[Frontiers in Bioscience 12, 282-296, January 1, 2007]

Gonadotropin-controlled mammal oocyte meiotic resumption

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TABLE OF CONTENTS

1. ABSTRACT

Fully grown mammalian oocytes resume meiosis as a consequence of rises in gonadotropin levels at the mid-cycle. The increase of cyclic adenosine 3',5'-monophosphate (cAMP) and the activation of protein kinase A (PKA), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) in cumulus cells are required for gonadotropins-induced meiotic resumption of oocytes. The various actions of cAMP activated by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) also include meiosis activating sterol (MAS), gonadal steroid hormones and epidermal growth factor (EGF) network during meiotic resumption. Another second messenger guanosine 3',5'-cyclic monophosphate (cGMP) induced by nitric oxide (NO) or atrial natriuretic peptide (ANP) also mediates gonadotropins-controlled mammalian oocyte meiotic resumption. The different actions of FSH and LH on meiotic resumption are discussed. We hope to provide a framework to understand how the initial signals generated by gonadotropins-stimulation control the expression of genes required for meiotic resumption.

2. INTRODUCTION

Mammalian oocytes are arrested at the diplotene stage, also called the germinal vesicle (GV) stage of the first meiotic division until shortly before ovulation. Following stimulation by FSH and LH, grown oocytes resume meiosis, followed by completion of meiosis I and another arrest in metaphase of meiosis II (except dog and horse). These meiosis II oocytes are ready for ovulation and subsequent fertilization. Morphologically, resumption of meiosis is characterized by the disappearance of nuclear membrane of oocytes, which is also called germinal vesicle breakdown (GVBD).

Molecular mechanisms by which gonadotropins induce oocyte meiotic resumption in the preovulatory follicle may involve the elimination of meiosis inhibiting factors and/or the accumulation or activation of oocyte maturation signals. Two major gonadotropins, FSH and LH, are used to study the signal pathways of oocyte maturation *in vivo* and *in vitro*. The actions of FSH and LH on mammalian oocyte meiotic resumption are believed to

be mediated in large part through increasing the production of cyclic adenosine 3',5'-monophosphate (cAMP), and subsequent activation of specific signaling pathways including protein kinase A (PKA), protein kinase C (PKC), calcium/calmodulin-dependent protein kinase II (CaMKII), and mitogen-activated protein kinase (MAPK) (1). However, it is becoming increasingly difficult to fully explain the divergent outcomes of cAMP activation in granulosa cells observed *in vitro* as well as the distinct patterns of gene expression induced by FSH and LH during meiotic resumption. Recent findings indicate that gonadotropins-inducing meiosis activating sterol (MAS), gonadal steroid hormones and epidermal growth factor (EGF)-like growth factors are also involved in the mediation of meiotic resumption. The presence of intact cumulus-oocyte connections are mandatory for gonadotropins-inducing meiotic resumption, since oocytes lack gonadotropins receptors (2, 3) and thus do not respond to follicle-stimulating hormone (FSH) and luteinizing hormone (LH) exposure (4-7). Recent studies suggest that the second messenger guanosine 3',5'-cyclic monophosphate (cGMP) induced by nitric oxide (NO) or atrial natriuretic peptide (ANP) also mediates gonadotropins-induced mammalian oocyte meiotic resumption (8-11).

The endocrine control of meiotic resumption by gonadotropins rests on a network of extracellular and intracellular molecular interactions. The purpose of this review is to summarize data from several laboratories, including ours, on the regulation of meiotic resumption in mammals. We attempt to provide a framework with which to understand how the initial signals generated by gonadotropins-stimulation control the expression of genes required for meiotic resumption.

3. GONADOTROPINS INDUCE MEIOTIC RESUMPTION VIA CUMULUS CELLS

It is well-known that fully grown mammalian oocytes arrested at the diplotene stage of first meiotic prophase resume meiosis as a consequence of preovulatory gonadotropin stimulation (12). Particularly, the endogenous LH peak initiates the specific alterations in follicular steroidogenesis in Graafian follicles, causes the changes in the cumulus cell-oocyte complex and subsequently oocyte meiotic resumption. The central role of gonadotropins in the regulation of meiotic resumption through the activation of G protein-coupled receptors mediated by the adenyl cyclase-cyclic AMP is now well established, but signal transduction following gonadotropins receptor activation is still poorly understood. The gonadotropins likely act through granulosa cells since oocytes lack gonadotropins receptors (2, 3) and do not respond to FSH and LH exposure (5-7). All cells within the follicular compartment are interconnected through a network of gap junctions allowing communication between somatic cells and oocytes. Through gap junctions, follicular somatic cells play an important role in gonadotropin-induced mammalian oocyte meiotic resumption (6, 13, 14). It is generally accepted that cAMP and MAPK in oocytes play an important role in this process.

3.1. Gonadotropins regulate the level of cAMP in the oocyte via cumulus cells

In preovulatory follicle oocytes with the competence of meiotic resumption, inhibitory signals derived from granulosa cells may be transported into oocytes through gap junctions and thus suppress the resumption of meiosis (15). On the other hand, it is needed for cumulus cells to be connected with oocytes for cytoplasmic maturation to occur (16). cAMP, produced endogenously in oocytes (17) or transported into oocytes from adjacent cumulus cells (18, 19), may be the meiotic inhibitor. The concentration of cAMP in oocytes plays a critical role in the regulation of oocyte meiotic resumption. Countless studies, *in vivo* and *in vitro,* have demonstrated that gonadotropins increase cAMP in granulosa cells and decrease cAMP in oocytes, which subsequently induce the resumption of meiosis (20, 21). Activation of type I PKA by elevated cAMP level in oocytes prevents spontaneous oocyte meiotic resumption, whereas FSH-stimulated activation of type II PKA within cumulus cells leads to meiotic resumption in cumulus-enclosed oocytes (CEOs) (22). Concomitant intra-oocyte cAMP degradation may be a prerequisite for meiotic resumption (23). Concurrently with meiotic resumption induced by FSH, a dramatic conformational change caused by the production and secretion of hyaluronic acid disperses the cumulus cells (24). The production of hyaluronic acid by cumulus cells is essential for the expansion of the cumulus oophorus before ovulation (25). It is reported that cumulus cells acquire the ability to expand before or possibly during the acquisition of meiotic competence (24). Cumulus expansion, required for normal ovulation (26), may disrupt the transfer of meiosis-arresting factors such as cAMP to oocytes. cAMP decrease in oocyte may be also resulted from closure of communication somatic cells and oocytes, since meiotic resumption induced by the preovulatory gonadotropin surge (possibly LH) is accompanied by disassembly of gap junctions (27, 28) and phosphorylation of gap junction protein connexin 43 (29).

The concentration of cAMP is determined not only by the activity of cyclases, but also the activity of phosphodiesterases (PDEs). Recent data have demonstrated that the regulation of PDE activity may be critical in shaping the cAMP signal in granulosa cells and in contribution to the specificity of the gonadortropin responses (30). It has been indicated that PDE subtypes are specifically localized to different compartments of bovine follicles: i.e. PDE3 in oocytes and PDE4 in granulosa cells (31, 32). Activity of PDE3 increases prior to resumption of meiosis in both spontaneous and gonadotropins-induced oocyte maturation, and a PDE3 inhibitor cilostamide inhibits meiotic resumption by increasing the levels of cAMP in oocytes (33). A PDE4 inhibitor rolipram increases the levels of cAMP in cumulus cells, which is a main contributor to cAMP content in oocytes (21). Studies on endocrine and nonendocrine cells have shown that cAMP-phosphodiesterases 4 (cAMP-PDE4s), expressed in granulosa and theca cells but not in oocytes (21), is part of a feedback control of cAMP levels after stimulated by gonadotropins (34). *In vivo*, PDE4D mRNA is induced by FSH in preantral follicles and by LH in periovulatory

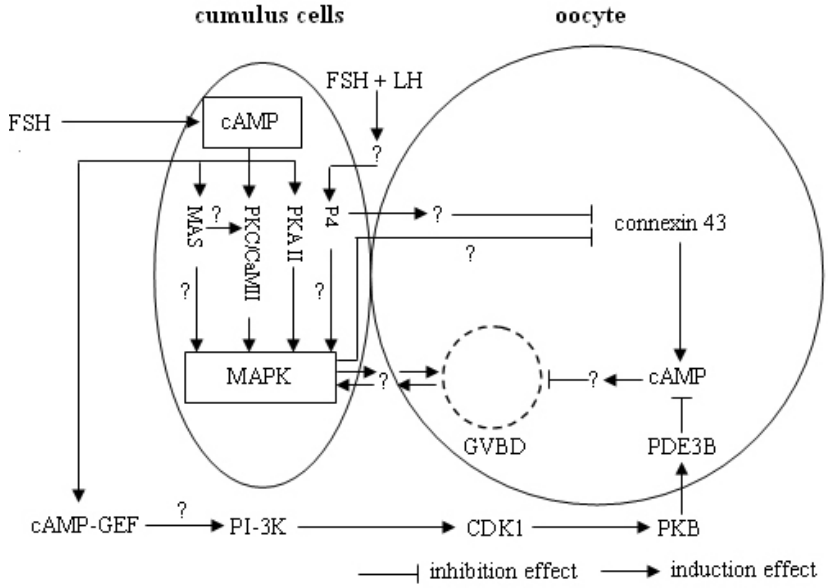

Figure 1. A proposed model for FSH-induced mammalian CEOs meiotic resumption. FSH binds to the G protein-coupled receptors in granulosa cells, resulting in activation of adenylyl cyclase and increased production of cAMP. The elevated cAMP in cumulus cells can activate MAPK possibly by PKA II, PKC/CaMII, MAS, and/or P4 (produced by FSH and LH), which in turn decreases cAMP level in oocyte and induces the release of a signal(s) that trigger meiotic resumption. The activation of cAMP-GEF may also involve in decreasing cAMP level in oocytes by PI-3K/CDK1/PKB/PDE3B pathway. FSH, follicle-stimulating hormone; LH, luteinizing hormone; CEOs, cumulus-enclosed oocytes; cAMP, cyclic adenosine 3',5'-monophosphate; MAPK, mitogen-activated protein kinase; PKA II, type II protein kinase A; PKC, protein kinase C; CaMKII, calcium/calmodulin-dependent protein kinase II; MAS, meiosis activating sterol; P4, progesterone; cAMP-GEF, cAMP-dependent guanine nucleotide exchange factor; PI-3K, phospatidil inositol 3 kinase; PDK1, phosphoinositide-dependent protein kinase-1; PKB, protein kinase B; PDE3B, phosphodiesterase 3B.

follicles (30). Stimulation of cultured rat granulosa cells with FSH causes a significant increase in PDE4s activity, which may in turn decrease the cAMP levels (35). Further studies show that the LH-regulated expression of PDE4D may contribute to desensitization in granulosa cells (30). Indeed, a rapid and transient cAMP response is followed by a decrease of cAMP after gonadotropins stimulation (35). The rapid increase of cAMP in cumulus cells may activate PDE3B and decrease cAMP level in oocytes, possibly by cAMP-dependent guanine nucleotide exchange factor (GEF)/phospatidil inositol 3 kinase (PI-3K)/ phosphoinositide-dependent protein kinase-1 (PDK1)/protein kinase B (PKB) pathway (34, 36).

3.2. Gonadotropins induce meiotic resumption via activation of MAPK pathway in cumulus cells

Xenopus oocytes remain in meiotic arrest after removal from the ovary, and can be induced *in vitro* to meiotic resumption by addition of various steroids. Steroids promote maturation through release of inhibition mechanisms whereby these constitutive repressive signals are antagonized, thus allowing meiosis to progress (37). In contrast to *Xenopus*, mammalian oocytes spontaneously mature when removed from ovary (38), suggesting that the primary signal maintaining meiotic arrest of mammalian oocytes comes from follicles rather than oocytes themselves. Thus, the gonadotropins-induced mammalian oocyte meiotic resumption probably involves not only termination of the flow of meiosis-arresting factors, but also the production of meiosis-promoting signals by granulosa cells (39, 40).

It is generally believed that the effects of FSH and LH on ovary are mediated in large part through increasing cAMP production, and subsequent activation of specific signaling pathways (1). More and more studies show that the activation of two isoforms of MAPKs, extracellular-regulated kinase 1 (ERK1) and ERK2, is indispensable for gonadotropins-inducing, but not spontaneous, meiotic resumption of mammalian oocytes as well as cumulus expansion (38), and that specific MAPK kinase (MEK) inhibitors block oocyte meiotic resumption and cumulus expansion induced by gonadotropins (41-43). Su et al (43) studied the role of MAPK in FSH-induced oocyte meiotic resumption and cumulus expansion using Mos^{tm1Ev}/Mos^{tm1Ev} (*Mos*-null) mice, and indicated that MAPK activation in oocytes was not necessary for FSH-induced resumption of meiosis. In contrast, a further study shows that cAMP-dependent activation of MAPK in cumulus cells is essential for FSH-induced porcine oocyte meiotic resumption (32). Inhibition of MAPK activity in cumulus by an MEK inhibitor, U0126, prevents gonadotropins-inducing meiotic resumption and cumulus expansion (43, 44). It is also reported that cumulus p38 MAPK, another member of the MAPK family, might be also involved in FSH-induced meiotic resumption of oocytes (45). These results indicate both meiotic resumption and cumulus expansion require the activation of MAPK in cumulus (Figure 1). Inhibition of MAPK activation prevents LH-stimulated resumption of meiosis as well as expression of two genes whose products are necessary for normal cumulus expansion, *Has2* and *Ptgs2* (44).

Gonadotropins-dependent activation of MAPK is downstream of elevated granulosa cell cAMP level and the activation of PKA (46-48). The cAMP-PKA pathway regulates functions of cumulus cells by activation of MAPK, since FSH-dependent elevation of cumulus cell cAMP level is not affected by MEK1/2 inhibitor U0126 (44). It is suggested that gonadotropins control mammalian oocyte meiotic resumption by decreasing cAMP level in oocytes, and increasing cAMP level and activating MAPK pathway in cumulus cells. More recently, it is suggested that MAPK mediates LH-induced GVBD by interrupting cell to cell communication within follicles, possibly through phosphorylation of connexin 43 (29).

3.3. Crosstalk of MAPK with other protein kinases during gonadotropin-induced meiotic resumption

PKC and CaMKII: The signal transduction of FSH is considered mainly through cAMP-PKA. Recently, the results of several studies show that PKC and intracellular calcium may also play an important role in the signal transduction of FSH (49, 50). While PKC activators, phorbol 12-myristate 13-acetate (PMA) and 1-oleoy-2-acetyl-sn-glycerol (OAG), inhibit denuded oocyte (DO) GVBD or meiosis progression (51, 52), the activation of PKC is implicated in hormone-induced oocyte meiotic resumption in rat (49), bovine (53) and porcine (50) through cumulus cells. Moreover, PKC inhibitors, staurosporine, sphingosine, calphostin C or chelerythrine, could inhibit the effect of FSH on stimulating porcine and mouse CEOs to resume meiosis (50, 54). It is reported that both PKC inhibitors and U0126 inhibit the FSH-induced GVBD of oocytes and MAPK activation in cumulus cells (54), suggesting that PKC might induce the meiotic reinitiation of CEOs by activating MAPK in cumulus cells. Interestingly, it is reported that FSH induces mouse oocyte maturation through the inhibition of PKC activity (55). Up todate, the signal transduction process of PKC in cumulus cells that leads to oocyte meiotic resumption is still not well known because many PKC subtypes exist, morever the results from above mentioned studies used both inhibitors and activators are not specific to different subtype of PKC.

In *Xenopus laevis*, oocytes meiotic resumption is induced when the intracellular Ca^{2+} concentration is raised in the presence of ionophore, and injection of calcium chelator BAPTA or CaMKII inhibitor autocamtide-2 related inhibitory peptide inhibits meiotic resumption (56). Similarly, calcium-dependent pathways are essential for FSH-induced oocyte meiotic resumption of mouse oocytes (57). Chelation of intracellular calcium blocks FSH-induced meiotic resumption in mouse and pig CEOs (58), and the FSH-induced meiotic resumption of mouse CEO, rather than spontaneous meiotic resumption, is inhibited by CaMKII inhibitors (59), indicating that CaM kinase II is required for FSH-activated oocyte meiotic resumption (59). Calcium probably interacts with calmodulin to regulate oocyte maturation (60, 61). However, some conflicting results have been reported, possibly due to the use of different systems and culture conditions (62, 63). CaM or CaMKII inhibitor suppresses the accumulation of cyclin B as well as the phosphorylation of MAPK at the same time of blocking meiotic resumption (64), suggesting that calcium-dependent mechanisms may participate in the initial activation of MAPK cascade during FSH-induced meiotic resumption. CaMKII may also be involved in the regulation of MPF activity in term of p34cdc2 dephosphorylation, which is more important than MAPK in controlling the meiotic initiation of mammalian oocytes (65).

MPF: In spite of MAPK activity, matured oocytes also exhibit elevated levels of maturation promoting factor (MPF) activity. MPF has been identified as heterodimeric protein kinase composed of a catalytic subunit p34cdc2 kinase and a regulatory subunit cyclin B (66), and acts as an important cell-cycle regulatory protein with kinase activity and is downstream to cAMP activation (67). In *Xenopus* oocytes, progesterone-dependent entry into meiosis I is accompanied by the synthesis of Mos (68) and the activation of the MAPK pathway (69), which leads to MPF activation (70). Meiotic competence of mammalian oocytes may be associated with the ability to synthesize and to activate MPF molecules. Recently, a general model of MPF formation during oocyte maturation in vertebrates has been proposed (71). Activation of MPF is also involved in mouse oocyte meiotic resumption (67). Furthermore, the degree of MPF activation is very low in porcine oocytes in the absence of MAPK activation (72), suggesting that MAPK may promote GVBD by increasing MPF activity.

3.4. Oocyte-somatic cell crosstalk

Gonadotropins, via activation of MAPK pathway in cumulus cells, induce meiotic resumption of mammalian oocyte. On the other hand, the function of cumulus cells is also regulated by oocytes. It is shown that oocytectomized mouse cumulus cell complexes do not produce hyaluronic acid and undergo expansion after FSH stimulation until addition of fully-grown oocytes (73, 74). Further studies indicate that mouse oocytes secrete a cumulus expansion-enabling factor that promotes cumulus expansion in response to FSH (24). This paracrine factor may be oocyte-specific growth differentiation factor-9 (GDF-9), since recombinant GDF-9 can promote *Has2* (a key gene for hyaluronic acid synthesis) expression and cumulus expansion (75). Moreover, mouse cumulus expansion requires gonadotropins-dependent increased expression of several key genes in cumulus cells, including *Has2 and Ptgs2* (75, 76). A recent study shows that denuded mouse oocytes greatly enhanced MAPK activity in cocultured cumulus cells after FSH stimulation, while MAPK activity is low in the absence of oocytes (44), and that mouse oocytes control the intercellular metabolic cooperativity between cumulus cells and oocytes, which is needed for energy production by granulosa cells and for oocyte and follicular development (77). All these results indicate that oocytes also play a key role in enabling or licensing the maturation of oocytes and cumulus oophorus. As that in oocyte, a likely path of gonadotropins may begin with the elevation of cAMP levels in granulosa cells to promote the activation of MAPK, then be followed by the events leading to meiotic resumption and cumulus expansion. Moreover, the activation of MAPK in cumulus also requires one or more paracrine factors secreted by the oocyte (44), suggesting that MAPK activation alone is not

sufficient to initiate these maturation processes. All these studies demonstrate a remarkable interaction between oocytes and cumulus cells, which is essential for gonadotropins-induced oocyte maturation processes.

In contrast, it seems that cumulus expansion in pig CEOs do not require a cumulus expansion-enabling factor, since pig oocytectomy does not change the ability of pig cumulus granulosa cells to respond to FSH and forskolin by increasing cAMP contents and hyaluronic acid synthesis (78). Furthermore, forskolin can induce cumulus expansion but inhibit oocyte maturation (79). However, compared to an almost full expansion induced by FSH in CEOs, the expansion stimulated by forskolin is limited to outer layers of cumulus cells in our studies and studies by others (11, 78, 79). Moreover, although FSH has no obvious effect on cumulus expansion but induces porcine oocyte maturation in porcine follicular fluid (pFF)-free culture system, the appearance of cumulus cells when stripped from oocytes indicates that the connection among the cumulus has been loosen in FSH-induced oocyte maturation (80). All these studies suggest that the normal interaction between oocytes and cumulus cells in pig may be also needed for the full cumulus expansion or incompact.

4. GONADOTROPINS INDUCE MEIOTIC RESUMPTION VIA OTHER HORMONES OR FACTORS

The primary signal emanating from gonadotropins stimulation is the accumulation of cAMP in theca and granulosa cells. However, additional signaling pathways may be activated during the action of FSH and LH. Recent studies show that gonadotropins-induced meiosis activating sterol (MAS), gonadal steroid hormones and epidermal growth factor (EGF)-like growth factors are also involved in meiotic resumption.

4.1. MAS

The gonadotropins-inducing signal has not yet been identified. A positive regulator produced by somatic cells may be transported to oocytes and induces meiotic resumption (6, 7, 40, 81). Byskov et al (82) reported that MAS in human follicular fluid (FF-MAS) and bull testicular tissue (T-MAS) might be a candidate signal molecule taking part in FSH induced meiotic resumption. Though the results are not always consistent, studies using follicle-derived or synthetic FF-MAS show that FF-MAS, in a dose-dependent manner, can induce meiotic resumption of roden oocytes *in vitro* in the presence of meiosis-inhibitors affecting different pathways (e.g. hypoxanthine (HX), isobutyl methylxanthine (IBMX) and dbcAMP) (83-87). FF-MAS also positively influences the survival rate of human oocytes and induces nuclear maturation *in vitro* (88, 89). It is hypothesized that MAS is responsible for many important aspects of meiosis signalling during oocyte maturation both nuclear and cytoplasmic maturation, chromatin condensation and spindle assembly (90).

Whether MAS is an obligatory downstream signal in FSH-induced oocyte meiotic resumption is currently controversy. It has been shown that the resumption of meiosis induced by MAS in rat oocytes was much slower, kinetically, than observed after LH stimulation (91). However, in our study, mice CEOs resumed maturation in a way similar to that of FSH in the media containing amphotericin B for 1 h, which accumulates MAS by inhibiting delta-7-reductase (92). It has been also reported that CYP51 inhibitor, azalanstat (50, 100 and 200 μM), does not prevent the LH-induced rat follicle enclosed oocytes (FEO) maturation at dose not leading to degenerate, and does not prevent spontaneous maturation of CEO (93, 94). However, we have successfully inhibited LH induced rat CEOs maturation by azalanstat at 60 μM, which did not significantly increase oocyte degeneration, and at 100 μM, which significantly induced oocyte degeneration (unpublished). In mice, we have demonstrated that both FSH and AY9944-A-7, which is used to accumulate FF-MAS, can significantly promote FEO maturation. Besides, FSH inducing FEO maturation can be inhibited by FF-MAS producing inhibitor, RS-21745 (95). In our recent study, FSH induced mouse FEO meiosis resumption can be significantly inhibited by intra-follicle injection of small interference RNA of *CYP51* gene (unpublished). However, RNA interference did not markedly decrease the rate of spontaneous FEO maturation *in vitro*, which is in accordance with the finding that spontaneous and FSH or MAS induced oocyte maturation are by different mechanisms (42, 58, 64, 92, 96, 97). All these results seem to support that the resumption of oocyte meiosis is the result of gonadotropins-inducing FF-MAS production by cumulus cells, by overcoming follicular inhibition of meiosis when transferred to oocytes.

Though FF-MAS levels increase in mouse CEOs after FSH stimulation (98) and in rabbit ovary stimulated by LH (90), and the secretion of FF-MAS by granulosa cells is a cAMP-dependent process, the precise mechanism by which gonadotropins inducing MAS accumulation has not yet been determined. An attractive hypothesis is that ovarian steroids cause a negative feed-back inhibition of steroidogenic enzymes (such as Δ14-reductase) and subsequently lead to MAS accumulation, reflecting events taking place during the mid-cycle surge of gonadotropins, when progesterone and 17α-hydroxyprogesterone accumulates (99-101).

MAS may have a membrane-bound receptor, since MAS-binding sites could be detected at the surface of mouse, cattle and marmoset denuded oocyte membrane (97) and the microinjection of FF-MAS does not lead to any induction of meiosis resumption (90). It is possible that MAS-mediated oocyte maturation is dependent on a G-protein coupled receptor mechanism. Addition of cholera toxin (CT), which results in activation of adenylate cyclase through G-proteins and increases cAMP, significantly inhibits MAS-mediated mouse oocyte maturation (84). Microinjection antibody of GTP binding protein G_s into oocytes of mouse FEO results in meiotic resumption, suggesting that G_s activation is vital for maintaining oocyte cycle arrest (102). Our resent results showed that MAS might induce porcine oocytes meiotic resumption via PKC signal pathway (unpublished). MAS receptor is

Table 1. A summary of gonadotropin-induced meiotic resumption of mammalian and amphibian (*Xenopus Laevis* as the representative) oocytes

Factors	**Mammals**	***Xenopus***
Meiotic inhibitor	cAMP, cGMP?	cAMP
Meiotic resumption	FSH and LH-induced meiotic resumption (functions in cumulus cells and theca cells, respectively)	Facilitate progesterone-induced meiotic resumption (functions in oocytes)
Gene expression	Yes	No
Signal pathway	Increasing MAPK activity in cumulus cells stimulated by PKA II, PKC, MAS, EGF worknet, and/or gonadal steroid hormones, and decreasing cAMP level in oocytes possibly through phosphorylation of connexin 43 and/or the activation of PDE3B	MAPK/MPF activated by decreasing cAMP level in oocytes

hypothesized to involve three pathways: the *mos*/MEK/MAPK pathways leading to MPF formation and stabilization, direct translation to cyclin B and decreasing cAMP and A-kinase activation (71, 103).

In summary, the exact functions of MAS, as well as its precise mode of action, need further investigation. Nevertheless, the confirmed beneficial action of MAS on follicle/oocyte quality and the degeneration of oocytes under inhibition of sterol synthesis are probably indicative of an important role(s) of sterols in oocyte growth and subsequent embryonic developmental potential (100, 104, 105).

4.2. Gonadal steroid hormones

The role of follicle cell steroids in gonadotropin-stimulating oocyte maturation in fish and amphibian oocytes has been well established (106, 107) (Table 1). Although gonadotropins-inducing increases in cAMP concentrations are associated with increased production of steroid hormones in mammalian follicle (108), and the regulation of oocyte maturation is quite similar to that in *Xenopus laevis* (17, 36, 102), little is known about the role of ovarian steroids on mammalian meiotic resumption. Earlier studies show that medium containing progesterone, testosterone, or androstenedione increases the incidence of mouse antral follicles remaining meiotically inactive, when compared to follicles cultured in steroid-free medium (109), and that Arimidex, a potent follicular aromatase inhibitor, reduces E_2 concentration in follicle culture medium and induces meiotic resumption in response to hCG (110). The effects of steroids on mouse oocyte meiotic resumption are inconsistent (14, 111, 112). The differences may be due in part to the background of spontaneous oocyte maturation upon removal from the ovary, as well as methods of oocyte removal that pre-expose oocytes to sex steroids.

Recently, it was shown that FSH and LH induced preogesterone production and progesterone receptor (PR) expression in porcine cumulus cells (113). Aminoglutethimide (AGT), an inhibitor of progesterone production, significantly suppresses gonadotropins-induced progesterone production and porcine oocyte meiotic resumption. Further study suggests that gonadotropins-induced expression and function of delta 14-reductase and delta 7-reductase (the members of the superfamily that converts acetyl-CoA to cholesterol) in cumulus cells contribute to porcine oocyte meiotic resumption via a progesterone-dependent pathway (114). A possible mechanism is decreasing connexin 43 in cumulus cells and cAMP level in oocytes (113). It is difficult to demonstrate that the maturation of mammalian oocytes is a steroid-mediating process. The drastic changes in the intrafollicular steroid concentrations induced by gonadotropins could adversely affect fertilization of *in vitro* grown oocytes (110). Thus, the role of steroids in mediating mammalian oocyte maturation remains to be elucidated.

4.3. EGF network

Although cAMP activated by gonadotropins clearly signals through PKA activation, additional signaling pathways are also activated during FSH and LH action. Recently, it is indicated that EGF network induced by LH is involved in the meiotic resumption. EGF receptors and EGF-receptor mRNA were detected on granulosa cells of many species (115). EGF induction of oocyte meiotic resumption and cumulus expansion has been observed in many species (116, 117), and the events *in vivo* are associated with the endogenous preovulatory surge of LH (6, 116, 118). EGF can only stimulate expansion of porcine CEOs from large follicles, indicating that EGF may operate as a mediator of signals elicited by the LH surge (117).

Recent studies have suggested that follicular EGF-like factors play a physiological role in the mediation of the ovulatory response to LH. LH/hCG stimulates the production of EGF-family members amphiregulin (AR), epiregulin (ER) and betacellulin (BTC) in pre-ovulatory follicles in many species and a transient expression of their receptor mRNAs in rat. Addition of EGF-like factors results in complete (with AR and ER) and partial (BTC) stimulation of the resumption of meiosis in mouse oocyte (119) and rat FEO (120). More importantly, meiotic resumption induced by LH is slower than that observed after ER and AR treatment (119), suggesting that these growth factors act downstream of LH. The three growth factors also induce cumulus expansion and the expression of *ptgs2*, *Has2*, and *Tnfaip6* genes that are critical for matrix remodeling in the CEOs (119, 121). EGFR kinase inhibitor AG1478 blocks the LH-induced meiotic resumption and cumulus expansion (120), suggesting that EGF-signal transduction is involved in the process. These findings can explain the observation that EGF

mimics some of the LH effects *in vitro*. The intracellular mechanisms by which growth factors affect oocyte meiotic resumption may involve tyrosine kinase (TK) and MAPK activation (115, 122, 123).

5. REGULATION OF cGMP IN GONADOTROPIN-INDUCED MEIOTIC RESUMPTION

The ac messenger cAMP, and subsequent activation of specific signalling pathways involving phosphorylation of MAPK (38, 124). Recent studies indicate that cGMP also influences a wide range of ovarian functions (108). The synthesis of cGMP is accomplished by two distinct classes of guanylyl cyclases, particulate and soluble, both of which appear to have relevance to oocyte maturation. Nitric oxide (NO) is a regulator of cGMP production via its action on soluble guanylyl cyclase, while natriuretic peptides activate receptors with intrinsic guanylyl cyclase activities (125). Activation of either form of guanylyl cyclase results in large increases in cGMP production, and subsequent activation of cGMP-dependent signaling pathways, including cGMP-dependent protein kinase activation, activation or inactivation of PDE, interaction with cyclic nucleotide gate (CNG) channels and phosphorylation of the cAMP response element binding protein (CREB) (108). It has been observed that ovarian cAMP increases during proestrous while cGMP decreases (126). These inverse changes in cyclic nucleotide concentrations were abolished when the preovulatory LH surges were blocked by phenobarbitone injection, but could be restored by administration of exogenous LH (126). cGMP induced by NO or ANP antagonizes the effects of FSH on oestradiol production and cAMP accumulation (108). All these studies suggest physiological roles of this second messenger in gonadortopin-induced oocyte maturation.

5.1. NO

NO is a chemical messenger enzymatically produced by enzymes known as nitric oxide synthases (NOS), which convert $_L$-arginine into NO and citrulline (127). Three isoforms of NOS have been identified: two constitutive isoforms, endothelial (eNOS) and neuronal NOS (nNOS), and an inducible isoform (iNOS). Constitutive isoforms are calcium- and calmodulin-dependent and, only small amount of NO are produced by these isoforms (108). iNOS, maintaining sustained synthesis of NO, is secreted by many types of cells (128). eNOS is expressed in ovarian thecal and stromal cells, in mural granulosa cells of maturing follicles, and in corpora lutea (129, 130). The expression of iNOS is primarily in immature follicles and apparently decreases with follicular growth (131, 132). In contrast, the expression of NOS isoforms in the ovary is consistent with physiological roles of NO in ovarian functions, such as sexual behavior, follicular development, steroidogenesis, oocyte meiotic resumption, ovulation and atresia, corpus luteum function, fertilization, implantation, embryo development and pregnancy (127).

It has been reported that NO donor, sodium nitroprusside (SNP), significantly stimulates meiotic maturation in CEOs, whereas NOS inhibitor $_L$-NAME suppresses resumption of meiosis and this inhibition is reversed by the addition of SNP (129, 133). In contrast, some reports show that NO inhibits rat meiotic maturation (9). Our studies showed that NO exerted dual function on mouse oocyte maturation (134, 135). High concentration of SNP (10^{-4}-10^{-3} M) could inhibit the spontaneous and FSH-induced meiotic resumption and cumulus expansion and, low concentration of SNP (10^{-5}-10^{-7} M) exhibited stimulatory effect on CEOs in the presence of HX, but no effect on DOs. A general trend highlighted is that NO plays biphasic role in reproduction. That is, NO, in a narrow range of and usually low concentrations, can stimulate oocyte maturation, but absence or too much NO has negative consequences (136). Further studies show that the stimulatory effect of NO on mouse oocyte meiotic resumption is via the cAMP pathway, while the inhibitory mechanism is through the cGMP pathway and, PKC possibly involves this process (10).

NO plays an important role in mammalian oocyte meiotic resumption (129, 137, 138). It has been reported that eNOS produces small quantities of NO (at nM) while iNOS produces NO at μM over a long period (128). The amount of NO produced under the condition of high concentration of SNP is equivalent to that produced by iNOS under physiological circumstance. Yamagata et al (133) indicated that iNOS expression significantly decreased after hCG injection, which induced a decrease of NO concentrations in preovulatory follicular fluid. Nakamura et al (9) also showed that hCG induces meiotic resumption in rat oocytes of preovulatory follicles, and the NO donor prevents this phenomenon. Moreover, aminoguanidine bicarbonate salt (AG), a pecific inhibitor for iNOS, induces meiotic resumption, which can be prevented by NO donor. All these results suggest that iNOS-derived NO may be an oocyte maturation inhibitor. A high-concentration of intrafollicular NO probably plays a role in meiotic arrest of oocytes, and the decrease of NO after hCG injection may be an important event for meiotic resumption. However, we find that AG strongly inhibits FSH-induced porcine oocyte meiotic resumption (139). The different roles of AG might be due to different experimental conditions, animals and stage of oocyte development.

Intracellular NO signaling pathway is quite complex and flexible. NO exerts functions by activating cyclooxygenase enzyme (COX) or PKC (127). It can also act throuth P53/Bax pathway. It has been indicated that the decrease of cGMP and cAMP in oocytes parallels spontaneous meiosis, and that microinjection of these substances into oocytes causes a delay in oocyte meiotic resumption (140, 141). Produced by iNOS-derived NO in granulosa cells and transported via gap junctions into oocytes, cGMP has an important role in maintaining the meiotic arrest of oocytes (141). cGMP maintains the meiotic arrest of preovulatory oocytes

mainly via two pathways: inhibition of oocyte cAMP phosphodiesterase to maintain cAMP level and activation of cGMP-dependent protein kinase in oocytes (141). It has been reported that PDE3 can be inhibited by NO and cGMP (142). Perhaps the most common pathway of cGMP action is mediated through activation of PKG, of which there are two types, PKGI and PKGII (143). PKGII expression is very low or non-existent in human ovarian tissue, whereas PKGI is detectable at significant concentrations in ovary (144). MAPK plays an important role in gonadotropins-induced mammalian oocyte meiotic resumption (29). Interestingly, NO can inhibit MAPK activity via generation of cGMP (145). All these results indicate NO, possibly produced by iNOS, maintains the intrafollicular cGMP level to inhibit meiotic resumption.

5.2 ANP

ANP is a small peptide and a member of a family of hormones mainly involved in the regulation of blood pressure, salt and water excretion, cell proliferation and body fluid homeostasis (146, 147). The diverse physiological responses of cells to ANP are manifested through binding of different cell-surface receptors. Four types of natriuretic peptide receptors have been identified using molecular cloning techniques: natriuretic peptide receptors-A (NPRA), natriuretic peptide receptors-B (NPRB), natriuretic peptide receptors-C (NPRC) and natriuretic peptide receptors-D (NPRD) (148). ANP exhibits high affinity to NPRA and activates particulate guanylate cyclase to produce physiological response in many tissues and cells (149). ANP also binds with high affinity to NPRC that has been thought to be responsible for clearance of plasma natriuretic peptides (150) and to play a role in the ANP-dependent inhibitory action on adenylate cyclase without intrinsic ability to generate cGMP (151).

Recently, it was found that ANP localizes in mammalian granulosa cells (152, 153) and oocyte (154). These results suggest that ANP may act as an autocrine and/or paracrine hormone that influences ovarian functions, including ovarian growth or steroidogenesis (155) and the regulation of oestradiol production after equine gonadotropin treatment of ovaries (156). ANP can also affect oocyte maturation by cGMP, i.e. ANP participates in ovum development by stimulation of cGMP accumulation and activation of cAMP-phosphodiesterase, thereby promotes *Xenopus* ovum maturation (157) and resumption of meiosis in hamster oocytes (158). ANP dose-dependently inhibits spontaneous maturation of rat oocytes via cGMP accumulation (159). These varied results suggest that there may be different signal pathways participating ANP-mediated action on mammalian oocyte meiotic maturation. The different responses to ANP may result from animal species, different pathways or experimental conditions.

In our studies, ANP inhibited FSH-induced, but not spontaneous, porcine oocyte meiotic resumption and cumulus expansion in a dose-dependent manner (80). ANP also obviously inhibited FSH-induced MAPK phosphorylation in both oocytes and cumulus cells (11). These effects is mimicked by 8-Br-cGMP and reversed by a PKG inhibitor KT5823, suggesting that cGMP/PKG signaling pathway is involved in FSH-induced porcine oocyte maturation. The inhibitory effect of ANP on FSH-induced porcine oocyte meiotic resumption and cumulus expansion might be via PKG/MAPK pathway, since FSH-induced MAPK phosphorylation in both oocytes and cumulus cells is necessary for pig oocytes maturation and cumulus expansion (38), and inhibition of MAPK activation prevents FSH-stimulated resumption of meiosis as well as cumulus expansion (44). ANP shows high affinity for NPRA receptors (160), and the concentrations of ANP used in our studies are in the same range as those used to regulate MAPK phosphorylation by ANP/NPRA system (149), suggesting that the inhibitory effect of ANP on FSH-induced pig oocytes maturation is mediated by its specific receptor NPRA.

Little is known about the physiological role of the ovarian ANP. Recently, granulosa cells from porcine large follicle shew strong expression of ANP (153). In addition, pregnant mare's serum gonadotropin (PMSG) increased the production of ANP and cGMP in rat ovary (161, 162), and LH results in decrease of cGMP by suppressing guanylate cyclase activity in rabbit ovary (161). Taken together, it can be hypothesized that accumulation of cGMP under FSH stimulation (during follicular growth) may serve to prevent untimely oocyte maturation until ovulation after the LH serge (Figure 2). The detailed roles of ANP in ovarian function need to be explored.

6. PERSPECTIVE

Gonadotropins induce mammal oocyte meiotic resumption mainly through cAMP/MAPK pathway. Recent studies suggest that MAS, gonadal steroid hormones and EGF network induced by FSH and/or LH are also involved in meiotic resumption. On the other hand, growing evidence indicates that cGMP-dependent signaling pathways exert a wide range of influences on gonadotropins-induced meiotic resumption (Figure 2). Although both FSH and LH use cAMP primary signaling pathway, the different downstream pathways may involve the regulation of meiotic resumption (36). FSH stimulates oocyte growth and LH receptor (LHR) expression in theca cells and cumulus cells (163), while a midcycle LH surge triggers meiotic resumption. Furthermore, the expression of LHR is essential for follicular maturation in the process from antral to preovulatory stage (164). It can be hypothesized that accumulation of cGMP under FSH stimulation (during follicular growth) may serve to prevent untimely oocyte maturation until ovulation after the LH serge**.** Further studies in this area may yield important new insights into the mechanisms regulating multiple aspects of oocyte maturation.tions of gonadotropins on mammalian oocyte meiotic resumption are believed to be mediated in

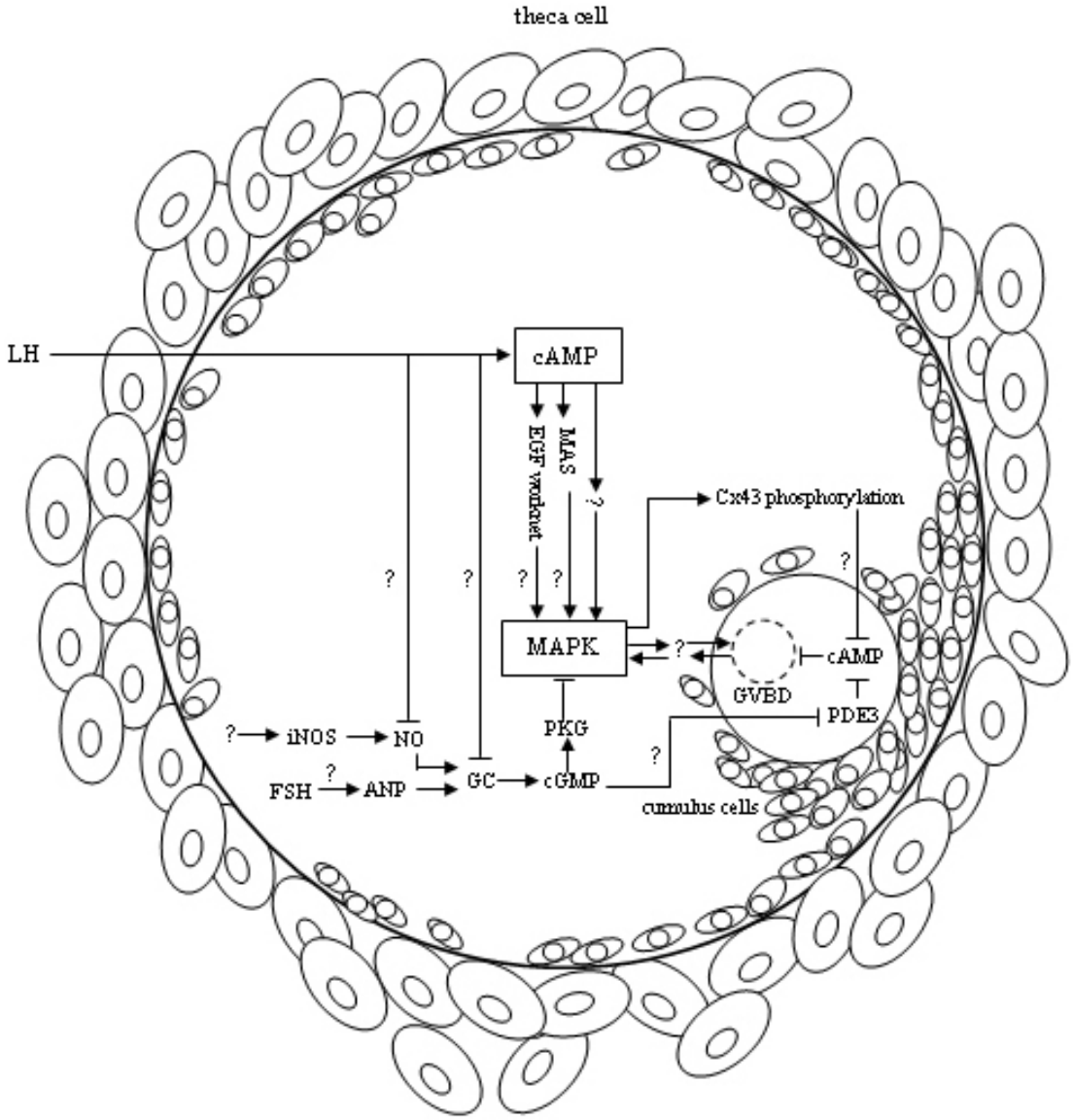

Figure 2. A proposed model for LH-induced mammalian oocyte meiotic resumption in preovulatory follicle. LH binds to the receptor in theca cells (or in cumulus cells), resulting in activation of adenylyl cyclase and increased production of cAMP. The elevated cAMP, possibly via MAS, EGF worknet, or other signal pathways, activates MAPK. MAPK, possibly through phosphorylation of Cx43 and decrease of cAMP in oocyte, trigger meiotic resumption. LH may also remove the negative effects of cGMP (produced by NO or ANP) on MAPK and PDE3 activity during gonadotropins-induced meiotic resumption. LH, luteinizing hormone; cAMP, cyclic adenosine 3',5'-monophosphate; MAS, meiosis activating sterol; EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; cGMP, guanosine 3',5'-cyclic monophosphate; Cx43, connexin 43; PDE3, phosphodiesterase 3; iNOS, inducible isoform of nitric oxide synthases; NO, nitric oxide; ANP, atrial natriuretic peptide; GC, guanylyl cyclase.

large part through increasing the production of the second

7. ACKNOLWEDGEMENT

This paper was supported by grants for National Outstanding Youth foundation of China (No. 30025032), National Natural Science Foundation of China (No. 30571358, No. 30470868) and Beijing Natural Science Foundation (No. 5052017). Meijia Zhang, and Guoliang Xia contributed equally to this manuscript.

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Key Words: Gamete Biology, Meiotic Resumption, Kinases, Signal Transduction, Review

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