivation occurs at the transition of metaphase I to anaphase I and again at fertilization. $P34^{cdc2}$ phosphorylation and cyclin degradation are both required for MPF inactivation. The significance of MPF in the maintenance of meiotic arrest has been demonstrated by the premature resumption of meiosis of primordial follicle oocyte nuclei after fusion with cells with higher MPF levels [25]. The direct actions of MPF during GVBD may involve dissolution of the nucleoli, chromosomal condensation, and reorganization of the microtubular complex to form a functional spindle apparatus.

c-mos proto-oncogene encodes a serine-threonine protein kinase, which is expressed in oocytes. In the oocyte, mos is involved in several aspects of oocyte maturation. Mos has been demonstrated to phosphorylate cyclin B and thereby affects cyclin stability and MPF activity [26, 27]. As part of the CSF complex, mos is involved in the maintenance of metaphase II arrest in mammalian oocytes. Mos has also been detected in human oocytes and its expression is restricted to the oocyte, as both the mos protein and

mRNA are degraded during embryonic development [28, 29]. Although the action of mos during human oocyte maturation *in vitro* has not been investigated directly, it is likely to play a role in the regulation of meiotic maturation by interacting with cyclin B and CSF to stabilize MPF and maintain meiotic arrest, as well as participate in the activation of MAP kinase.

Mitogen activated protein (MAP) kinase, which is alternatively known as extracellular regulated kinase, is a serine-threonine kinase that is activated via a protein kinase cascade at the onset of oocyte maturation in mammals [30, 31]. MAP kinase is not necessarily required for GVBD in mouse oocytes although activated at the onset of oocyte maturation in mice [32]. Nevertheless, MAP kinase activity is associated with a plethora of cytoplasmic events including the regulation of microtubule dynamics, spindle assembly and chromosome condensation [31,33]. In humans, MAP kinase is inactive in immature oocytes, active in mature oocytes, and the activity decreases after pronuclei formation after fertilization [34]. Therefore, the MAP kinase pattern of activation in the cell cycle appears to be similar to that of other mammalian species.

Oocyte Maturation *In Vitro*

Significance of in vitro maturation

In humans, several pathological conditions cause anovulatory infertility, and different

ovulation induction protocols have been developed during IVF programs to solve the problem, but these fertility therapies also have some undesirable side-effects. The potential benefits of developing an effective *in vitro* maturation (IVM) program as an alternative clinical strategy to conventional IVF are many: it would not only substantially reduce both the costs of drug treatment and wastage of immature eggs collected during standard IVF, but could also lessen the risks of hyperstimulation syndrome. Additionally, IVM may provide a valuable model for investigating the causes of meiotic aberrations and aneuploidies which are remarkably common in mature human oocytes [35]. Finally, IVM might open the door to oocyte cryopreservation if freeze storage of germinal vesicle stage oocytes avoids the problem of damaging the meiotic spindle of metaphase II oocytes. Therefore, IVM deserves rigorous experimental evaluation to determine whether it is practicable in reproductive medicine.

The potential clinical benefits of IVM are profound. Many young women with cancer undergo potentially sterilizing chemotherapy or radiotherapy treatments. These life-saving therapies frequently cause a premature menopause. If ovarian tissue could be cryopreserved before such treatment, the patient's own oocytes could be conserved for future child-bearing. Oocytes could be matured and fertilized *in vitro* before embryo transfer. It is possible that such preserved ovarian tissue may be autografted once cancer treatment is concluded. Once the problems of IVM are solved, donated ovarian tissue could provide a rich source of donor oocytes for patients who undergo premature ovarian failure or those with gonadal dysgenesis. Ultimately, IVM could be useful for many infertile patients undergoing IVF.

Methods for IVM of human oocytes were developed by Trounson *et al.* for the recovery of oocytes from the ovaries of patients with polycystic ovary syndrome (PCOS) in which the dominance of a particular follicle fails to occur and the cohort of growing follicles accumulates in the cortex [36]. Most of these follicles are about 5 mm in diameter (range 3–8 mm) and remain under an androgen-dominated environment due to increased thecal cell secretion of androgens and a blockage of aromatization in the granulose cell compartments [37]. When oocytes are recovered by transvaginal guided ultrasonography, the oocytes undergo nuclear maturation in a number of different culture media, undergo fertilization after insemination or intracytoplasmic sperm injection (ICSI), and begin cleavage in culture [36, 37]. Compared with oocytes recovered from regularly cyclic non-PCOS women, oocyte maturation, fertilization, and embryo development in culture are significantly retarded [36,38]. Possible explanations of these disappointing results include: the poor quality of oocytes recovered from the patients with polycystic ovaries, suboptimal culture conditions, and the timing of embryo development in relation to the physiological stage and degree of development of the endometrium. In none of these studies, however, were the ovaries exposed to gonadotropin stimulation prior to oocyte collection.

IVM has been used successfully to treat patients in whom human chorionic gonadotropin (hCG) has been withheld both unintentionally because of patient forgetfulness and intentionally in individuals at risk of severe ovarian hyperstimulation syndrome [39, 40], but the prime candidates for IVF are women with PCOS [9, 22, 41]. This group of patients are most suitable because they have the highest risk of hyperstimulation from gonadotropins and they have a large crop of follicles for oocyte harvest. Whereas there is evidence

that GV oocytes removed from women with PCOS have a lower maturation potential than oocytes harvested from women with regular menstrual cycles, acceptable pregnancy rates have been achieved in this particular group of patients [22, 40, 41].

Priming with FSH or hCG

Immature oocytes retrieved from untreated ovaries can be matured, fertilized and developed *in vitro*, but the rate of implantation of the cleaved embryos is low [42]. The maturation rate of immature oocytes retrieved from patients with PCOS is impaired compared with that in women with regular cycles [9, 22, 41]. An alternative approach, which has been shown to improve the harvest, quality, and developmental competence of oocytes, is to provide a truncated course of ovarian stimulation with follicle-stimulating hormone (FSH) before oocyte collection [43, 44]. The data from these studies support the idea that human oocytes only acquire full meiotic competence late in follicular development after the follicles have been exposed to supra-threshold levels of FSH.

In contrast, several studies of the treatment of women for 1 or 3 days with recombinant human FSH (rhFSH) early in the follicular phase showed no difference in the recovery rate of oocytes, or oocyte maturation, fertilization or development in culture [37, 45]. Mikkelsen *et al.* have demonstrated that treatment with rhFSH at a dose of 150IU/day for 3days (day 3–5) results in no improvement in any parameter of oocyte recovery, maturation or developmental competence. The failure to achieve a substantial improvement in the number and developmental competence of oocytes by pre-treatment with FSH indicates that maturation *in vitro* is not limited by the growth phase of follicles in the ovaries. Normally one or two follicles become dominant in the ovulatory cycle, and the other antral follicles cease growth and enter atresia as the follicular phase progresses. There is no detectable difference in the maturational and developmental competence of oocytes recovered from the dominant follicle or those from atretic follicles, a surprisingly common observation that was first made by Moor and Trounson in sheep follicle culture [46]. Pre-treatment of women with FSH to collect immature oocytes may have no clinical application, unless a very substantial benefit is demonstrated for maturation and development to term.

Chian *et al.* have reported pregnancies after the treatment of unstimulated patients with PCOS with hCG 36 hours before immature oocyte collection and IVM [47]. They found that hCG priming of PCOS patients could improve the developmental competence of the recovered oocytes, leading to improved maturation rates and higher pregnancy rates. No discernible differences were detected in fertilization, cleavage and embryo quality between hCG-treated and non-treated patients. These findings suggest that the effect of hCG may act through the inhibition of oocyte maturation inhibitor and the loss of communication between the oocyte and the cumulus complex. The positive effect of hCG may be derived from communication initiated at the level of the theca interstitial cells and progressing to the granulosa syncytium [48]. Potential effects include androgenation of the follicle which is associated with atresia; alteration of oocyte metabolism through increases in glycolytic activity, mitochondrial glucose oxidation, and glutamine within cumulus-enclosed oocytes; and/or direct signaling of the granulosa to differentiate [48].

Conclusions

Complete maturation of oocytes is essential for the developmental competence of embryos. Any interventions in the growth phase of the oocyte and the follicle in the ovary will affect oocyte maturation, fertilization and subsequent embryo development. Immature oocyte collection and *in vitro* oocyte maturation have been successfully applied to laboratory animals and are now a tempting treatment method for human infertility. Creating a consistent *in vitro* follicular environment may provide numerous advantages by having access to the female gamete with the potential to improve all aspects of human IVF through improved embryogenesis. Oocyte maturation *in vitro* can be accomplished in humans, but is associated with a loss of developmental competence unless the oocyte is near completion of its preovulatory growth phase. This loss of developmental competence is associated with the absence of specific proteins in ooctyes cultured to metaphase II *in vitro*. The better understanding of the regulation and production of all the different factors implied in follicle development will help in elucidating the problems connected with unexplained forms of infertility as well as in the search of the optimal conditions to obtain fertilizable oocytes to be used in programs for assisted reproduction.

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