CHAPTER IV

DISCUSSION

The quality of *in vitro* matured oocytes was suboptimal because the fertilization rate resulting from these oocytes was lower than that of *in vivo* matured oocytes (Eppig et al., 1996 ; Goud et al., 1998). Many important factors regulating the nuclear and cytoplasmic maturation of oocytes maturing *in vitro* have to be considered in order to maintain the oocyte's fertilizability, such as the process of immature oocyte preparation, oocyte characterization (denuded or cumulus cell-enclosed oocytes), maturation medium, and maturation duration. Results from previous studies indicated that PMSG could be used as the hormone to induce superovulation in the mouse model for the purpose of IVM/IVF studies (Eppig and Downs, 1987 ; Downs et al., 1988 ; Hirao et al., 1990 ; Downs et al., 1995). However, various concentrations of PMSG, such as 5, 7.5, and 10 IU, were used in many investigations. This experiment was designed to investigate the most suitable concentration of PMSG for the stimulation of immature female mice to obtain immature oocytes for the IVM/IVF study in this laboratory.

However, a previous study demonstrated that PMSG and hCG were gonadotropic hormones used for *in vivo* maturation of oocytes in mice for the

IVF studies (Schultz et al., 1983 ; Downs and Eppig, 1987 ; DeMeestere et al., 1997). Some investigators suggested that immature 3 - 4 week-old female mice have the antral follicles in their ovaries, and have FSH and LH receptors on the follicular membrane (Greenwald, 1994 ; Eppig et al., 1996). In addition, the results from this laboratory have shown that a significant increase was observed in the percentage of oocytes undergoing the MII stage in maturation medium with hCG compared with medium without hCG. Thus, the effects of hCG (LH agonist) on *in vitro* oocyte maturation and fertilization were examined in this experiment and compared with the effects of PMSG on those parameters.

The present study has shown that gonadotropins, PMSG and hCG, increase nuclear and cytoplasmic maturation of both *in vivo* and *in vitro* matured oocytes, and confirm previous reports that gonadotropins were clearly beneficial for the acquisition of competence to resume meiosis and improve fertilization rate of the oocytes (Schroeder et al., 1988 ; Jinno et al., 1990 ; Saeki et al., 1991 ; Eppig et al., 1992). However, the results of previous studies have shown that oocyte maturation comprises three stages of nuclear maturation, i.e., the GV, GVBD or MI, and the MII stage (Downs et al., 1988 ; Buccione et al., 1990 ; Eppig, 1991). The oocytes at the GV and GVBD stage are immature oocytes in which are not suitable for fertilization by sperm because they have a diploid number of chromosomes (2n) in their nucleus (Eppig, 1991 ; Eppig et al., 1996).

The oocytes at GV-IV were at the latest GV stage before developing to the GVBD and the MII stage after the preovulatory gonadotropin surge during *in vivo* maturation of oocytes. In the present study, oocytes at the GV stage were obtained from both PMSG- and hCG-primed mice undergoing maturation at the MII stage (87.4 ± 2.4% for PMSG- primed mice, 86.3 ± 3.2% for hCG- primed mice) in maturation medium for 24 hours and thus significantly increasing (P<0.05) the percentage of *in vitro* fertilization (47.0 ± 3.4% for PMSG- primed mice, 45.5 ± 1.7% for hCG-primed mice. Results from this study suggest that the nuclear morphology of oocytes at the GV stage changes from GV-X to GV-III or GV-IV in PMSG- and hCG-primed immature female mice. These data indicate that gonadotropins, PMSG and hCG, enhance nuclear and cytoplasmic maturation of oocytes to be mediated by FSH and LH receptors, respectively, and confirm previous reports on FSH and LH receptors being present on the follicular membrane of mice older than 15 days postpartum (Sorensen and Wassarman, 1976 ; Greenwald, 1994). However, the mature oocytes obtained from either 7.5 IU PMSG or hCG stimulated groups were increased in the percentage of *in vitro* fertilization compared to those of the 5 and 10 IU PMSG or hCG stimulated groups. These data suggest 7.5 IU as the optimum concentration of gonadotropins to induce nuclear and cytoplasmic maturation of oocytes. In addition, some investigators suggested that high concentrations of PMSG to cause parthenogenesis and incomplete preimplantation embryo development in the thus primed animals (Edwards, 1965 ; Gianfortoni and Gulyas, 1985). However, in this study the fertilization

rate was determined by the percentage of 2-cell embryos but not stained with 1% orcein to examined the incidence of oocytes penetrated normally by assessment of monospermy and male pronuclear formation. Thus, the success rate of *in vitro* fertilization may be included the parthenogenetic activation of oocytes.

In this study the overall higher rates of maturation and fertilization in cumulus cell-enclosed oocytes than in denuded oocytes indicate the quality of nuclear and cytoplasmic maturation to be superior in the presence of the attached cumulus cells. The beneficial effects of cumulus cells on oocyte maturation and early development are well known in different species (human : Kennedy and Donahue, 1969; mouse : Cross and Brinster, 1970; rat : Vanderhyden and Armstrong, 1989; and bovine : Sirard et al., 1988 ; Fukui, 1990). The cumulus cells increase the surface area:volume ratio of the oocyte which increases the rate of entry of small molecules (OMS, such as growth factors) into the oocytes. The cumulus cells are also intimately connected with the oocyte through long microvilli that traverse the zona to contact the oolemma to form gap junctions and desmosomes (Motta et al., 1994 ; Legge, 1995). The gap junctions help mediate the transport of certain molecules necessary for oocyte metabolism and maturation (Haghinat and Van Winkle, 1990 ; Sherr, 1996). In addition to these effects, the granulosa cells also appear to exert some of their effects on the oocyte via paracrine signals which may explain the beneficial effect of co-culture of granulosa cells, especially when the latter were

primed with FSH (Dandekar et al., 1991 ; Schramm and Bavister, 1996). A similar effect was also seen when cumulus-denuded oocytes were co-cultured with Vero cells (Janssenswillen et al., 1995). Recently, a novel 'apocrine' mechanism for the storage and release of growth factors and certain proteins was demonstrated in follicular fluid and cumulus cells (Antczak et al., 1997). Some of these proteins are expressed and selectively distributed in mature oocytes and developing embryos, which is suggestive of their role during embryogenesis (Antczak and Van Blerkom, 1997). These mechanisms may at least partly explain some of the beneficial effects of cumulus cells seen in this study. The present study supports the idea of a positive maturation signal generated in the cumulus cells in response to PMSG and hCG stimulation.

Many investigators suggested that the percentage of denuded oocytes that has been matured and fertilized *in vitro* was lower than that of cumulus cell-enclosed oocytes, and this reduction was probably caused by changes in the ZP that prevent sperm penetration (Schroeder and Eppig, 1984 ; Gianfortoni and Gulyas, 1985 ; Downs et al., 1988). These changes in the ZP are retarded by cumulus cells. The cumulus cells produce some substances that prevent ZP hardening during oocyte maturation and increase oocyte fertilizability. However, some investigators have shown that the immature oocytes obtained from gonadotropin stimulated immature female mice for IVM/IVF studies were cumulus cell-enclosed oocytes (Downs et al., 1988 ; Eppig et al., 1994 ; Eppig et al., 1996). Denuded oocytes were prepared by repeated pipetting of cumulus

cell-enclosed oocytes through a sterile Pasteur pipet ; they were then washed and transferred to maturation medium. In this study, the immature oocytes obtained from gonadotropin stimulated immature female mice were denuded oocytes (appoximately 70%). This might due to various different factors such as strain of mice and technique applied to collect immature oocytes.

In this study, the effects of PMSG and hCG on *in vivo* maturation of mouse oocytes have shown that hCG plays a more crucial role as to nuclear maturation of *in vivo* matured oocytes than PMSG. The results of this study confirm those of a previous report in that FSH produces first an inhibition of oocyte maturation and it has been proposed that cAMP may play a role in the resumption of meiosis in oocytes. However, the highest percentage of *in vivo* oocyte maturation observed in hCG-primed mice was arrested at the GVBD stage rather than the MII stage, whereas in PMSG-primed mice it was arrested at the GV stage . These data suggested that hCG caused incomplete nuclear maturation of oocytes in the antral follicles of immature mice. Some investigators indicated that oocytes from small antral follicles are often only "partially competent" to complete nuclear maturation ; the progression of meiosis becomes arrested at the GVBD (MI) rather than the MII stage (Schramm et al., 1993 ; De Smedt et al., 1994 ; Eppig et al., 1994 ; Eppig et al., 1996). The follicles in the ovaries of mice used in this experiment may contain many small antral follicles. Nevertheless, these oocytes could be fertilized and developed to the late blastocyst stage.

In IVM/IVF studies, one of the most important factors for maintaining the oocyte's fertilizability is oocyte maturation duration. The results of this study indicated that the quality of *in vitro* matured oocytes is time dependent and suggested that the optimum oocyte maturation duration for IVM/IVF studies in the mouse model to optimize the oocyte's fertilizability and embryo development is 15 hours. The results of this study confirm previous reports in that *in vitro* oocyte maturation can lead to a premature discharge of cortical granule contents and a reduced incidence of fertilization (Ducibella et al., 1990 ; Hinsch et al., 1997). *In vitro* matured oocytes for 21-24 hours in maturation medium have shown increased time required for ZP digestion by α-chymotrypsin associated with a decreased fertilizability.

The ZP of mammalian oocytes and preimplantation embryos is an acellular matrix composed of sulfated glycoproteins that serve several biological functions during fertilization and development (Dean, 1992). Detailed characterization of the mouse ZP revealed three distinct glycoproteins (ZP1, ZP2, and ZP3). An important biochemical change in ZP2 occurs immediately after fertilization (Bleil et al., 1981). Proteases (e.g., peroxidase) released by the oocyte's cortical granules cause proteolysis of the ZP2 molecule into ZP2f molecules, identified by electrophoretic analysis (Ducibella et al., 1990), which remain joined by intramolecular disulfide bonds (Moller and Wasserman, 1989). The postfertilization modification of ZP2 has been associated with inhibition of sperm binding that is believed responsible for the

prevention of polyspermic fertilization (Bleil et al., 1988 ; Meduri et al., 1992 ; Wasserman, 1994).

The postfertilization physical change in the ZP has been commonly referred to as "zona hardening" because the ZP2f disulfide linkages make the ZP more resistant to dissolution by proteases, reducing agents, and acid treatment (Schiewe et al., 1995). However, ZP hardening also occurs during the spontaneous maturation of oocytes in defined medium. The degree of spontaneous ZP hardening is directly correlated with the failure of the oocytes to become fertilized *in vitro* (Schroeder et al., 1990). Thus the process of ZP hardening, which is essential for normal development after fertilization, can prevent fertilization when it occurs prematurely. The results of this study are similar to those described above for ZP hardening *in vitro* in that the degree of hardening is time dependent, inversely correlated to oocyte fertilizability and that there is no correlation between ZP hardening and ZP thickness of *in vitro* matured oocytes from PMSG- and hCG-primed mice. However, in *in vitro* matured oocytes obtained from hCG-primed mice less degree of ZP hardening occurred during culture in medium than in *in vitro* matured oocytes obtained from PMSG-primed mice. These results suggest that hCG may some how affect the hardening process of the ZP by inducing a decrease of intracellular cAMP. In addition, along with a decrease of intracellular cAMP in *in vitro* matured oocytes obtained from hCG-primed mice after culturing for 9 - 18 hours, an increase in the percentage of *in vitro* fertilization, and blastocyst development

of these oocytes was observed. These results suggest that a decrease of intracellular cAMP may induce an increase in nuclear and cytoplasmic maturation of oocytes for maintaining the oocyte's fertilizability.

Various factors can inhibit ZP hardening during the spontaneous maturation of mammalian oocytes: cumulus cells, granulosa cells, follicular fluid, sulfated glycosaminoglycans, and serum (DeFelici et al., 1985 ; Downs et al., 1986 ; Eppig and Schroeder, 1986 ; Choi et al., 1987 ; Vanderhyden and Armstrong, 1989). Serum from various sources is effective in preventing ZP hardening with different degrees of efficacy: FBS, mouse serum, and newborn calf serum > bovine calf serum > human fetal cord serum (Eppig and Schroeder, 1986 ; Schroeder et al., 1990 ; Funahashi and Day, 1993). Serum must contain factors such as fetuin, a major glycoprotein in FBS, that inhibit the protease of cortical granules released during maturation because serum prevents the conversion of ZP2 to ZP2f (Ducibella et al., 1990).

In the present experiments, supplementing the maturation medium with 10% FBS did not completely preserve fertilizability of the *in vitro* matured oocytes. Associated with this reduced fertilizability was a failure of FBS to prevent spontaneous hardening of the ZP and it was shown that 10% FBS may not be a suitable concentration. Some the previous reports indicated that increasing the serum concentration from 5% to 10% had no effect on fertilization or ZP digestion time (Eppig and Schroeder, 1986 ; George et al.,

1992). Serum deprivation, even for a short period of time, at the onset of culture significantly reduced the fertilizability of cumulus-cell enclosed oocytes and increased the ZP digestion time (Downs et al., 1986 ; Vincent et al., 1991). In addition, some reports indicated that protection against this ZP hardening effect of the prematurely discharged cortical granule contents can be achieved by addition of 20% FBS to the medium (Vincent et al., 1991 ; George and Johnson, 1993). Thus, in this study 10% FBS supplemented to the medium may have been below the concentration to prevent ZP hardening complete in our observation.

Advanced release of cortical granules occurs during oocyte maturation *in vivo* or *in vitro* (Ducibella et al., 1988 ; Ducibella et al., 1990). Hardening of the ZP, however, does not occur during maturation *in vivo*, suggesting that substances are present within ovarian follicles that prevent conversion of ZP2 to ZP2f during oocyte maturation (Schroeder et al., 1990). Another protein often found in plasma, α_2-macroglobulin, has been demonstrated in small antral and preovulatory rat ovarian follicles (Gaddy-Kurten et al., 1989). This glycoprotein prevents conversion of ZP2 to ZP2f (Moller and Wassarman, 1989) and, therefore, probably functions to prevent precocious ZP hardening *in vivo*. Although α_2-macroglobulin is present in small antral and preantral follicles, α_2-macroglobulin mRNA is not detectable in the granulosa cells of these follicles. Nevertheless, this mRNA is present in luteinized follicles of rats 12 hours after injection of hCG (Gaddy-Kurten et al., 1989). The α_2-macroglobulin in the

small antral follicles and preovulatory follicles can be detected in follicular fluid but not in granulosa cells, hence it is not clear whether α_2-macroglobulin originates in granulosa cells or extrafollicular sources. Accordingly, the findings reported here suggest that hCG, to a greater extent than PMSG, may increase the α_2-macroglobulin activity in antral follicles to trigger developmental or physiological processes required for complete cytoplasmic maturation before fertilization and subsequent embryogenesis.

The effects of intraoocyte cAMP on nuclear and cytoplasmic maturation, and preimplantation embryo development have been shown in this study. The results of this study confirm previous reports in that a drop in intraoocyte cAMP concentration results in resumption of meiosis (Cicirelli and Smith, 1985 ; Smith, 1989 ; Downs and Hunzicker-Dunn, 1995). In addition, the studies presented here demonstrated that a decrease of intraoocyte cAMP causes an increase in the percentage of *in vitro* fertilization and preimplantation embryo development, and prevents ZP hardening. Noh and Han (1998) reported that two well-known transmembrane signaling pathways, the adenylyl cyclase and phosphatidylinositol pathways, have been studied extensively and are thought to play crucial roles during the meiotic maturation and fertilization of oocytes. It has been generally accepted that a transient decrease in the level of intraoocyte cAMP is the main signal triggering gonadotropin to induce oocyte maturation. Some investigators indicated that higher levels of the cyclic nucleotide would preferentially activate type I PKA and suppress oocyte

maturation, whereas lower levels achieved in response to the preovulatory gonadotropin surge would activate the type II holoenzyme and lead to GVBD and cumulus expansion (Downs and Hunzicker-Dunn, 1995 ; Noh and Han, 1998). In this study hCG-primed mice, to a greater extent than PMSG-primed mice, induce a decrease in the level of intraoocyte cAMP and an increase in the percentage of *in vitro* oocyte maturation and fertilization, and blastocyst development during an oocyte maturation period of between 9 and 18 hours. Therefore, the recent study suggests that hCG rather stimulates the activity of type II PKA than that of type I PKA, and is a very important inducer of complete nuclear and cytoplasmic oocyte maturation.

The result of previous studies has demonstrated that FSH produces first an inhibition of oocyte maturation, followed by a stimulation of GVBD (Downs et al., 1988). It has been proposed that FSH stimulates the production of a positive signal by a cAMP-dependent process and that the initial rise in intraoocyte cAMP activates type I PKA until a threshold level of stimulator is reached that induces GVBD. This study proposes that PMSG (FSH agonist) may stimulate the activity of type I PKA and suppress nuclear and cytoplasmic maturation, whereas hCG may preferentially activate type II PKA and stimulate nuclear and cytoplasmic maturation. In addition, some analogues of cAMP, such as 8-thiomethyl-cAMP and 8-bromo-cAMP, selectively activate type II PKA in mouse oocyte-cumulus cell complexes and induce an increase in the number and activity of LH receptors (Sanders and Midgley, 1983 ; Ogreid et

al., 1989 ; Downs and Hunzicker-Dunn, 1995). In this experiment, evidence has been presented to suggest that the induction of LH receptors by hCG is mediated through depletion of the second messenger cAMP and that this process causes an increase of *in vitro* oocyte maturation and fertilization, blastocyst development, and prevents ZP hardening by activation of type II PKA.

In addition to the involvement of intraoocyte cAMP, recent evidence has demonstrated that the phosphatidylinositol pathway might also be an important signaling component during hormonally induced oocyte maturation. It has been shown that the treatment of oocytes with 12-O-tetradecanoylphorbol 13-acetate (TPA), an activator of protein kinase C (PKC), induces GVBD in the absence of hormone (Wasserman et al., 1990 ; Han et al., 1992). In addition, PKC has been identified in mouse oocytes and microinjection of a cDNA coding for PKC into the oocyte induced migration of the GV (Muramatsu et al., 1989). Recently, Stith et al. (1992) have reported a detailed analysis of inositol 1,4,5-triphosphate (IP3) changes in maturing mouse oocytes and demonstrated that the level of IP3 is increased in progesterone-stimulated oocytes. Furthermore, changes in the level of diacylglycerol (DG), another second messenger generated from phosphoinositol lipid hydrolysis, have been monitored in response to progesterone stimulation at resumption of meiosis (Chien et al., 1991 ; Stith et al., 1991 ; Han et al., 1992). Finally, Noh and Han (1995) showed that reducing phosphatidylinositol 4,5-biphosphate (PIP2) hydrolysis

by microinjecting PIP2 antibody into the mouse oocytes inhibited meiotic maturation. These data indicated that PIP2 hydrolysis and subsequent generation of second messengers is involved in the maturation of mouse oocytes.

As a result of the present studies, suggests that hCG may exert its positive effect directly on the oocyte after binding to its receptors thus increasing the level and / or activity of IP3 and DG in oocytes more than PMSG. The activation of LH receptors (by hCG) suppresses adenylyl cyclase by means of inhibitory G-protein (Giα). Stimulation of these receptors in oocytes reduces the cAMP level, increases the activity of type II PKA, and increases the level of IP3 and DG. This evidence suggests the possibility that a decrease in cAMP-induced phosphorylation and production of IP3 and DG may be necessary and suffice as the signal to initiate the nuclear and cytoplasmic maturation of mouse oocytes.

This result is agreed to the findings of Suzuki et al. (1998) that (1) meiotic maturation was significantly inhibited in oocytes injected with anti-phosphotyrosine monoclonal antibody, (2) oocytes cultured with genistein, an inhibitor of tyrosine protein kinase, showed a decreased rate of the first polar body emission, and (3) the second polar body emission, pronuclei formation, and cortical granule exocytosis were associated with calmodulin-dependent kinase (CaMK) of mouse oocytes. These data suggest that activation of protein

tyrosine phosphorylation and CaMK is crucial during maturation, fertilization, and embryo development of mouse oocytes.

However, the developing ovarian follicle is one of the most rapidly proliferating normal tissues *in vivo*. Mesenchymal-epithelial cell interactions between theca cells and granulosa cells are essential for this follicular expansion. Ovarian hormones, such as estrogens and LH, may promote follicular development by regulating the local production of mesenchymal inducer proteins that mediate theca cell-granulosa cell interactions (Rao et al., 1978 ; Skinner and Osteen, 1988 ; Koji et al., 1994). Recently, theca cells have been shown to produce keratinocyte growth factor (KGF) that can act in a paracrine fashion to stimulate granulosa cell growth (Parrott et al., 1994 ; Rubin et al., 1995 ; Izumi et al., 1996). Parrott and Skinner (1998) reported that both estrogen and hCG stimulate KGF gene expression in theca cells. These results suggest that estrogen and hCG may promote follicular growth and oocyte maturation by stimulating the local production of KGF. The current results suggest that hCG might increases nuclear and cytoplasmic maturation of oocytes potentially by stimulating the local production of growth factors, such as KGF.

In addition, many investigations have suggested that cell cycle progression and proliferation are controlled by a balance of positive and negative regulators converging on cell cycle kinase cascades (Rempel et al.,

1995 ; Taieb and Jessus, 1996 ; Taieb et al., 1997). Interestingly, specific roles

for cell cycle regulatory molecules in the control of granulosa cell proliferation

and differentiation during follicular development have been elucidated by the

altered ovarian phenotypes brought about in mice by cyclin D2, cyclin E, and

p27^{Kip1} (Hirshfield, 1991 ; Richards, 1994 ; Tikoo et al., 1997). Cyclin D2 acts

as a proliferative regulatory molecule of the cell cycle by binding cyclin

dependent kinases (cdks) 4 or 6 and thereby activating follicular development

(Wu and Wolgemuth, 1995 ; Sherr, 1996). Cyclin E also acts as a proliferative

regulatory molecule of the cell cycle by binding and activating cdk2 (Rhee and

Wolgemuth, 1995 ; Wu and Wolgemuth, 1995). In contrast, p27^{Kip1} acts as a

differentiative regulatory molecule of the cell cycle by inactivating these same

cdk cascades and inducing oocyte maturation (Tikoo et al., 1997 ; Robker and

Richards, 1998).

In the ovary, estradiol, FSH and LH, are essential signals for the growth

of preovulatory follicles and their subsequent terminal differentiation into

corpora lutea. Each hormone acts via specific receptors and intracellular

signaling pathways (Robker and Richards, 1998). Additionally, FSH acts via

FSH receptors and induces high levels of intraoocyte cAMP, and also increases

the activity of cyclin D2 and E. In contrast, the low levels of intraoocyte cAMP

generated in response to LH profoundly enhance the activity of p27^{Kip1}

(Richards, 1994 ; Tikoo et al., 1997). Thus, high levels of intraoocyte cAMP,

cyclin D2 and E or low levels of p27^{Kip1} lead to termination of oocytes

maturation. In this study, hCG-primed mice enhance *in vitro* oocyte maturation and fertilization, and preimplantation embryo development to a larger extent than PMSG-primed mice. This might due to the effect of hCG to decrease the activity of cyclin D2 and E, and increase p27^{Kip1} activity by activating LH receptors (Figure 4.1).

In summary, has demonstrated in these studies that

1. the suitable concentration of gonadotropins for the stimulation of immature female mice to obtain immature oocytes for IVM/IVF studies in the mouse model is 7.5 IU.

2. the GV stage of cumulus cell-enclosed oocytes more readily proceeds to the MII stage than the GV stage of denuded oocytes.

3. hCG, more than PMSG, plays a major role in nuclear maturation (increasing oocyte maturation), cytoplasmic maturation (increasing the fertilization rate and decreasing the ZP digestion time), and preimplantation embryo development (increasing blastocyst development) on the optimum maturation duration.

4. the optimum maturation duration of the GV stage oocytes for IVM/IVF studies in the mouse model is 15 hours.

5. ZP hardening of *in vitro* matured oocytes is not associated with ZP thickness in the mouse model.

6. hCG-primed mice, more than PMSG-primed mice, induce a decrease in the level of intraoocyte cAMP.

7. a decrease in the level of intraoocyte cAMP has a direct effect on nuclear and cytoplasmic maturation, and preimplantation embryo development of mouse oocytes.

PMSG   hCG

⇓   ⇓

--------------®®®®®®----------------------------------⊗⊗⊗⊗⊗⊗------------

(FSH receptors)   (LH receptors)

⇓   ⇓

↑ ACase activity   ↓ PLC activity   ↓ ACase activity   ↑ PLC activity

⇓   ⇓

↑ cAMP level   ↓ IP3 and DG   ↓ cAMP level   ↑ IP3 and DG

⇓   ⇓

↑ PKA I   ↓ PKC and   ↑ PKA II   ↑ PKC and

activity   CaMK activity   activity   CaMK activity

⇓   ⇓

↓ α2-macroglobulin, KGF, and p27^{Kip1} ↑ α2-macroglobulin, KGF, and p27^{Kip1}

activity, and   activity, and

↑ cyclin D2 and E activity   ↓ cyclin D2 and E activity

⇓   ⇓

negative effects on   **positive effects on**

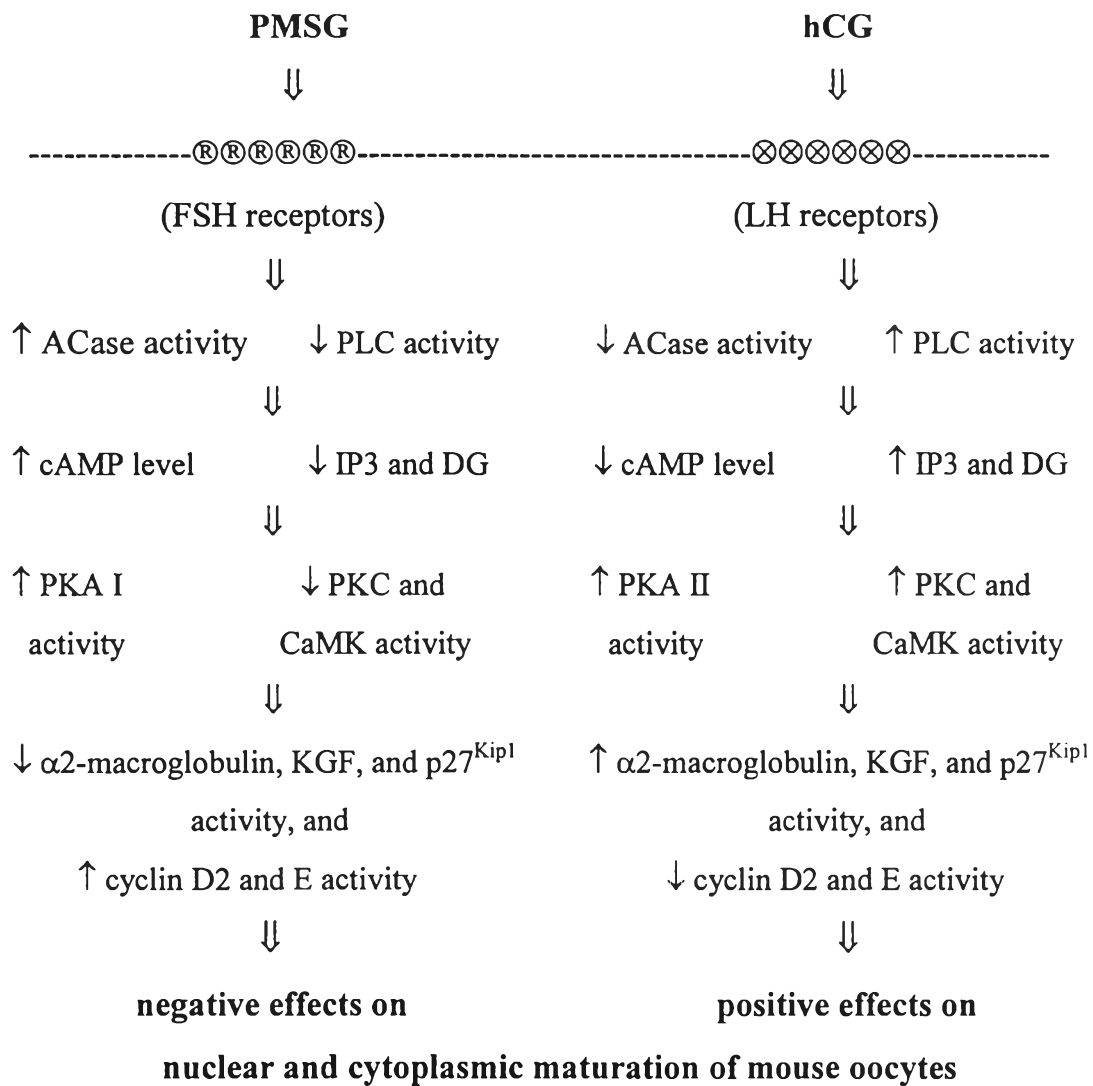**nuclear and cytoplasmic maturation of mouse oocytes**

Figure 4.1 Possible signal transduction pathways during maturation, fertilization, and preimplantation embryo development of mouse oocytes after PMSG and hCG stimulation.