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Oocyte Maturation: A story of arrest and release

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1. ABSTRACT

The release of a mature healthy egg for fertilization is the center of the entire reproductive process. From the time of embryonic development till fertilization, the oocyte undergoes several stop-and-go periods. In most animals, oocytes are held in meiotic arrest in prophase I prior to ovulation. The ovulatory luteinizing hormone (LH) surge promotes the resumption of meiosis of the arrested oocytes and their progression through the second meiotic cycle, only to be arrested again at metaphase II until fertilization. This review addresses the underlying mechanisms involved in maintaining the oocyte in meiotic arrest as well as the signaling pathways responsible for releasing it from the arrested phase just prior to ovulation until the completion of meiosis at the time of fertilization.

2. INTRODUCTION

Mammalian oocytes undergo first meiotic progression during embryonic development, and at the time of birth, they become arrested in the diplotene stage of prophase I. This meiotic arrest of oocytes is maintained until shortly before ovulation. During each reproductive cycle, the pre-ovulatory LH surge triggers the resumption of meiosis and its progression to metaphase II (MII), a process commonly termed *oocyte maturation*. Following resumption of meiosis I, there is organized disassembly of the nuclear envelope (germinal vesicle, GV) referred to as germinal vesicle breakdown (GVBD), followed by chromosome condensation, spindle formation and extrusion of the first polar body. Thereafter, the oocyte enters meiosis II and again gets arrested at metaphase II stage until fertilization.

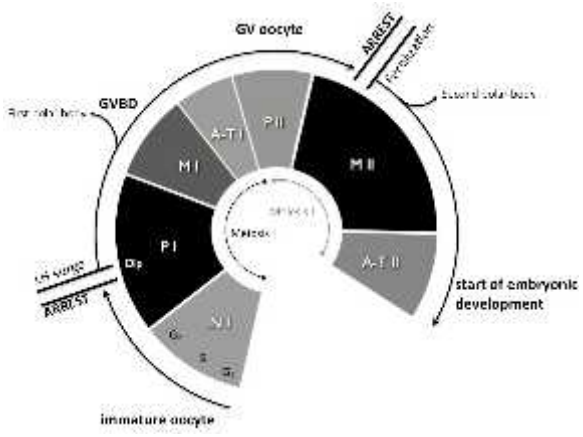

Figure 1. Stages of meiosis during oocyte maturation. Meiosis I is subdivided into Interphase (IN), Prophase (P), Metaphase (M), Anaphase and Telophase (A-T). The immature oocyte enters the first meiotic cycle during embryonic development where it passes through different stages of the cell cycle (G_1-S-G_2) in the Interphase (INI) and then gets arrested at the diplotene stage (Dip) of prophase I (PI). LH serves as a stimulus to induce completion of meiosis I, formation of first polar body followed by germinal vesicle breakdown (GVBD). The mature oocyte is now referred as germinal vesicle (GV) occyte. At metaphase II (MII) the oocyte is arrested again for the second time until fertilization. Fertilization reactivates meiosis II and completes maturation to start embryonic development.

Upon fertilization, the oocyte resumes meiosis II and extrudes the second polar body, thereby completing maturation (1, 2) (Figure 1). Oocyte maturation is one of the major steps for the oocyte to attain competence for successful fertilization and subsequent embryonic development. Acquisition of this developmental competence of the oocyte involves multiple factors regulated by different signaling pathways at various stages prior to fertilization (3, 4). In humans, failure of or incomplete oocyte maturation results in infertility that is attributed to a poorly-defined phenomenon known as "oocyte factor"(5-8). This article reviews recent progress in understanding the various signaling pathways and underlying intracellular mechanisms involved in maintaining the meiotic arrest and regulation of oocyte maturation that is essential for mammalian oocyte competence.

3. REGULATION OF MEIOTIC ARREST OF OOCYTES AT PROPHASE I STAGE

The segregation of the germ cell lineage from the somatic lineage is an event occurring very early during development of both invertebrates and vertebrates (reviewed by A. McLaren (9)). In mouse, primordial germ cells (PGC) are derived from the embryonic ectoderm during gastrulation that migrates to the genital ridges, where sex determination occurs through a retinoic acid (RA) dependent mechanism. In the embryonic ovaries, retinoic acid induces the progression of PGCs through meiosis by an undefined mechanism. In contrast, in the fetal testis, P450 cytochrome enzyme CYP26B1 degrades RA, retarding the onset of meiosis until after birth; as a consequence, oogenesis is prevented in favor of spermatogenesis (10, 11). Once the PGC has committed to oogenesis and enters meiosis, it progresses through the diplotene stage of prophase I, where the first meiotic cycle is arrested through mechanisms described below.

In the mammalian ovary, oocytes within the primordial or primary follicles are meiotically incompetent and will not mature if isolated from the follicle (12-16). This is largely due to lack of or low concentration of cell cycle proteins that are essential for oocyte maturation (14, 16-18). In contrast, the oocytes arrested in the diplotene stage of prophase I of antral follicles are fully competent to complete meiosis when taken out of the follicular environment (3, 19-22) and produce an egg that is perfectly capable of fertilization and can undergo embryonic development (1, 23-25). This spontaneous resumption of meiosis in oocytes is not observed in all species. For example, *Xenopus* oocytes do not undergo spontaneous resumption of meiosis upon removal from their follicular environment (26). During follicular development, oocytes grow in size and acquire competence for maturation by expressing cell cycle proteins that remain in an inhibited state at prophase I, resulting in the arrest of the oocyte (7, 12, 17, 27). In mammals, for *in vitro* maturation, oocyte size [~ 3mm diameter in pig (25, 28), ~75μm diameter in mouse (17, 21, 29) and ~100μm in human (3)] is an important factor for resumption of meiosis. Oocytes that do not attain the appropriate size may remain arrested at prophase I stage, or if cultured *in vitro*, may only mature to metaphase I stage (6, 12, 13). In fact, in cat (30), pig and cattle (12), oocyte competence for *in vitro* maturation depends on the follicular size from which the oocyte is isolated. However, oocyte size is not the sole factor that determines oocyte competence, and even fully grown oocytes can fail to resume meiosis when cultured *in vitro* and remain arrested in prophase I or in metaphase II (5, 31). There are reported cases in humans of oocytes being developmentally immature even when isolated from follicles of desired size. This incompetence of fully-grown oocytes to undergo oocyte maturation has been proposed to be due to defects in the molecular mechanisms responsible for regulation of oocyte maturation (31-33).

3.1. High cAMP levels are essential for meiotic arrest: role of G-protein coupled receptors and gap junctions

In mammalian oocytes, meiotic arrest is regulated by high intracellular cAMP levels (1, 3, 34) (Figure 2). In oocytes isolated from the antral follicles, a steady decrease in cAMP levels occur in parallel with meiotic resumption (35) and this spontaneous oocyte maturation can be prevented by cAMP analogues or cAMP phosphodiesterase (PDE) inhibitors (36-41). Furthermore, in culture, where cumulus-oocyte complexes (COCs) are maintained in meiotic-arrest, resumption of meiosis can be triggered by treatment with a cAMP antagonist like mycophenolic acid (42). Evidence supports two hypotheses to explain how elevated cAMP levels are maintained in oocytes. First is that the granulosa cells surrounding the oocyte produce

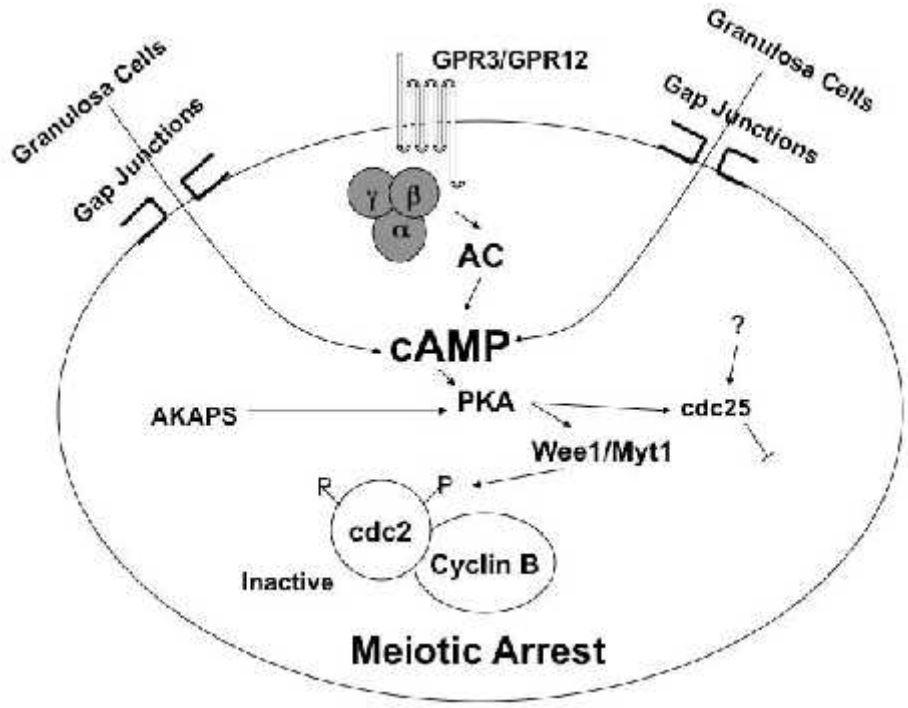

Figure 2. Meiotic arrest in mammals. High level of cAMP, required for oocyte arrest at prophase I, is maintained by synthesis and diffusion of cAMP in the oocyte via GPCRs and gap junctions, respectively. High cAMP levels activate PKA and the spatial and temporal localization of the later is regulated by AKAPs. PKA in turn regulates the kinase activity of the downstream maturation promoting factor (MPF) inhibitory proteins like Wee1/Myt1 that phosphorylates cdc2 thereby maintaining MPF in its inactive state resulting in meiotic arrest. Additionally, PKA (as well as other unknown factors) phosphorylates cdc25 phosphatase preventing it from localizing in the nucleus and activating the MPF.

high levels of cAMP that diffuses into the oocyte through gap junctions (2, 43, 44). A volume of recent studies in mouse oocytes support a second hypothesis that proposes that the cAMP that maintains meiotic arrest is generated at least in part within the oocyte (3, 34, 45). In fact, all components essential for cAMP production like G-proteins and adenylyl cyclase (AC) are present in mouse oocytes (46-49).

In a study by Mehlmann *et. al*., microinjection of an antibody directed against Gs into follicle-enclosed mouse oocytes resulted in resumption of meiosis, indicating the role of the Gs activity in meiotic arrest (50). Furthermore, in the rodent oocyte two Gs-coupled receptors, GPR3 (49, 50) and GPR12 (46) have been identified as important regulators of meiotic arrest. GPR3 is an orphan G-protein coupled receptor (51, 52) that was shown to be highly expressed in the mouse oocyte using a mouse oocyte cDNA library search (50). GPR3 constitutively activates Gs protein within the oocyte resulting in elevated cAMP levels (49, 50, 53, 54). Whether this constitutive activation of Gs protein by GPR3 occurs in a ligand-dependent or independent fashion is still unclear (3, 48, 52, 54). GPR3 knockout (KO) female mice are sub-fertile and the majority of antral follicle oocytes undergo spontaneous resumption of meiosis that can be reversed by GPR3 RNA injection (49, 50, 55). Also, GPR3 KO mice show premature ovarian aging and may prove to be an important model system for studying ovarian aging in humans (55). The GPR3-Gs protein dependent mechanism for maintaining meiotic arrest is only required in antral follicles (50). In GPR3 KO mice, oocytes in preantral follicles remain arrested in the prophase I stage but undergo spontaneous resumption of meiosis upon antrum formation (48, 50). How the oocytes in primordial or primary follicles remain arrested is poorly understood but is attributed to low concentration of downstream cell cycle proteins (discussed later) that are essential for meiosis.

GPR12 is another Gs-coupled receptor that is highly expressed in rat oocytes and is involved in increased production of cAMP (46). Even though both GPR3 and GPR12 are expressed in rat and mouse oocytes, GPR3 is the predominant receptor in mouse while GPR12 is expressed at higher levels in the rat (46). Also, the spontaneous oocyte meiotic maturation phenotype seen in GPR3 KO mice is not observed in GPR12 KO mice (46, 50). Recently GPR3 has also been cloned in the *Xenopus* and was demonstrated to be associated with meiotic arrest (56, 57). Furthermore, a study in human oocytes demonstrated that GPR3 (but not GPR12) is expressed at least at the RNA level and might be responsible for meiotic arrest, suggesting the intriguing hypothesis that human oocytes could maintain meiotic arrest prior to the LH surge using a signaling pathway similar to that of rodent oocytes (58).

In addition to different G-protein coupled receptors, different isoforms of AC have been demonstrated to be expressed in rodent oocytes (34, 47). Adenylyl cyclase 3 is predominantly expressed in rat oocytes while AC 1, 9 and 3 are expressed in mouse oocytes (47). Also, AC isoforms 1, 3, 4, 6 and 9 have been identified in bovine cumulus cells and have been shown to be highly regulated at different stages of oocyte development (59).

The significance of GPR3 as the sole inhibitor of meiosis is debatable. The argument against GPR3 is that since oocytes from wild-type animals expressing GPR3 undergo spontaneous maturation when removed from follicular environment, the self-generation of cAMP (via GPR3) by the oocyte is insufficient to maintain meiotic arrest (44, 60). Moreover, GPR3 KO mice are sub-fertile, but not infertile and. two preliminary studies in Premature Ovarian Failure (POF) patients from China and North America have found no perturbations in the GPR3 coding region, suggesting that mutations of GPR3 are likely not linked specifically to POF (61, 62). Thus, it is proposed that cAMP produced in the surrounding cumulus granulosa cells diffuses into the oocyte through gap junctions resulting in maintenance of meiotic arrest (44). In support of the above hypothesis, *in vitro* and *in vivo* studies in rat oocytes have demonstrated that disruption and/or blocking of the gap junction results in resumption of meiotic maturation that is accompanied by a decrease in cAMP levels (63-65). A more detailed discussion of the role of gap junction disruption and the underlying mechanisms involved in meiotic maturation has been reviewed in the next section. However, recent genetic evidence using a PDE3A and GPR3 double knockout mouse model demonstrates that both GPR3 and PDE3A are primary factors involved in maintaining meiotic arrest in mouse oocytes (45). In this study GPR3 down regulation and/or inactivation resulted in oocyte maturation in the PDE3A-null mouse that is known to be infertile due to lack of cAMP hydrolysis and significant accumulation of cAMP in the oocyte. In contrast, in the PDE3A and GPR3 double knockout mouse in which gap junctions and other receptors like GPR12 are unaffected, oocyte maturation was restored (45). Based on these results it has been suggested that gap junctions and/or other receptors are unable to sustain high cAMP levels to maintain oocyte meiotic arrests (45). However, it is not implausible that the high levels of cAMP required to arrest oocytes at prophase I is dependent on both the synthesis of cAMP within the oocyte as well as on diffusion of cAMP from cumulus cells through gap junctions into the oocyte.

3.2. Role of cell cycle proteins (Maturation Promoting Factors-MPF) in maintaining oocyte meiotic arrest

The intra-cellular mechanism by which high levels of cAMP prevent meiotic maturation is poorly understood (Figure 2). As mentioned previously, for oocytes to be competent to undergo meiotic maturation, expression of cell cycle proteins like cdc2/cyclin B complex commonly called maturation promoting factor (MPF) is absolutely essential (4). In rodents (66, 67), bovine (68-71) and pig (72-74), inhibition of MPF activation prevents GVBD. Similarly, when oocytes from MI or MII phase (containing very high MPF levels) are fused with germinal vesicle stage oocytes, GVBD is instantly induced (75-77). MPF is a heterodimer consisting of a catalytic CDK1 (cdc2) and a regulatory subunit (cyclin B). The activity of MPF is highly regulated by different proteins and depends on its state of phosphorylation (4). During oocyte meiotic arrest at the prophase 1 stage, MPF is phosphorylated by inhibitory proteins, like Wee1/Myt1 kinases at two highly conserved residues, Thr14 and Tyr15 of CDK1 that keep the heterodimer in an inactive pre-MPF state (1, 4, 78). In contrast, at the time of oocyte maturation, a dual-specific phosphatase, cdc25 dephosphorylates the CDK1 at the same sites thereby releasing it from its inhibitory state (4, 79). There are three isoforms of cdc25 (A, B and C), all of which are expressed in the mouse oocyte (80, 81) but cdc25B is indispensable for resumption of meiosis (82, 83). In cdc25B KO mice, the oocytes are arrested in the prophase I stage and fail to resume meiosis. This phenotype can be reversed by injection of cdc25B mRNA (82).

Also, the inactive pre-MPF is spatially localized to the cytoplasm and upon activation is translocated into the nucleus resulting in GVBD (84). In mouse (17, 84) and pig (16) the concentrations of CDK1 and cyclin B increase with oocyte growth and it is speculated that meiotic competence of oocytes may be dependent on a threshold of these proteins. In mouse, both competent and incompetent oocytes have higher concentrations of cyclin B1, cdc25 and Wee1 proteins than CDK1 and an increase in CDK1 amounts during oocyte growth is rate-limiting for acquirement of meiotic competence (17, 84). However, supplemention of CDK1 to growing incompetent oocytes fail to make them meiotically competent (85) and thus it has been proposed that a balance between the phosphorylated and unphosphorylated states of MPF as well as its spatial localization within the oocyte may underlie the event of oocyte competence. In addition to suppression of MPF activation, high cAMP levels also inhibit the expression of cyclin B thereby decreasing the availability of pre-MPF (86).

3.3. Role of cAMP-activated PKA in regulating MPF activity during oocyte arrest

As mentioned, Wee1 and Myt1 are the major downstream substrates of PKA involved in maintaining meiotic arrest (78, 79). During oocyte meiotic arrest, the high cAMP levels activate PKA which in turn regulates the kinase activity of the downstream MPF inhibitory proteins like Wee1/Myt1 thereby maintaining MPF in its inactive state (78, 79, 87) (Figure 2). There are differences in the expression patterns and isoforms of Wee1 among species. For example, Wee1A and 1B are expressed in *Xenopus* oocytes while only Wee1B is expressed in mouse oocytes (79, 84). Also, in *Xenopus*, Wee1A mRNA is present in GV oocytes but the protein is expressed only at MII stage. By early embryonic stage the Wee1A protein is degraded and Wee1B is expressed (88). In contrast, in mouse Wee1B protein is expressed in GV oocytes and plays an important role in maintaining the meiotic arrest (78, 79). In fact, ablation of Wee1B mRNA by RNAi results in meiotic maturation in mouse. PKA phosphorylates a Ser15 residue

of Wee1B in mouse thereby activating the latter to phosphorylate cdc2 of the MPF in order to maintain it in an inactive state for meiotic arrest (78). In *Xenopus* (89) and *Bufo* (90), PKA also phosphorylates cdc25 which results in the sequestration of cdc25 by 14-3-3 protein in the cytoplasm, thus preventing the cdc25-induced MPF activation (89), and exerting a fine-tune spatial control contributing to the maintenance of meiotic arrest. The same mechanism has also been reported in mouse oocytes (91).

The activity of PKA in oocytes is dependent primarily on two factors: (1) cAMP sensitivity and (2) sub-cellular localization. A recent study in rat oocytes has demonstrated the expression of two regulatory subunits (R1 and R2) of PKA that vary in cAMP responsiveness and subcellular localization (87). While PKA R1 subunit is highly sensitive to cAMP levels and is expressed predominantly in growing oocytes, PKA R2 subunit is expressed at higher levels in fully-grown oocytes and is less sensitive to cAMP levels (87). This study further suggested that a high basal activity of PKA in growing oocytes may be maintained due to relatively high abundance of PKA R1, which is more sensitive to low cAMP levels and/or its subcellular compartmentalization in close proximity of both its downstream and upstream effectors. The spatial and temporal localization of PKA to its site of action plays a crucial role in both meiotic arrest and maturation and is regulated by a set of proteins called A Kinase Anchoring Protein (AKAP). It is proposed that during meiotic arrest, binding of PKA in the cytoplasm to an isoform of AKAP results in trans-localization of PKA subunits to its site of action (near Wee1B and cdc25), thereby increasing the efficiency of meiotic arrest. While, during resumption of meiosis and GVBD, PKA binds to another isoform of AKAP, AKAP1 and is re-localized to the mitochondria away from its site of action by mechanisms that are yet to be identified (92). In rat oocytes a splice variant of AKAP 1 called AKAP140, has been identified in the mitochondria (93-95). Thus, AKAPs are involved in both meiotic arrest and maturation by regulating the spatial and temporal localization of PKA to its site of action. Moreover, the AKAP1 KO mouse is sub-fertile and more sensitive to cAMP levels. In AKAP1 KO mice, PKA does not get re-localized to the mitochondria and thus oocyte maturation process is defective (92).

Thus, the spatial and temporal localizations of factors regulating cAMP levels, like PDEs, (discussed later) and compartmentalization of kinases and phosphatases that regulate MPF is also critical for the control of meiotic arrest in addition to cell cycle re-entry in the mouse oocyte, as demonstrated by experiments with Wee1B and cdc25B mutants (96). The two proteins shuttle in and out of the nucleus during maturation by means of different mechanisms and different temporal patterns. The correct nuclear relocation of cdc25B, which is dependent on PKA inactivation, and cytoplasmic relocation of Wee1B following active NES-dependent nuclear export are essential for the activation of nuclear MPF and the subsequent onset of GVBD (96). The majority of the above-described mechanisms of MPF regulation involved in maintaining meiotic arrest at the prophase 1 stage have been identified in the rodent model, where as the expression and role of Wee1/Myt1 in other mammalian species is yet to be demonstrated. Even though there is some evidence for the requirement of CDK1 phosphorylation during meiotic arrest in porcine oocytes (7, 97), further comprehensive studies are required to extend the above observations to other species. The role of cAMP and downstream-activated signaling pathways in maintaining oocyte arrest as discussed above are summarized in figure 2.

Of note, the meiotic-arrested oocytes are vulnerable to DNA damage and therefore detection of DNA damage is essential to oocyte competence and fertility. A homologue of the tumor suppressor gene p53, known as p63 has been identified to be constitutively expressed in arrested oocytes and is proposed to be essential in the mechanism of DNA damage-induced oocyte death (98). Furthermore, it is interesting to note that some of the mechanisms controlling meiotic arrest at prophase I and resumption of meiosis I during maturation share common features with DNA damage-induced mitotic G2-checkpoint arrest and checkpoint recovery, respectively, including (but not limited to) cdc25B-dependent cell cycle resumption (99).

4. OOCYTE MATURATION

In mammals, the LH surge induces oocyte maturation by decreasing cAMP levels in oocytes (3, 100, 101) as depicted in figure 3 and 4. However, the mechanism by which LH stimulation initiates meiotic maturation is very complex and poorly understood. In humans (5, 6), failure to resume meiosis *in vivo* is believed to be due to: 1) absence of or incomplete LH effect, 2) defective signaling from the surrounding cumulus cells and 3) intrinsic oocyte factors. In order to understand the underlying mechanisms of oocyte maturation we have identified two major areas and tried to review them with respect to the latest findings.

4.1. LH-induced resumption of meiosis in oocytes

4.1.1. Role of epidermal growth factor (EGF)-related proteins

In granulosa cells, LH via a cAMP-dependent pathway induces the expression of different epidermal growth factors (EGF) like proteins (101) (Figure 3). Expression of EGF related proteins such as amphiregulin (AREG), epiregulin (EREG) and beta-cellulin (BTC) have been demonstrated in rodents (101-104), humans (105, 106), non-human primates (107), bovine (108) and porcine (109, 110) granulosa cells. These EGF-like proteins act on mural and cumulus granulosa cells in an autocrine and paracrine manner through EGF-receptors (EGFRs), respectively (102-104, 110, 111), as outlined in figure 3. In fact, it is thought that these EGF-like proteins are cleaved and released by matrix metalloproteinases (MMPs) in response to LH (112), as EGF activity can be detected in the follicular fluid of human (106, 113, 114) and porcine (115) ovaries and are implicated in fertilization competence and oocyte quality. Furthermore, *in vitro* studies have shown EGFR to be important for resumption of meiosis

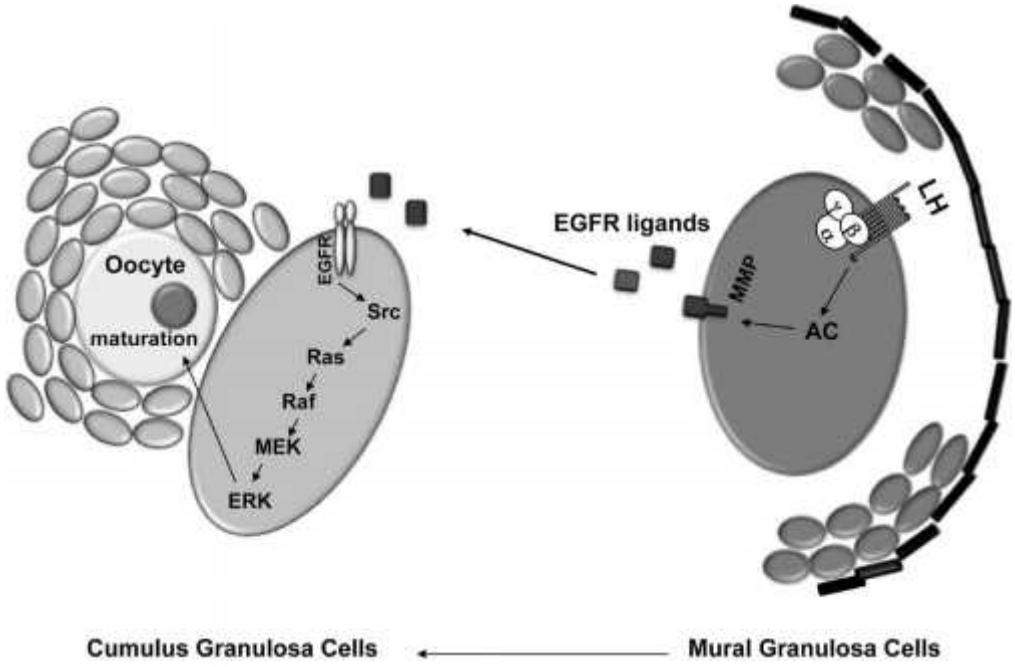

Figure 3. Different signaling pathway activated in mural versus cumulus granulosa cells. In Luteinizing hormone receptor (LHR) expressing mural granulosa cells, LH via a cAMP-dependent pathway activates the release of membrane-bound Epidermal Growth Factor (EGF) ligands. These ligands act in autocrine and paracrine fashion on the cumulus granulosa cells via EGFR to activate downstream signaling pathways including MAPK, promoting oocyte maturation

and increasing the efficiency of *in vitro* oocyte maturation (101, 104). Studies involving AREG, EREG and EGFR inhibitors and KO mouse model have established that EGF-like growth factors acting via EGFR are the mediators of LH action involved in oocyte maturation (102-104, 111). Ablation of the AREG and / or EGFR in mouse either delays or blocks LH-induced oocyte maturation, cumulus expansion and ovulation (111). Even though expression of EGFR as well as direct effects of EGF on oocytes has been reported (104, 116-119), whether the action of these proteins in meiotic maturation is direct or indirect (through cumulus cells) is still not clear. However, a functional bi-directional communication between the oocyte and granulosa cells is essential for maturation, and it has been recently demonstrated that the oocyte can influence expression of EGFR in cumulus cells via the paracrine action of oocyte-derived growth factors GDF9 and BMP15 (120). Given the importance of EGF-like proteins in oocyte maturation, it is thought that these proteins may be likely targets for the improvement of *in vitro* oocyte culture and fertility in humans. This hypothesis is further supported by reports of positive effects of EGF addition in culture medium on *in vitro maturation* (IVM) and *in vitro fertilization* (IVF) in goat, pig, dog and non-human primates (121-124). Recently, the expression of the type III neuregulin NRG1 has been reported in mouse granulosa cells during ovulation, suggesting an involvement of other EGFR-family receptors (like HER2-HER3) in maturation-inducing signaling. NRG1 enhances AREG-induced ERK1/2 phosphorylation in both granulosa cells and cumulus cells, as well as increasing progesterone production and enhancing developmental competence of COC (120).

4.1.2. Role of steroids in oocyte maturation

In fish (125) and amphibians (126), gonadotropin-induced steroids act as mediators in stimulation of meiosis (127, 128). Prior to this finding, it was believed that steroids had little or no role in resumption of meiosis in mammalian oocytes (3). However, this hypothesis is now being challenged. In fact several recent studies have shown that the oocyte maturation process is highly conserved from fish to *Xenopus* to mammals as androgens, estrogens and progestins trigger oocyte maturation both *in vitro (129)* and *in vivo (130)*. Studies in mouse have demonstrated that EGF-EGFR in cumulus granulosa cells can stimulate steroidogenesis via the regulation of steroidogenic acute regulatory protein (StAR) activity. The steroids, produced, in turn trigger oocyte maturation that appears to be mediated by classical steroid receptors (131). Also, studies involving *in vitro* oocyte maturation have demonstrated that like *Xenopus*, in mouse, androgen (especially testosterone) treatment, via a non-genomic pathway, can decrease cAMP levels as well as activate MAPK and CDK1 signaling thereby stimulating oocyte maturation (129). Inhibition of androgen receptor (AR) or KO of ARs in mouse oocytes significantly compromises oocyte maturation, suggesting a role for androgens in oocyte maturation and follicle development (129, 132, 133). Recently, it has been shown that testosterone can also trigger oocyte maturation in porcine oocytes (134). A positive role of progesterone in induction of GVBD via breakdown of gap junctions has also been

demonstrated in porcine oocytes (135, 136). In mouse, progesterone can completely rescue AG-1478 and Galardin mediated inhibition of EGF and LH-induced maturation (130) while in non-human primates progesterone and/or androgens can trigger oocyte maturation *in vivo* (137). Our group has shown that both progesterone and testosterone can attenuate constitutive G protein activity and induce maturation in *Xenopus* oocytes acting via either PR or AR (138). Both steroids also induce maturation in a mouse model of follicle-enclosed oocytes (130). In fact, oocytes express the cytochrome P450 enzyme CYP17 that converts progesterone to its androgen metabolite androstenedione (139, 140). Consequently, incubation of oocytes with progesterone *in vitro* is equivalent to adding two different ligands, and discerning their separate individual actions is no easy task. The thought is that progesterone may be the physiologic mediator of maturation, while testosterone may only be important in diseases of androgen excess. Also, many different groups have shown (141-143) that inhibition of steroidogenesis in follicles does not prevent LH-induced maturation, although two other papers reported evidence for the opposite effect (144, 145).

It has also been shown in mouse that FSH via a PKA-CREB dependent pathway induces the expression of AR and Cytochrome P450 lanosterol 14α-demethylase (CYP51), a key enzyme in the biosynthesis of sterols and steroids that are involved in oocyte maturation (146). All the above experiments support the physiological significance of steroids in the mammalian oocyte maturation process. However, earlier studies demonstrated that inhibition of steroidogenesis did not completely suppress the gonadotropin-induced oocyte maturation thereby suggesting the existence of other mechanisms (131, 142, 147, 148), although two different studies demonstrated the opposite (136, 149). The differences between these studies may be due to variable culture conditions that can affect steroid-triggered maturation. However, many different recent reports from various groups have provided compelling evidence (both *in vitro* and *in vivo*) that steroids contribute to promoting oocyte maturation (129, 131, 134, 137, 146). Other mechanisms, including disruption of gap junctions and/or attenuation of G-protein mediated signaling responsible for meiotic arrest may also play a major role in other aspects of oocyte maturation (130).

4.2. Underlying mechanisms of LH-induced signals involved in decrease of cAMP levels in the oocyte

4.2.1. Role of gap junctions in oocyte maturation

The role of gap junctions in maintaining high cAMP levels during meiotic arrest has been discussed previously (44). Gap junctions are composed of proteins from the connexin (Cx) family and Cx43 is the most abundant Cx in the ovarian follicles. According to the model depicting the role of gap junctions in meiotic arrest and oocyte maturation, LH induces the inhibition of Cx43 translation thereby causing breakdown of gap junctions between cumulus and oocytes that prevents diffusion of cAMP into the oocyte, lowers cAMP levels and triggers initiation of oocyte maturation (44, 63-65). Studies in rodents have demonstrated that LH via the cAMP/PKA/MAPK pathway rapidly phosphorylates Cx43, which in turn causes breakdown of the gap-junctional communication. In addition, with time LH also inhibits Cx43 translation, ultimately leading to elimination of gap junctions (44, 64, 65, 150). During *in vitro* maturation of mouse COCs, Cx43 clusters to lipid rafts contributing to the early stage of gap junction breakdown through a functional inactivation followed by the removal of Cx43 from the cell surface (151). Studies in pig COCs have shown that the breakdown of gap junctions is dependent on phosphorylation of Cx43 by PKC and PI-3K, but is independent of MAPK (152, 153). In bovine, Cx43 mRNA has been proposed as a marker of oocyte developmental competence, because Cx43 mRNA levels are significantly lower in poor quality COCs when compared to good quality COCs (154). Other types of connexins have also been implicated in oocyte maturation in different species: Cx37 and Cx26 are expressed in mouse and they may be responsible for impaired maturation and poor pregnancy outcomes associated with diabetes (155), while Cx45 and Cx60 are the main connexins expressed in porcine oocytes during folliculogenesis, (156). Thus the diversity in connexin genes expressed in mammalian oocytes could account for differential roles of gap junction communication during oocyte maturation in different species. Different connexins also play different roles in the same species: in the mouse follicle, Cx37 is the predominant connexin expressed at gap junctions between the oocyte and granulosa cells, while Cx43 is mainly expressed in gap junctions between granulosa cells. Furthermore, oogenesis can be rescued in Cx37-null mutant mice (which are sterile) by oocyte-specific replacement with Cx43 (157), suggesting that both connexins may be involved in important maturation-promoting gap junctional communication, independent of their spatial localization in the follicle.

Pharmacological closure of gap junctions by carbenoxolone (CBX) mimics LH–induced oocyte maturation in a LH-MAPK independent manner, strengthening the fact that breakdown of cell-cell communication is one of the factors involved in resumption of meiosis (65). The precise cascade of events following LH surge that leads to oocyte maturation is still unclear and needs further investigation. However, there still exists the controversy of whether the inhibition of intra-oocyte cAMP production or the prevention of granulosa cell-derived cAMP diffusion into oocytes is responsible for resumption of meiosis in oocytes. It is likely that both of these possibilities are not mutually exclusive, as LH-induced signals like EGF, AREG, EREG and BTC may act on cumulus cells to cause cumulus expansion, gap junction breakdown and trigger an active signal, all of which together decrease cAMP levels resulting in resumption of meiosis. There is evidence that EGF-EGFR signaling induces several genes like Cox-2, hyaluron synthase 2 (HAS-2) and tumor necrosis factors-α-induced protein 6 (TSG-6) that are known to play important roles in cumulus expansion and ovulation (111). Nevertheless, the precise mechanism of the above proposed model remains to be established.

4.2.2. Role of G-proteins in decreasing cAMP levels in the oocyte for resumption of meiosis

Following LH surge, the events that lead to a decrease in cAMP level in the oocyte are poorly understood (3). This decrease in cAMP can be due to the disruption of gap junctions (as discussed above) or by inhibition of the G-protein coupled receptor-AC pathway that is responsible for intra-oocyte cAMP synthesis or by an increase in expression and / or activity of cAMP PDE. In other cell types, both G_i proteins and elevated Ca^{2+} levels are known to be induced by LH and can decrease cAMP levels by inhibiting AC expression and / or activity (100). Thus, the role of G_i proteins and elevated Ca^{2+} levels in LH-induced mouse oocyte maturation have been investigated lately by injecting pertusis toxin (PTX), a potent inhibitor of G_i proteins or treatment with Ca^{2+} chelators like EGTA (100). Both PTX and EGTA have been reported to have no effect on LH-induced meiotic resumption, thereby suggesting that neither of these well-established cAMP-regulating pathways is involved in LH-induced meiotic resumption in mouse oocyte. This study further hypothesized that LH may activate PDEs by lowering cGMP or by phosphorylating PDEs in the oocyte thereby decreasing cAMP levels (100). A more recent study, also in mouse oocyte, has reported that unlike *Xenopus*, the Gβγ-signaling decreases cAMP levels and can induce meiosis (158). The Gβγ-induced decrease in cAMP levels does not involve a constitutive Gβγ-signaling but rather is mediated by partially inhibiting the Gsα-stimulated rise in cAMP levels. Moreover, possibility of additional Gβγ-induced mechanisms like inactivation of adenyl cyclase and /or activation of PDE have also been proposed (158). However, further studies are still needed to identify the physiological factors responsible for activation of this Gβγ-signaling pathway.

4.2.3. Role of phosphodiesterase (PDE) in oocyte maturation

It has been known for long that PDE inhibitors like IBMX can prevent spontaneous meiotic maturation, but the identity of the PDE isoforms or the underlying mechanism(s) involved in stimulating the PDEs were unclear (34). Studies with PDE3A specific inhibitors (cilostamide, milrinone) in different species [rodents (159, 160), bovine (161, 162), porcine (163, 164), macaque (165) and humans (166)] have established the importance of this PDE isoform in the process of oocyte maturation. The effects of cAMP in the follicle are somewhat contradictory. For example, it is well established that in granulosa cells LH-induced effects are mediated via an increase in cAMP while in the oocyte, LH surge triggers a decrease in cAMP levels. It is hypothesized that cAMP levels and its actions are highly compartmentalized within the two separate cells types, the oocyte and the granulosa cells, and may also be differentially regulated (34, 167, 168). In mouse (34, 160), porcine (169) and bovine (162), two different PDE isoforms, PDE3A and PDE4 are expressed exclusively in the oocyte and granulosa cells, respectively. Also, inhibition of PDE4 in granulosa cells induces resumption of oocyte meiosis (162) while inhibition of PDE3A blocks meiotic maturation in bovine(162), mouse (170) and non-human primates(171), supporting different roles for cAMP to promote or repress meiosis resumption in granulosa cells or oocytes (respectively). COCs retrieved from unstimulated human ovaries and cultured with a specific PDE3 inhibitor and forskolin displayed a delayed spontaneous meiotic progression, reduced GVBD and increased oocyte developmental competence (172). Moreover, recent work has shown that PDE3 is transcriptionally up-regulated via a cAMP-dependent mechanism in cumulus follicular cells (173), further demonstrating how increased cAMP in the follicle can contribute to decrease cAMP levels in the occyte to promote resumption of meiosis. PDE3A KO mouse has normal folliculogenesis and ovulation but is infertile (167). The knockout study has revealed that PDE3A is indispensable for resumption of meiosis and its activity is not compensated by other PDE isoforms like PDE3B. Also, in the PDE3A KO mouse, oocyte maturation can be restored by inhibiting PKA or increasing the expression of cdc25, suggesting that in this KO mouse ablation of PDE3A results in elevated cAMP-PKA activity that in turn inhibits the MPF/MAPK pathway involved in resumption of meiosis (167).

It has been proposed that the decrease in cAMP levels is the primary signal for resumption of meiosis. The decrease in cAMP level lowers the concentration of active PKA that in turn releases the inhibitory effects of Wee1B/Myt kinase on CDK1, while cdc25B dephosphorylates and subsequently activates MPF (78, 79). In *Xenopus*, PKA directly phosphorylates cdc25 and sequesters it via 14-3-3 protein and inactivation of PKA activates cdc25 (89), a mechanism that has also been described in mammals (90, 91, 99). In addition, Akt/PKB by an unknown mechanism activates PDE3, which further lowers cAMP levels enabling the oocyte to resume meiosis.

Another mechanism contributing to decreasing cAMP levels in the oocyte, and also involving communication between cumulus cells and the oocyte, is mediated by guanine 3', 5' monophosphate. cGMP produced in the follicle passes through gap junctions into the oocyte, where it inhibits hydrolysis of cAMP by PDE3A (174). This inhibition contributes to the high concentrations of intra-oocyte cAMP to maintain the oocyte in the prohpase I stage. LH reverses the inhibitory signal by lowering cGMP levels in the follicle and by closing gap junctions between the cumulus cells, consequently contributing to the decrease in oocyte cAMP, leading to meiosis resumption (174, 175). However, it is still not completely clear how cGMP contributes to the onset of maturation, since gonadotropins are able to induce the expression and activity of both cGMP-specific PDEs and guanylate cyclase-A (176, 177). Consequently, the effect of the LH surge on cGMP needs further clarification.

4.3. Signaling pathways involved in resumption of meiosis and GVBD

4.3.1. MAP Kinase pathway

In the *Xenopus*, steroid-stimulated MAPK signaling is essential for activation of MPF and resumption of meiosis (139, 178-180). The MAPK pathway is activated before GVBD through an upstream serine/thronine oocyte

specific protein kinase called c-mos that in turn activates other downstream MAPK proteins, MEK and Erk2 (34, 180). The latter in turn through a positive feedback loop activates mos via paxillin thus amplifying the kinase signaling cascade (181). Ribosomal S6 protein kinase p90rsk is a major substrate for Erk2 and activated p90rsk inactivates Myt1 kinase (182) thereby inducing MPF activation and entry into meiosis I (126, 178, 179). On the contrary, other studies have shown a reverse hierarchy, where MPF activates MAPK pathway and in turn is itself activated by MAPK-independent mechanisms (183-186). Recently, the RSK2 protein kinase has been identified as another key player involved in MPF activation via direct phosphorylation of cdc25C independently of MAPK. In fact, while p42 MAPK phosphorylates cdc25C at the N-terminus, RSK2 acts on three C-terminally located serine/threonine residues of cdc25C (187). The authors of this study further suggest that additional biochemical events may be required to fully activate cdc25C, implying the action of one (or possibly more) kinases other than MAPK and RSK2 (187).

In mammals, while it is widely accepted that the MAPK pathway is essential for oocyte maturation, the chronology of MAPK activation and GVBD is still unclear. There are reports suggesting that in mammals c-mos activated MAPK pathway may not be involved in GVBD (179, 188). In rodents, some studies show that MAPK activation occurs after GVBD (179) and experiments involving MAPK inhibitors (189-191) and a mos KO mouse model (192, 193) demonstrate that resumption of meiosis and extrusion of first polar body can take place in absence of the MAPK pathway yet the ability of these oocytes to become arrested at MII stage is impaired (194, 195). In goat (196) MAPK exists in an inactive form in the GV stage and is activated after GVBD, while in bovine (197, 198) and equine (199) oocytes, MAPK is activated before GVBD. However, inhibition of MAPK activity by injection of a dual specific phosphatase (MKP1) mRNA into GV stage bovine oocytes did not have any effect on the resumption or progression of meiosis but prevented MII arrest (200). Thus, at least in farm animals, it is believed that MAPK activation may not be necessary for resumption of meiosis (200), but necessary for MII arrest. However, the exact timing of MAPK activation in relation to GVBD might not be precisely measurable because the techniques used to visualize GVBD and to quantify MAPK phosphorylation have different sensitivities. Thus, the observation of GVBD before Erk phosphorylation might be more a function of sensitivity than chronology. In the pig (7, 97, 197, 201-203) MAPK activation has been shown to occur around GVBD but inhibition of c-mos by siRNA (204) or MAPK inhibitors (205-208) does not have any effect on GVBD in denuded oocytes but prevented GVBD in cumulus-enclosed oocytes. These studies suggested that in the pig, MAPK activation may not be important for spontaneous MAPK activation of denuded oocyte but may be essential for gonadotropin-induced meiotic maturation of cumulus-enclosed oocytes (205). However a very recent study shows that in pig oocytes testosterone-induced MAPK activation occurs before GVBD (134). Testosterone-induced GVBD was mediated via the AR that in turn activated MAPK signaling by interacting with the Src family tyrosine kinases, and notably U0126, a well-established MAPK inhibitor blocked testosterone-induced GVBD. Also, other studies have shown that artificial activation of MAPK pathway is sufficient to induce GVBD, and that injection of active MAPK into GV accelerates GVBD in pig oocytes (209). In rodents and pig there are reports of partial activation of p90rsk before GVBD in a MAPK independent fashion followed by a MAPK dependent pathway after GVBD for full activation of p90rsk (205, 210, 211). However, both MAPK and p90rsk are not essential factors for meiotic resumption of oocytes (97, 205). Thus, whether MAPK activation is required for initiation or progression of meiotic resumption is still unclear, but it is believed that in mammals other signaling pathways may also be involved in this process.

4.3.2.Other signaling pathways

In pig COCs, PI3K-PKC activation in cumulus cells disrupts gap junctions leading to a decrease in oocyte cAMP level (153, 207, 212) as well as activation of MAPK and MPF activity in the oocytes, but how this signal is transmitted from the cumulus cells to the oocyte is poorly understood (153, 207). Whether the activation of MAPK and MPF in porcine oocytes is dependent or independent of each other is not known. In rodents, it has been demonstrated that MPF is upstream of MAPK pathway (95, 213). Inhibition of MPF by an inhibitor of MPF activation, roscovitine (189) as well as ablation of cdc2 by siRNA techniques prevents MAPK activation via inhibition of mos mRNA polyadenylation (214). Since cAMP inhibits MPF, there is a linear relationship between cAMP levels, MPF and MAPK activation (95, 213).

In vitro experiments in bovine oocytes with roscovitine have also revealed the involvement of other signaling pathways like EGF-EGFR, JNK, PI3K/Akt and Aurora-A in regulation of oocyte maturation in cattle (119). This study has shown that EGF-EGFR induced meiotic maturation involving activation of Aurora-A and JNK proteins is MPF-independent while the activation of PI3K-Akt pathway is MPF-dependent (119). Thus, the Akt/PKB activation may be a secondary effect of decreased cAMP levels and MPF activation. Furthermore, *in vitro* maturation studies in bovine have shown that recombinant human FSH via PKA and PKC pathways significantly increases oocyte competence and thus has been proposed to have significant clinical relevance given the wide use of r-hFSH in assisted reproduction protocols (215). More comprehensive studies are required to understand the hierarchy of events and cross-talk between different signaling pathways that lead to resumption of meiosis and GVBD.

4.3.3. Regulation of MPF complex for resumption of meiosis and GVBD- Species difference

Although all species have MPF complex there exists some basic difference in its regulation. Resumption of meiosis and GVBD in rodents can take place without *de novo* protein synthesis (79). In contrast, in *Xenopus* and farm animals protein synthesis is indispensable during resumption of meiosis. In *Xenopus*, the major isoform of cdc25 regulating MPF is cdc25C, while in mammals it is

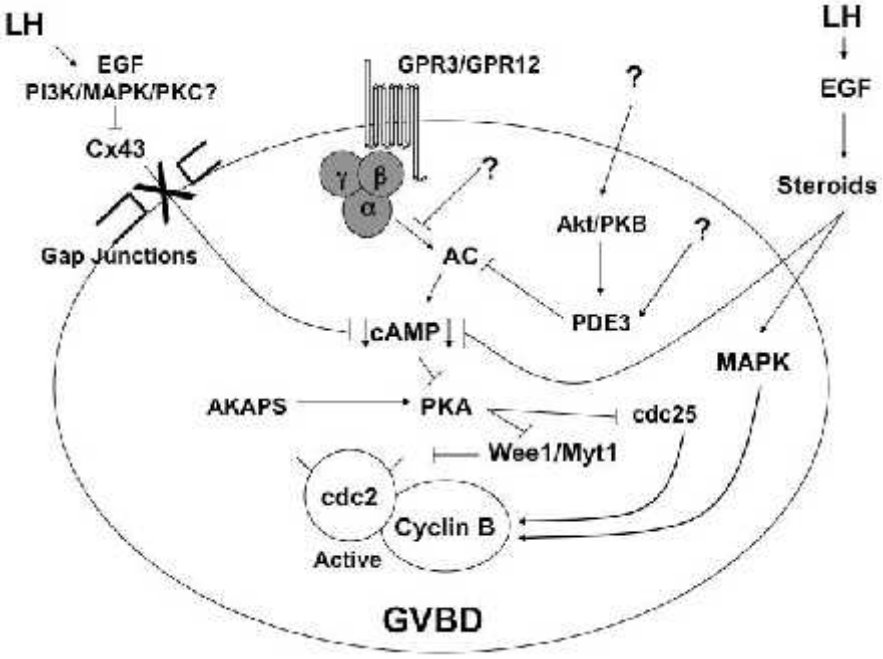

Figure 4. Signaling pathways involved in GVBD in mammals. In mammals, resumption of meiosis and germinal vesicle breakdown (GVBD) is a complex process involving cross talk between different signaling pathways. Luteinizing hormone (LH-) stimulated Epidermal Growth Factor (EGF) proteins in the cumulus cells induces various signaling pathways that lead to disruption of gap junctions as well as steroidogenesis. The steroids in a transcription-independent manner decrease cAMP levels and activate MAPK and CDK1 signaling. Also activation of PDE3A by Akt/PKB or by some unknown factors and/or inhibition of the Gs-induced pathway by a mechanism yet to be identified, decrease cAMP levels in oocytes. This decrease in cAMP and regulation of PKA localization by AKAPs lower the level of activated PKA that inhibits the phosphorylation of cdc2 by Wee1/Myt1. At the same time cdc25 dephosphorylates cdc2 thereby causing activation of maturation promoting factor (MPF) and GVBD. The components and interactions represented with a "?" are either controversial or remain to be discovered.

cdc25B (79). However recently in mouse oocytes, it was reported that cdc25A also plays a major role in meiotic resumption, MI spindle formation as well as in MI-MII transition (216). Also, in *Xenopus*, the steroid-mos-MAPK-p90rsk inactivates Myt1 resulting in MPF activation (34, 178, 179), while in mammals, the underlying mechanism involved in MPF activation is more complex and may involve various signaling pathways. In mammals, the activation of MPF may involve a decrease in cAMP (3, 44), regulation of PKA localization by AKAPs (92, 95), activation of PDE3A by Akt/PKB (168, 217) or by some unknown factors along with steroid-triggered MAPK activation (129, 130, 134), as outlined in figure 4. Further studies are required in mammals to understand the various gaps in the mechanism of meiotic resumption and GVBD.

4.4. Meiosis I and transition from Meiosis I to Metaphase II stage

Following GVBD, MPF activation and resumption of meiosis, there is meiotic spindle formation and extrusion of the first polar body. During MI metaphase, the homologous chromosomes arrange at the spindle equator and after recombination, the homologous pairs are pulled apart by microtubules that are attached to the centrosomal chromatins via the kinetochore (4). Completion of the first meiotic division is marked by the segregation of the homologous chromosomes between the oocyte and the first polar body while the sister chromatids remain attached (4, 218). Thereafter, the ooctye undergoes immediate transition into MII, where it remains arrested until fertilization (219, 220). The progression of oocytes through meiosis I and the transition to metaphase II stage involve the activity of different proteins. In mammals [rodents (4, 189), pig (7, 97) and bovine (221)] the action of MPF occurs in an oscillatory pattern where MPF activity increases at the time of GVBD, declines at the time of MI completion and again reaches a high level of activity at MII and continues to remain elevated until fertilization (219) (Figure 5).

4.4.1. Meiosis I (MI) completion: Mechanism of MPF decline by proteosomal degradation

The decline of MPF during MI completion is not due to re-phosphorylation of CDK1 but rather proteosomal degradation of cyclin B1 (4, 95, 213). In rat oocytes, use of proteosomal inhibitor and/or microinjection of methylated ubiquitin that impairs the proteosomal degradation process, prevent the transition from MI to MII, thus demonstrating the importance of the proteosomal degradation of cyclin B1 for completion of MI (222). The polyubiquitination of cyclin B1 for proteosomal degradation is caused by a multi-subunit E3 ligase complex called anaphase-promoting complex/cyclosome (APC/C) (219). APC targets mitotic

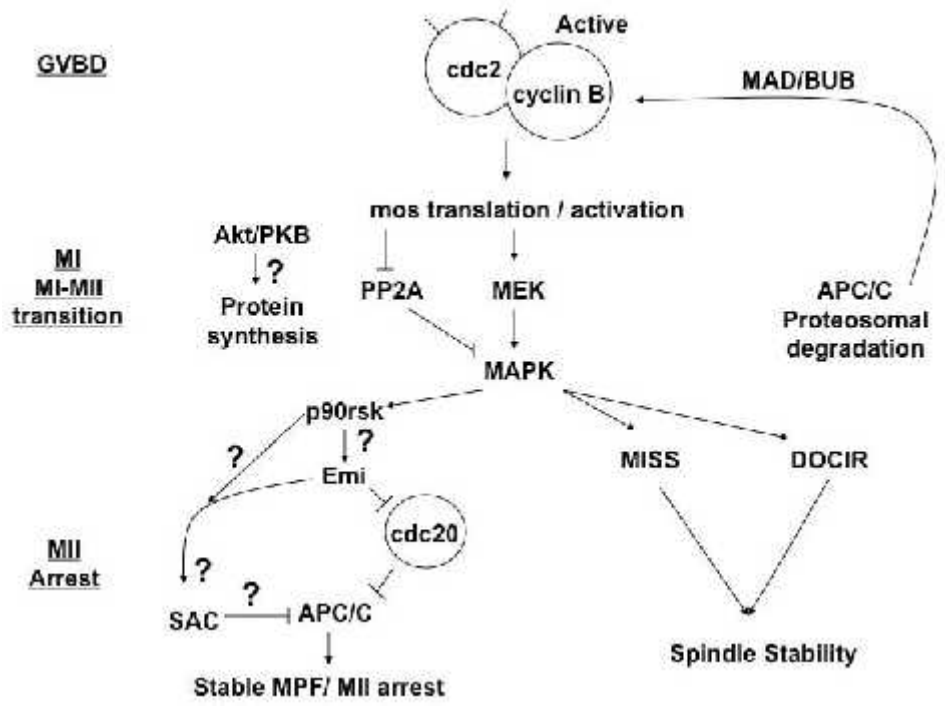

Figure 5. Intra-cellular mechanisms involved in MI, MI-MII transition and MII arrest in mammals. Following GVBD, activated MPF induces translation and activation of the mos-MAPK pathway. During MI completion there is a decline in MPF activity by proteosomal degradation of cyclin B1 regulated by APC/C via MAD/BUB proteins. Also, Akt/PKB regulates protein translation at MI-MII transition. During transition of MI-MII and at MII arrest, MPF activity is reestablished by mos-MAPK-p90rsk-Emi pathway that inhibits APC activity thereby preventing cyclin B degradation and stabilizing MPF resulting in MII arrest. Two more proteins, MISS and DOC1R are substrates for the MAPK pathway and are involved in spindle stability during the MII arrest. The components and interactions represented with a "?" are either controversial or remain to be discovered.

regulators for degradation and is activated by phosphorylation and association with APC/C activators like CDC20 and FZR1. These activators bind to specific sequences including the destruction box (D-box) for CDC20 and D-box, KEN-box and CRY-box for FZR1 found on target proteins thereby labeling them for degradation (223-225).

APC/C activators - CDC20 and FRZ1: Both CDC20 and FRZ1 have been reported to be expressed in mouse and porcine oocytes. FZR1 is shown to be involved in meiotic arrest in GV oocytes by maintaining low levels of cyclin B1 thereby preventing MPF activation (226, 227). Levels of CDC20 are also regulated by ADC (FZR1). During M-phase, activated MPF inhibits FZR1 by phosphorylation (228) to increas cyclin B levels and further promote MPF activation. After meiotic resumption, as the oocytes progress through MI, CDC20 accumulates while MPF phosphorylates and activates APC to bind to CDC20 and form an APC (CDC20) complex (229-231). The latter then degrades cyclin B and inactivates MPF, resulting in progression of oocytes from MI to first meiotic anaphase (231).

The major difference between mouse and porcine oocytes is that in pigs FZR1 regulates CDC20 levels during prophase 1 arrest while in mouse it is after GVBD (227, 231). CDC20 hypomorphism *in vivo* results in chromosome missegregation and formation of aneuploid gametes. As a consequence, mice expressing low CDC20 are healthy and have normal folliculogenesis as well as standard fertilization rates, but produce little or no offspring (232). The timing and spatial organization of cyclin B1 degradation is very important and is regulated by a number of different proteins. For example, based on the observation that APC expression and activity is predominantly nuclear in mouse oocytes, Holt *et al.* proposed a spatial model of G2 arrest in which nuclear APC-mediated proteasomal activity guards against any cyclin B1 accumulation mediated by nuclear import, while still maintaining extra-nuclear cyclin B necessary for subsequent progression through the cell cycle (233).

Spindle assembly checkpoint proteins (SAC): In *Xenopus*, a group of proteins called SAC proteins (spindle assembly checkpoint proteins) regulate the timing of cyclin B1 degradation by inhibiting premature APC/C activation thus preventing mis-segregation during MI. The main SAC proteins are mitotic arrest deficient (MAD) and budding inhibited by benzimidazole (BUB) (4, 218, 219, 234). While the expression and functions of these cell-cycle checkpoint proteins during somatic cell-cycle is well-studied in yeast (235), *Xenopus* (236), mouse as well as humans (237), their roles during oogenesis in mammals

remain elusive (218). In *Xenopus*, SAC proteins have been implicated to act downstream of mos-MAPK-p90rsk pathway (180, 220, 238), but in mammals the precise mechanism of how these SAC proteins are activated is unclear. Recent studies in mouse oocytes have identified the expression and involvement of SAC proteins during MI (239-241). Studies involving inhibition and knockdown of SAC proteins in mouse oocytes have shown the importance of these proteins in regulating cell-cycle progression and maintaining fidelity of chromosome segregation during MI (240, 241). The role of SAC proteins during MI of oocytes in other mammalian species is also unknown. However, in humans, low expressions of MAD and BUB have been proposed to be associated with oocyte aging and hypothesized to be a factor for aneuploidy in older women (242).

Aurora kinase A (AURKA): Another protein called Aurora Kinase A (AURKA), a centrosome-localized serine/threonine kinase, has been reported to be expressed in *Xenopus* (243), mouse (244), porcine (245) and bovine (246) oocytes. In *Xenopus* oocytes, Eg2 (the *Xenopus* homolog of AURKA) is phosphorylated soon after progesterone stimulation and is directly involved in activation of MAPK and MPF to promote GVBD. Activated Eg2 binds to CPEB and promotes polyadenylation and translation of mos mRNA (247, 248). Eg2 is activated following a biphasic pattern that mirrors that of MPF activation. In fact, dephosphorylation of Eg2 after GVBD is essential for degradation of cyclin B and progression through meiosis I: oocytes injected with a constitutively active form of AURKA remain arrested in meiosis I with high levels of activated MPF and highly condensed metaphase-like chromosomes, but lack organized microtubule spindles (249). Experiments with both over-activation (249) or inhibition of AURKA confirmed that the protein kinase is involved in the progression from MI to MII (250). In mouse oocytes (251) AURKA is expressed throughout the GV-stage but is activated only after GVBD and is localized to microtubule organizing centers. Recently it was reported that in mouse oocytes AURKA may be involved in regulation of microtubule organizing centers, resumption of meiosis, spindle microtubule dynamics and organization of the metaphase spindle (244, 251). It has also been suggested that AURKA may regulate or interact with SAC proteins (251), a hypothesis which was recently confirmed with the use of a specific AURKA inhibitor, which is able to rescue SAC arrest, as well as rescue cyclin B1 degradation induced by nocodazole (252). Unlike *Xenopus*, the activation of AURKA in mouse oocytes is PI3K-Akt and CDK1 independent (251) but the signaling pathways regulating AURKA activity is still unknown.

4.4.2. Meiosis I to metaphase II transition

Reactivation of MPF is absolutely necessary for the transition of MI-MII and this process takes place in a MAPK-independent manner in rats (95, 189). Many studies have been conducted in understanding the interplay between MPF and MAPK pathway during oocyte maturation. In rodents it has been demonstrated that MPF activates mos, the upstream protein kinase responsible for activation of the MAPK pathway (95, 189, 214, 253). In addition to directly phosphorylating and activating downstream MEK1, mos also inactivates Ser/Thr phosphatases like PP2A, thereby inhibiting the dephosphorylation of MAPK proteins (254). Studies in mouse reveal that the mos-MAPK pathway regulates the migration of the spindle to the cortex and chromatin condensation during the MI-MII transition (255). However, since in the mos-KO mouse oocytes reach the metaphase II stage, it has been suggested that the asymmetric division during MI-MII transition takes place in a mos-dependent as well as independent manner in the mouse (192-195, 255).

In farm animals, MAPK pathway is inactive at GV but gets activated around GVBD (depending on species) and its activity steadily increases, reaching its maximum at MII (7, 97, 256). In mammals, MAPK pathway is known to be involved in spindle formation (188). Phosphorylated MAPK and p90rsk are associated with microtubule assembly at different stages of oocyte maturation (188, 201). In porcine occytes, inhibition of MAPK at the time of MI-MII transition prevents chromosome condensation, first polar body extrusion and MII spindle formation (201). It is hypothesized that the actions of MAPK during the above-mentioned events take place via p90rsk (201). Moreover, studies in bovine oocytes using the Akt inhibitor SH6, have proposed a role for Akt/PKB in MI-MII transition and hypothesized that Akt may regulate protein translation during oocyte maturation (257). Further studies are needed to elucidate the physiological role of different signaling pathways and their differences among various species during MI and during MI-MII transition.

5. METAPHASE II STAGE ARREST

After completion of MI, the oocytes enter MII and become arrested until fertilization due to the activity of the cytostatic factor (CSF) (4, 218-220). The underlying mechanism of MII arrest involves the ability of the oocyte to maintain high MPF levels/activity by inhibiting APC/C activity thereby preventing the proteasomal degradation of cyclin B (220) (Figure 5). The CSF activity can occur by different pathways: 1) by increasing MPF synthesis or 2) by directly inhibiting APC/C activity via activating SAC proteins or by activating Emi, a protein that inhibits the ability of CDC20 to activate APC/C through mos-MAPK pathway or 3) by regulation of proteasomal activity that prevents the degradation of cyclin B (219). In mouse, though increase in cyclin B1 levels has been demonstrated during meiotic maturation (258), it is believed that this process is unimportant for MII arrest (259, 260). Unlike *Xenopus* (220), where the mos-MAPK-p90rsk activated SAC proteins inhibit APC/C in MII arrest (261), it is proposed that SAC proteins do not play any role in MII arrest in the mouse (262, 263). Microinjections of dominant negative BUB and MAD into mouse eggs has no effect on MII arrest but accelerates MI (263). These experiments demonstrate that SAC proteins may play a role in MI (239) but not in MII arrest.

It is proposed that a protein called Emi1 may be the likely downstream substrate for the mos-MAPK-p90rsk pathway to promote MII arrest (4, 219). However, in

mouse, the role of p90rsk-Emi1 in MII arrest is controversial. Similar to *Xenopus* (220), a study using mouse oocytes demonstrated that p90rsk, the downstream mediator of mos-MAPK pathway, directly phosphorylates Emi1 to inhibit CDC20 activation of APC/C and establishes CSF activity (264). In contrast, another study with mouse oocyte showed that p90rsk does not interact with Emi1 and is not involved in CSF activity (262). This study proposed that even though the upstream mos-MAPK pathway is similar between *Xenopus* and mouse for MII arrest, the signaling pathway diverges at the level of p90rsk in these species (262). Thus, though the mos-MAPK pathway plays an essential role in MII arrest in Xenopus and mammals (7, 218, 220), how the mos-MAPK pathway causes MII arrest in mammals is still unclear. Interestingly, injection or removal of mos from *Xenopus* (265-267) and mouse (268) eggs cause induction or inhibition of MII arrest, respectively. Furthermore, studies involving mos KO mice and mos siRNA have demonstrated that MII arrest is impaired in these eggs (192, 193, 269). In pig and bovine oocytes, similar experiments with MEK inhibitor and mos-siRNA techniques have also established the essential role of mos-MAPK pathway in MII arrest in these species (204, 205, 270). However, understanding of how the mos-MAPK pathway mediates the MII arrest in mammals remains inadequate and is controversial.

Two additional proteins, MISS (MAPK-interacting and spindle stabilizing protein) and DOC1R are substrates for the MAPK pathway and have been demonstrated to be involved in spindle stability during the MII arrest (271, 272). MISS is stable only in MII-arrested oocytes while expression of DOCR1 occurs at the time of meiotic maturation and depletion of either of these two proteins result in spindle defects. Interestingly, a similar signaling pathway involving mos, MAPK and Emi2, is also present in male gametes where it plays a role in late spermatogenesis (273).

Prolonged arrest at MII stage results in deterioration of oocyte quality, a phenomenon termed “oocyte aging” that can significantly impact fertility (274). In farm animals, aged oocytes have a higher tendency for spontaneous activation (275), decreased ability to fertilize and develop (276), high rate of fragmentation and low MPF activity (275). The aged oocyes have a high level of inactive pre-MPF that is proposed to be caused by an imbalance between kinase and phosphatase activities (277). Studies with porcine oocyte using caffeine and vanadate to artificially induce MPF activity by manipulating Wee 1/Myt 1 and cdc25, have demonstrated that a decrease in active MPF levels may be one of the major causes of oocyte aging (274). In humans too, degradation of cell cycle checkpoint proteins has been proposed to be a possible cause of oocyte aging (242). The exact mechanism involved in oocyte aging in mammals is yet to be elucidated and awaits further investigation.

6. CALCIUM (Ca^{2+}) -INDUCED COMPLETION OF MEIOSIS

Fertilization triggers an increase in intracellular Ca^{2+} levels resulting in release of the oocyte from MII arrest and progression through meiosis II (219). The completion of meiosis is marked by the extrusion of the second polar body (4, 219). The underlying mechanism of sperm-induced Ca^{2+} signaling is poorly understood, but recent evidence suggests that following sperm-oocyte fusion, a soluble sperm factor is introduced into the oocyte that activates the phosphoinositide (PI) pathway resulting in Ca^{2+} efflux and egg activation (278, 279). There are contradicting reports regarding the identity of the sperm factor and the most likely candidates are Phospholipase C (PLC) zeta or a truncated form of c-kit receptor that activates the PLC pathway via fyn (a src-family kinase) (280-284). Phospholipase C catalyses the hydrolysis of phosphatidyl inositol 4,5-bisphosphate (PIP2) into inositol 3-phosphate (IP3) and diacyl glycerol (DAG). DAG is localized to the membrane and is involved in PKC activation while IP3 is released in the cytosol where it binds to an IP3 receptor (IP3R) located on the membrane of the endoplasmic reticulum (ER). The IP3R is a tetrameric ligand gated Ca^{2+} -channel and binding of IP3 to IP3R results in Ca^{2+} efflux (285). There are three isoforms of IP3R expressed in mammalian eggs and ovarian cells with IP3R1 being the most abundant isoform in mammalian oocytes (286-289). During oocyte maturation, the oocyte acquires enhanced IP3 sensitivity by increased IP3R1 expression and spatial redistribution of IP3R1 from perinuclear region during the GV stage to the cortex at MII arrest. This acquisition of activation-competence by the oocyte enables higher Ca^{2+} release from the ER during fertilization (290-292). Inhibition of IP3R1 by either antibody or competitive IP3R antagonist (heparin) or injection of non-hydrolyzable IP3 analogue (adenophostin) in eggs significantly impairs Ca^{2+} release during fertilization in mammals, suggesting the importance of IP3R1 in fertilization (293-295). Lack or absence of IP3R significantly compromises oocyte competence. In addition to IP3R, another Ca^{2+} release channel receptor, called ryanodine receptor (RyR) has been proposed to be involved in Ca^{2+} release following fertilization in mammals (296-298). The exact role of this receptor in oocytes is yet to be established; however, its involvement in Ca^{2+} release during fertilization has been recently addressed in the frog *Bufo arenarum*. This study demonstrated that caffeine, a well known specific RyR agonist, was able to trigger oocyte activation in a dose-dependent manner, while ruthenium red, a specific RyR blocker, was able to inhibit oocyte activation induced either by sperm or caffeine (298).

In mammals, the Ca^{2+} signal is oscillatory and lasts for several hours after fertilization (4, 219). This prolonged oscillatory Ca^{2+} signal is necessary for ablation of cyclin B1 levels as the oocyte has the ability to continually synthesize new cyclin B1 (260). In aged oocytes, that lack the ability to synthesize cyclin B1, a single Ca^{2+} increase can be an effective parthenogenetic stimulus (4). The downstream mediators of this Ca^{2+} signal is not yet fully identified but studies have shown that Ca^{2+} activates CaMKII (299, 300) and PKC (301, 302). Inhibition of both of these proteins significantly impairs egg activation (303, 304). Studies in mouse egg have reported that PKC may play a role in maintaining the oscillatory Ca^{2+} signal while CaMKII is involved in resumption of meiosis (305, 306). A recent study has shown that the γ3 isoform of CaMKII is expressed in mouse oocytes and is necessary and sufficient to transduce

the oscillatory Ca^{2+} signal into cell cycle resumption (307). The substrates of CaMKII in mammals are still debatable, but studies in mouse have shown that CaMKII may activate APC/C thereby resulting in cyclin B1 destruction (219, 263) and releas of the oocyte from MII arrest. PKCs, which are activated by concomitant action of Ca^{2+} and DAG, may also play a pivotal role during fertilization through two different mechanisms: first, they contribute to inhibition of polyspermy by promoting actin de-polymerization and cortical degranulation. Secondly, they allow the PKC action to diffuse and act on specific intracellular components of the oocyte through the cleavage and release of the non-membrane-bound carboxy-terminal catalytic subunit of PKC (named PKM), (308). Further studies are required to address the issues of how and where Ca^{2+} acts in mammalian oocytes during fertilization.

7. CONCLUSIONS

In summary, oocyte maturation is a complex process involving several "stop and go" steps which are tightly regulated through out the reproductive cycle. Oocyte maturation has been extensively studied in several model organisms as diverse as starfish, frogs, cattle and mouse; however our understanding of the various signaling pathways, their cross talk and regulation, still remains elusive and needs further investigation. While species-specific differences exist, some crucial components of the signaling pathways involved in oocyte maturation are conserved from amphibians to mammals. The central paradigm of meiotic maturation in vertebrate oocytes is the fine-tuning of the spatial and temporal regulation of intracellular cAMP to control the activation of the MPF. Although the role of MPF is ubiquitous, different species-specific pathways contribute to its inhibition and/or activation, involving both intra-oocyte pathways and paracrine signaling between the oocyte and the surrounding granulosa and cumulus cells. Human oocyte contain the same cell cycle regulatory proteins as mouse and other species (309), and the few studies published so far suggest that maturation of human oocytes might be regulated at least in part by mechanisms similar to those of rodent oocytes (310-312). Consequently, a further understanding of how oocyte maturation is regulated in mouse and other mammalian species could be crucial in understanding the pathophysiology of disease states characterized by reduced fertility, as well as aiding in the technical development of *in vitro* maturation (IVM) of human oocyte.

8. REFERENCES

1.J. J. Eppig, M. Viveiros, C. Bivens and R. De La Fuente: Regulation of Mammalian Oocyte Maturation. . *Chapter 7. The Ovary. Second Edition. Editors: Peter C.K. Leung & Eli Y. Adashi.*, 113-129 (2004)

2.N. Dekel: Molecular control of meiosis. *Trends Endocrinol Metab*, 6, 165-169 (1995)

3.L. M. Mehlmann: Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation. *Reproduction*, 130(6), 791-799 (2005)

4.K. T. Jones: Turning it on and off: M-phase promoting factor during meiotic maturation and fertilization. *Mol. Hum. Reprod.*, 10(1), 1-5 (2004)

5.D. Levran, J. Farhi, H. Nahum, M. Glezerman and A. Weissman: Maturation arrest of human oocytes as a cause of infertility: Case report. *Hum. Reprod.*, 17(6), 1604-1609 (2002)

6.M. Mrazek and J. Fulka, Jr.: Failure of oocyte maturation: Possible mechanisms for oocyte maturation arrest. *Hum. Reprod.*, 18(11), 2249-2252 (2003)

7.Q.-Y. Sun and T. Nakai: Molecular Mechanisms Underlying Pig Oocyte Maturation and Fertilization. *Journal of Reproduction and Development*, 49, 347-359 (2003)

8.G. Marteil, L. Richard-Parpaillon and J. Z. Kubiak: Role of oocyte quality in meiotic maturation and embryonic development. *Reprod Biol*, 9(3), 203-24 (2009)

9.A. McLaren: Primordial germ cells in the mouse. *Developmental Biology*, 262(1), 1-15 (2003)

10.J. Bowles, D. Knight, C. Smith, D. Wilhelm, J. Richman, S. Mamiya, K. Yashiro, K. Chawengsaksophak, M. J. Wilson, J. Rossant, H. Hamada and P. Koopman: Retinoid signaling determines germ cell fate in mice. *Science*, 312(5773), 596-600 (2006)

11.J. Koubova, D. B. Menke, Q. Zhou, B. Capel, M. D. Griswold and D. C. Page: Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proc Natl Acad Sci USA*, 103(8), 2474-9 (2006)

12.J. Motlik and J. Fulka: Factors affecting meiotic competence in pig oocytes. *Theriogenology*, 25, 87-96 (1986)

13.J. Motlik, N. Crozet and J. Fulka: Meiotic competence *in vitro* of pig oocytes isolated from early antral follicles. *J Reprod Fertil*, 72, 323-8 (1984)

14.Y. Hirao, Y. Tsuji, M. Miyano, A. Okano, M. Miyake and S. Kato: Association between p34cdc2 levels and meiotic arrest during early growth. *Zygote*, 3, 325-32 (1995)

15.T. Fair, P. Hyttel and T. Greve: Bovine oocyte diameter in relation to maturational competence and transcriptional activity. *Mol Reprod Dev*, 42, 437-42 (1995)

16.L. Christmann, T. Jung and R. Moor: MPF components and meiotic competence in growing pig oocytes. *Mol Reprod Dev*, 38, 85-90 (1994)

17.M. Kanatsu-Shinohara, R. M. Scultz and G. S. Kopf: Acquisition of meiotic competence in mouse oocytes: Absolute amounts of p34cdc2,cyclin B1 and wee1 in

meiotically incompetent and competent oocytes. *Biol Reprod*, 63, 1610-1616 (2000)

18.L. Gall, S. Ruffini, D. Le Bourhis and C. Boulesteix: Cdc25C expression in meiotically competent and incompetent goat oocytes. *Mol Reprod Dev*, 62(1), 4-12 (2002)

19.G. Pincus and E. V. Enzmann: The comparative behavior of mammalian eggs *in vivo* and *in vitro*: I. The activation of ovarian eggs. *J. Exp. Med*, 62, 665-675 (1935)

20.R. Edwards: Maturation *in vitro* of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature*, 208, 349-351 (1965)

21.R. Sorensen and P. Wassarman: Relationship between growth and meiotic maturation of the mouse oocyte. . *Dev Biol.*, 50, 531-536 (1976)

22.G. F. Erickson and R. Sorensen: *In vitro* maturation of mouse oocytes isolated from late, middle, and pre-antral graafian follicles. *Journal of Experimental Zoology*, 190(1), 123-127 (1974)

23.J. J. Eppig: Growth and development of mammalian oocytes *in vitro*. *Arch Pathol Lab Med*, 116(4), 379-82 (1992)

24.J. J. Eppig, M. O'Brien and K. Wigglesworth: Mammalian oocyte growth and development *in vitro*. *Mol Reprod Dev*, 44(2), 260-73 (1996)

25.T. Miyano: Bring up small oocytes to eggs in pigs and cows. *Theriogenology*(59), 61-72 (2003)

26.G. Arvind, M. Jamnongjit and S. R. Hammes: Androgens promote maturation and signaling in mouse oocytes Independent of transcription: A release of inhibition model for mammalian oocyte meiosis. *Mol Endocrinol*, 18, 97-104 (2004)

27.J. Fulka, N. First and R. Moor: Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. *Mol. Hum. Reprod.*, 4, 41-49 (1998)

28.R. Marchal, C. Vigneron, C. Perreau, A. Bali-Papp and P. Mermillod: Effect of follicular sizeon meiotic and developmental competenceof porcine oocytes. *Theriogenology*, 57, 1523-1532 (2002)

29.R. M. Schultz: Oogenesis and control of meiotic maturation. Cambridge University Press, New York (1986)

30.K. Uchikura, M. Nagano and M. Hishinuma: Evaluation of Follicular Development and Oocyte Quality in Pre-pubertal Cats. *Reproduction in domestic animals = Zuchthygiene* (2010)

31.M. S. Neal, L. Cowan, J. P. Louis, E. Hughes, W. A. King and P. K. Basrur: Cytogenetic evaluation of human oocytes that failed to complete meitic maturation *in vitro*. *Fertil. Steril*, 77, 844-845 (2002)

32.M. Bergere, R. Lomborso, M. Gombault, R. Wauner and J. Selva: An idiopathic infertility with oocytes metaphase I maturation block. *Hum. Reprod.*, 16, 2136-2138 (2001)

33.D. Levran, J. Farhi, H. Nahun, M. Glerezman and A. Weissman: Maturation arrest of human oocytes as a cause of infertility. *Hum. Reprod.*, 17, 1604-1609 (2002)

34.M. Conti, C. Andersen, F. Richards, C. Mehats, S.-Y. Chun, K. Horner, C. Jin and A. Tsafriri: Role of cyclic nucleotide signaling in oocyte maturation. Mol Cell Endo, 187, 153-159 (2002)

35.J. Tornell, H. Billing and T. Hillensjo: Resumptionof rat oocyte meiosis is paralleled by decrease in guanosine 3',5'-cyclic monophosphate and is inhibited by microinjection of cGMP. Acta Physiol. Scand(139), 511-517 (1990)

36.R. M. Schultz, R. R. Montgomery and J. R. Belanoff: Regulation of mouse meiotic maturation: implication of a decrease in oocyte cAMP and protein dephosphorylation in commitment to resume meiosis. Dev Biol., 97, 264-273 (1983)

37.W. K. Cho, S. Stern and J. D. Biggers: Inhibitory effect of dibutyryl cAMP on mouse oocyte maturation in vitro. J.Exp. Zool, 187, 383-386 (1974)

38.J. J. Eppig, P. F. Ward-Bailey and D. L. Coleman: Hypoxanthine and adenosine in murine ovarian follicular fluid: concentrations and activity in maintaining oocyte meiotic arrest. Biol Reprod, 33, 1041-1049 (1985)

39.H. Aktas, M. B. Wheeler, C. F. Rosenkrans, N. First and M. L. Leibfried-Rutledge: Maintenance of bovine oocytes in prophase of meiosis-1 by high [cAMP](i). *J. Reprod. Fertil*, 105, 227-235 (1995)

40.N. Dekel, T. S. Lawrnce, N. B. Gilula and W. H. Beers: Modulation of cell-to-cell communication in the cummulus-oocyte complex and the regulation of oocyte maturation by LH. *Dev Biol.*, 86, 356-362 (1981)

41.E. Vivarelli, M. Conti, M. De Felici and G. Siracusa: Meiotic resumption and intracellular cAMP levels in mouse oocytes treated with compounds which act on cAMP metabolism. *Cell Differentiation*, 12, 271-276 (1983)

42.J. J. Eppig: Maintenance of meiotic arrest and the induction of oocyte maturation in mouse oocyte-granulosa cell complexes developed *in vitro* from preantral follicles. *Biol Reprod*, 45, 824-830 (1991)

43.A. Tsafriri and N. Dekel: Molecular mechanisms in ovulation. Academic Press, San diego (1994)

44.I. Edry, S. Sela-Abramovich and N. Dekel: Meiotic arrest of oocytes depends on cell-to-cell communication in the ovarian follicle. *Mol Cell Endo*, 252, 102-106 (2006)

45.S. Vaccari, K. Horner, L. M. Mehlmann and M. Conti: Generation of mouse oocytes defective in cAMP synthesis and degradation: Endogenous cyclic AMP is essential for meiotic arrest. *Developmental Biology*, 316(1), 124 (2008)

46.M. Hinckley, S. Vaccari, K. Horner, R. Chen and M. Conti: The G-protein-coupled receptors GPR3 and GPR12 are involved in cAMP signaling and maintenance of meiotic arrest in rodent oocytes. *Dev Biol.*, 287, 249-261 (2005)

47.K. Horner, G. Livera, M. Hinckley, K. Trinh, D. Storm and M. Conti: Rodent oocytes express an active adenylyl cyclase required for meiotic arrest. *Dev Biol.*(258), 385-396 (2003)

48.L. M. Mehlmann, T. L. Z. Jones and L. A. Jaffe: Meiotic arrest in the mouse follicle maintained by a Gs protein in the oocyte. *Science*, 297, 1343-1345 (2002)

49.L. Freudzon, R. P. Norris, A. R. Hand, S. Tanaka, Y. Daeki, T. L. Z. Jones, M. M. Rasenick, C. H. Berlot, L. M. Mehlmann and L. A. Jaffe: Regulation of meiotic prophase arrest in mouse oocytes by GPR3, a constitutive activator of the Gs G protein. *J Cell Bio*, 171, 255-265 (2005)

50.L. M. Mehlmann, Y. Saeki, S. Tanaka, T. J. Brennan, A. V. Evsikov, F. L. Pendola, B. B. Knowles, J. J. Eppig and L. A. Jaffe: The Gs-Linked Receptor GPR3 Maintains Meiotic Arrest in Mammalian Oocytes. *Science*, 306(5703), 1947-1950 (2004)

51.D. Eggerickx, J. F. Denef, O. Labbe, Y. Hayashi, S. Refetoff, G. Vassart, M. Parmentier and F. Libert: Molecular cloning of an orphan G-protein-coupled receptor that constitutively activates adenlate cyclase. *Biochemical Journal*, 309, 837-843 (1995)

52.K. Unlenbrock, H. Gassenhuber and E. Kostenis: Sphingosine 1-phosphate is a ligand of the human gpr3,gpr6 and gpr12 family constitutively activate G protein coupled receptors. *Cellular Signalling*, 14, 941-953 (2002)

53.L. M. Mehlmann: Oocyte-specific expression of Gpr3 is required for the maintenance of meiotic arrest in mouse oocytes. *Dev Biol.*, 288, 397-404 (2005)

54.R. R. Kalinowski, C. H. Berlot, T. L. Jones, L. F. Ross, L. A. Jaffe and L. M. Mehlmann: Maintenance of meiotic prophase arrest in vertebrate oocytes by a Gs protein-mediated pathway. *Dev Biol*, 267, 1-13 (2004)

55.C. Lendent, I. Demeestere, D. Blum, J. Petermans, T. Hamalainen, G. Smits and G. Vassart: Premature ovarian aging in mice deficient for Gpr3. *Proc Natl Acad Sci USA*, 102, 8922-8926 (2005)

56.J. Deng, S. Lang, C. Wylie and S. R. Hammes: The Xenopus laevis Isoform of G Protein-Coupled Receptor 3 (GPR3) Is a Constitutively Active Cell Surface Receptor that Participates in Maintaining Meiotic Arrest in X. laevis Oocytes. *Mol Endocrinol*, 22(8), 1853-1865 (2008)

57.D. Ríos-Cardona, R. R. Ricardo-González, A. Chawla and J. E. Ferrell: A role for GPRx, a novel GPR3/6/12-related G-protein coupled receptor, in the maintenance of meiotic arrest in Xenopus laevis oocytes. *Developmental Biology*, 317(1), 380-8 (2008)

58.A. DiLuigi, V. N. Weitzman, M. C. Pace, L. J. Siano, D. Maier and L. M. Mehlmann: Meiotic arrest in human oocytes is maintained by a Gs signaling pathway. *Biol Reprod*, 78(4), 667-72 (2008)

59.M. Lastro, S. Collins and W. B. Currie: Adenylyl cyclases in oocyte maturation: A characterization of AC isoforms in bovine cumulus cells. *Molecular Reproduction and Development*, 73, 1202-1210 (2006)

60.E. A. Bornslaeger and R. M. Schultz: Regulation of mouse oocyte maturation: effect of elevating cumulus cell cAMP on oocyte cAMP levels. *Biol Reprod*, 33, 698-704 (1985)

61.E. Kovanci, J. L. Simpson, P. Amato, J. Rohozinski, M. J. Heard, C. E. Bishop and S. A. Carson: Oocyte-specific G-protein-coupled receptor 3 (GPR3): no perturbations found in 82 women with premature ovarian failure (first report). *Fertil Steril*, 90(4), 1269-71 (2008)

62.S. Zhou, B. Wang, F. Ni, J. Wang, Y. Cao and X. Ma: GPR3 may not be a potential candidate gene for premature ovarian failure. *Reprod Biomed Online*, 20(1), 53-5 (2010)

63.Y. Kalma, I. Granot, D. Galiani, A. Barash and N. Dekel: Luteinizing hormone-induced connexin 43 down-regulation: inhibition of translation. *Endocrinology*, 145, 1617-1624 (2004)

64.S. Sela-Abramovich, E. Chorev, D. Galiani and N. Dekel: Mitogen activated protein kinase mediates luteinizing hormone-induced break-down of communication and oocyte maturation in rat ovarian follicles. *Endocrinology*, 146, 1236-1244 (2005)

65.S. Sela-Abramovich, I. Edry, D. Galiani, N. Nevo and N. Dekel: Disruption of gap junctional communication within the ovarian follicle induces oocyte maturation. *Endocrinology*, 147, 2280-2286 (2006)

66.A. Sanfins, C. E. Plancha, E. W. Overstrom and D. F. Albertini: Meiotic spindle morphogenesis in *in vivo* and *in vitro* matured mouse oocytes: insights into the relationship between nuclear and cytoplasmic quality. *Hum. Reprod.*, 19(12), 2889-2899 (2004)

67.T. Choi, F. Aoki, M. Mori, M. Yamashita, Y. Nagahama and K. Kohmoto: Activation of p34cdc2 protein kinase activity in meiotic and mitotic cell cycles in mouse oocytes and embryos. *Development*, 113, 789-795 (1991)

68.M. Kubelka, M. J., R. M. Schultz and A. Pavlok: Butyrolactone I reversibly inhibits meiotic maturation of bovine oocytes, without influencing chromosome condensation activity. *Biol Reprod*, 62, 292-302 (2000)

69.P. Mermillod, M. Tomanek, R. Marchal and L. Meijer: High developmental competence of cattle oocytes maintained at the germinal vesicle stage for 24 hr in culture by specific inhibition of MPF kinase activity. *Mol Reprod Dev*, 55, 89-95 (2000)

70.N. I. Ponderato, I. Lagutina, G. Crotti, P. Trurini, C. Galli and G. Lazzari: Bovine oocytes treated prior to *in vitro* maturation with a combination of butyrolactone I and roscovitine at low doses maintain a normal devlopmental capacity. *Mol Reprod Dev*, 60, 579-585 (2001)

71.C. Vigneron, C. Perreau, R. Dalbies-Tran, C. Joly, P. Humblot, S. Uzbekova and P. Mermillod: Protein synthesis and mRNA storage in cattle oocytes maintained under meiotic block by roscovitine inhibition of MFP activity. *Mol Reprod Dev*, 69, 457-465 (2004)

72.G. M. Wu, Q.-Y. Sun, J. D. Mao, L. Lai, T. C. McCauley, K. W. Park, R. S. Prather, B. A. Didion and B. N. Day: High developmental competence of pig oocytes after meiotic inhibition with a specific M-phase promoting factor kinase inhibitor, butyrolactone. *Biol Reprod*, 67, 170-177 (2002)

73.B. Meinecke and C. Krischek: MAPK/ERK kinase (MEK) signaling is required for resumption of meiosis in cultured cumulus-enclosed pig oocytes. *Zygote*, 11, 7-16 (2003)

74.D.-X. Zhang, W.-J. Park, S.-C. Sun, Y.-N. Xu, Y.-H. Li, X.-S. Cui and N.-H. Kim: Regulation of Maternal Gene Expression by MEK/MAPK and MPF Signaling in Porcine Oocytes During *In vitro* Meiotic Maturation. *The Journal of reproduction and development*, 57(1), 49-56 (2011)

75.J. M. J. F. N. C. J. Fulka Jr: Inhibition of nuclear maturation in fully grown porcine and mouse oocytes after their fusion with growing porcine oocytes. *Journal of Experimental Zoology*, 235(2), 255-259 (1985)

76.M. Mattioli, G. Galeati, M. L. Bacci and B. Barboni: Changes in maturation-promoting activity in the cytoplasm of pig oocytes throughout maturation. *Mol Reprod Dev*, 30, 119-125 (1991)

77.R. Prochazka, J. Motlik and J. Fulka: Activity of maturation promoting factor in pig oocytes after microinjection and serial transfer of maturing cytoplasm. *Cell Differ Dev*, 27, 175-181 (1989)

78.S. J. Han, R. Chen, M. P. Paronetto and M. Conti: Wee1B is an oocyte-specific kinase involved in the control of meiotic arrest in the mouse. *Current Biology*, 15, 1670-1676 (2005)

79.S. J. Han and M. Conti: New pathways from PKA to the cdc2/cyclin B complex in oocytes. *Cell Cycle*, 5(3), 227-231 (2006)

80.I. Nilsson and I. Hoffmann: Cell cycle regulation by the cdc25 phosphatase family. *Prog Cell Cycle Res*, 4, 107-114 (2000)

81.S. Wu and D. J. Wolgemuth: The distinct and developmentally regulated patterns of expression of members of the mouse cdc25 geme family suggests differential functions during gametogenesis. *Dev Biol*, 170, 195-206 (1995)

82.A. J. Lincoln, D. Wickramasinghe, P. Stein, R. M. Schultz, M. E. Palko, M. P. De Miguel, L. Tessarollo and P. J. Donovan: Dcd25b phosphatase is required for resumption of meiosis during oocyte maturation. *Nat Genet*, 30, 446-449 (2002)

83.M. S. Chen, J. Hurov, L. S. White, T. Woodford-Thomas and H. Piwnica-Worms: Absence of apparent phenotype in mice lacking cdc25C protein phosphatase. *Mol Cell Endo*, 21, 3853-3861 (2001)

84.J. Mitra and R. M. Schultz: Regulation of the acquisition of meiotic competence in the mouse: changes in the subcellular localization of cdc2, cyclin B1, cdc25C and wee1, and in the concentration of these proteins and their transcripts. *J Cell Sci*, 109(9), 2407-2415 (1996)

85.C. de Vantery, A. Stutz, J. D. Vassalli and S. Schorderet-Slatkine: Acquisition of meiotic competence in growing mouse oocytes is controlled at both translational and post-translational levels. *Dev Biol*, 187, 43-54 (1997)

86.L. B. Josefberg, D. Galiani, S. Lazar, O. Kaufman, R. Seger and N. Dekel: Maturation-promoting factor governs mitogen-activated protein kinase activation and interphase suppression during meiosis of rat oocytes. *Biol Reprod*, 68, 1282-1290 (2003)

87.M. Kovo, M. Kandil-Cohen, M. Ben-Haim, D. Galiani and D. W. Carr: An active protein kinase A (PKA) is involved on meiotic arrest of rat growing oocytes. *Reproduction*, 132, 33-43 (2006)

88.W. I. Leise and P. Mueller: Multiple Cdk 1 inhibitory kinases regulate the cell cycle during development. *Dev Biol*, 249, 156-173 (2002)

89.B. C. Duckworth, J. S. Weaver and J. V. Ruderman: G2 arrest in Xenopus oocytes depends on phosphorylation of cdc25 by protein kinase A. *Proc Natl Acad Sci USA*, 99, 16794-16799 (2002)

90.G. S. Toranzo, F. Bonilla, M. C. G. Bühler and M. I. Bühler: Participation of MAPK, PKA and PP2A in the regulation of MPF activity in Bufo arenarum oocytes. *Zygote (Cambridge, England)*, 1-9 (2010)

91.G. Pirino, M. P. Wescott and P. J. Donovan: Protein kinase A regulates resumption of meiosis by

phosphorylation of Cdc25B in mammalian oocytes. *Cell Cycle*, 8(4), 665-70 (2009)

92.K. J. Newhall, A. R. Criniti, C. S. Cheah, K. C. Smith, K. E. Kafer, A. D. Burkart and G. S. McKnight: Dynamic anchoring of PKA is essential during oocyte maturation. *Current Biology*, 16(3), 321-327 (2006)

93.M. Kovo, R. V. Schillace, D. Galiani, L. B. Josefberg, D. W. Carr and N. Dekel: Developmental regulation of cAMP-dependent protein kinase A (PKA) and A-kinase anchoring proteins (AKAPs) during meiosis in rat oocytes. *Mol Cell Endo*, 192, 105-113 (2002)

94.R. L. Brown, T. Ord, S. B. Moss and C. J. Williams: A-kinase anchoring proteins as potential regulators of protein kinase A function in oocytes. *Biol Reprod*, 67, 981-987 (2002)

95.N. Dekel: Cellular, biochemical and molecular mechanisms regulating oocyte maturation. *Mol Cell Endo*, 234, 19-25 (2005)

96.J. S. Oh, S. J. Han and M. Conti: Wee1B, Myt1, and Cdc25 function in distinct compartments of the mouse oocyte to control meiotic resumption. *J Cell Biol*, 188(2), 199-207 (2010)

97.J. Ye, P. Flint, M. Luck and K. Campbell: Independent activation of MAP kinase and MPF during the initiation of meiotic maturation in pig oocytes. *Reproduction*, 125, 645-656 (2003)

98.E.-K. Suh, A. Yang, A. Kettenbach, C. Bamberger, A. H. Michaelis, Z. Zhu, J. A. Elvin, R. T. Bronson, C. P. Crum and F. McKeon: p63 protects the female germ line during meiotic arrest. *Nature*, 444(7119), 624 (2006)

99.P. Solc, R. M. Schultz and J. Motlik: Prophase I arrest and progression to metaphase I in mouse oocytes: comparison of resumption of meiosis and recovery from G2-arrest in somatic cells. *Mol Hum Reprod*, 16(9), 654-64 (2010)

100.L. M. Mehlmann, R. R. Kalinowski, L. F. Ross, A. F. Parlow, E. L. Hewlett and L. A. Jaffe: Meiotic resumption in response to luteinizing hormone is independent of a Gi family G protein or calcium in the mouse oocyte. *Developmental Biology*, 299(2), 345 (2006)

101.M. Conti, M. Hsieh, J.-Y. Park and Y. Q. Su: Role of epidermal growth factor network in ovarian follicles. *Mol Endocrinol*, 20(4), 715-723 (2006)

102.J.-Y. Park, Y. Q. Su, M. Ariga, E. Law, C. Jin and M. Conti: EGF-like growth factors as mediators of LH action in the ovulatory folicle. *Science*, 303, 682-684 (2004)

103.A. Tsafriri, X. Cao, H. Ashkenazi, S. Motola, M. Popliker and S. H. Pomerantz: Resumption of meiosis in mammals: On models, meiosis activating sterols, steroids and EGF-like factors. *Mol Cell Endo*, 234, 37-45 (2005)

104.H. Ashkenazi, X. Cao, S. Motola, M. Popliker, M. Conti and A. Tsafriri: Epidermal Growth Factor Family Members: Endogenous Mediators of the Ovulatory Response. *Endocrinology*, 146(1), 77-84 (2005)

105.S. Freimann, I. Ben-Ami, A. Dantes, R. Ron-El and A. Amsterdam: EGF-like factor epiregulin and amphirgulin expression is regulated by gonadotropins/cAMP in human ovarian follicular cells. *Biochem Biophys Res Commun*, 324, 829-834 (2004)

106.A. M. Zamah, M. Hsieh, J. Chen, J. L. Vigne, M. P. Rosen, M. I. Cedars and M. Conti: Human oocyte maturation is dependent on LH-stimulated accumulation of the epidermal growth factor-like growth factor, amphiregulin. *Hum Reprod*, 25(10), 2569-78 (2010)

107.K. N. Fru, M. Cherian-Shaw, M. Puttabyatappa, C. A. VandeVoort and C. L. Chaffin: Regulation of granulosa cell proliferation and EGF-like ligands during the periovulatory interval in monkeys. *Hum. Reprod.*, del519 (2007)

108.C. Robert, D. Gagne, D. Bousquet, F. L. Barnes and M. A. Sirard: Differential display and suppressive subtractive hybridization used to identify granulosa cell mRNA associated with bovine oocyte develpmental competence. *Biol Reprod*, 64, 1812-1820 (2001)

109.Z. Ellederova, P. Halada, P. Man, M. Kubelka, J. Motlik and H. Kovarova: Protein Patterns of Pig Oocytes During *In vitro* Maturation. *Biol Reprod*, 71(5), 1533-1539 (2004)

110.R. Prochazka, P. Kalab and E. Nagyova: Epidermal Growth Factor-Receptor Tyrosine Kinase Activity Regulates Expansion of Porcine Oocyte-Cumulus Cell Complexes *In vitro*. *Biol Reprod*, 68(3), 797-803 (2003)

111.M. Hsieh, D. Lee, S. Panigone, K. Horner, R. Chen, A. Theologis, D. C. Lee, D. W. Threadgill and M. Conti: Luteinizing hormone-dependent activation of the epidermal growth factor network id essential for ovulation. *Molecular and Cellular Biology*, 27(5), 1914-1924 (2005)

112.M. Conti, M. Hsieh, J.-Y. Park and Y.-Q. Su: Role of the Epidermal Growth Factor Network in Ovarian Follicles. *Mol Endocrinol*, 20(4), 715-723 (2006)

113.N. Reeka, F. D. Berg and C. Brucket: Presence of transforming growth factor alpha and epidermal growth factor in human ovarian tissue and follicular fluid. *Hum. Reprod.*, 13, 2199-2205 (1998)

114.M. Ozörnek, P. Bielfeld, J. Krüssel, J. Hirchenhain, R. Jeyendran and U. Koldovsky: Epidermal growth factor and leukemia inhibitory factor levels in follicular fluid. Association with *in vitro* fertilization outcome. *J Reprod Med*, 44, 367-369 (1999)

115.J. Hsu, S. D. Holmes and J. M. Hammond: Ovarian epidermal growth factor-like activity. Concentrations in porcine follicular fluid during follicular enlargement. *Biochem Biophys Res Commun*, 147, 242-247 (1987)

116.J. Smitz, R. Cortvrindt and Y. Hu: Epidermal growth factor combined with recombinant human chorionic gonadotrophin improves meiotic progression in mouse follicle-enclosed oocyte culture. *Hum. Reprod.*, 13, 664-669 (1998)

117.L. Gall, N. Chene, M. Dahirek, S. Ruffini and C. Boulesteix: Expression of epidermal growth factor receptor in the goat cumulus-oocyte complex. *Mol Reprod Dev*, 67(439-445) (2004)

118.K. Das, L. Stout, H. Hensleigh, G. Tagatz, W. Phipps and B. Leung: Direct positive effect of epidermal growth factor on the cytoplasmic maturation of mouse and human oocytes. *Fertil. Steril*, 55, 1000-1004 (1991)

119.C. P. J. D. S. U. C. P. P. M. Céline Vigneron: Several signaling pathways are involved in the control of cattle oocyte maturation. *Molecular Reproduction and Development*, 69(4), 466-474 (2004)

120.Y.-Q. Su, K. Sugiura, Q. Li, K. Wigglesworth, M. M. Matzuk and J. J. Eppig: Mouse oocytes enable LH-induced maturation of the cumulus-oocyte complex via promoting EGF receptor-dependent signaling. *Molecular endocrinology (Baltimore, Md)*, 24(6), 1230-9 (2010)

121.M. V. A. Saraiva, R. Rossetto, I. Brito, J. Celestino, C. Silva, L. Faustino, A. Almeida, J. Bruno, D. Magalhes, M. H. Matos, C. Campello and J. Figueiredo: Dynamic Medium Produces Caprine Embryo From Preantral Follicles Grown *In vitro*. *Reproductive sciences (Thousand Oaks, Calif)*, 17(12), 1135-43 (2010)

122.H. J. Song, E. J. Kang, G. H. Maeng, S. A. Ock, S. L. Lee, J. G. Yoo, B. G. Jeon and G. J. Rho: Influence of epidermal growth factor supplementation during *in vitro* maturation on nuclear status and gene expression of canine oocytes. *Research in veterinary science*, 91(3), 439-45 (2010)

123.O. Y. Tkachenko, S. Delimitreva, E. Isachenko, R. R. Valle, H. W. Michelmann, A. Berenson and P. L. Nayudu: Epidermal growth factor effects on marmoset monkey (Callithrix jacchus) oocyte *in vitro* maturation, IVF and embryo development are altered by gonadotrophin concentration during oocyte maturation. *Hum Reprod*, 25(8), 2047-58 (2010)

124.S. J. Uhm, M. K. Gupta, J. H. Yang, H.-J. Chung, T. S. Min and H. T. Lee: Epidermal growth factor can be used in lieu of follicle-stimulating hormone for nuclear maturation of porcine oocytes *in vitro*. *Theriogenology*, 73(8), 1024-36 (2010)

125.Y. Nagahama and M. Yamashita: Regulation of oocyte maturation in fish. *Development, growth & differentiation*, 50 Suppl 1, S195-219 (2008)

126.S. R. Hammes: Steroids and Oocyte Maturation--A New Look at an Old Story. *Mol Endocrinol*, 18(4), 769-775 (2004)

127.M. Rasar and S. Hammes: The physiology of the Xenopus laevis ovary. *Methods Mol Biol.*, 322, 17-30 (2006)

128.Y. Zhu, C. D. Rice, Y. Pang, M. Pace and P. Thomas: Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. *Proc Natl Acad Sci USA*, 100(5), 2231-6 (2003)

129.A. Gill, M. Jamnongjit and S. R. Hammes: Androgens Promote Maturation and Signaling in Mouse Oocytes Independent of Transcription: A Release of Inhibition Model for Mammalian Oocyte Meiosis. *Mol Endocrinol*, 18(1), 97-104 (2004)

130.M. Jamnongjit, A. Gill and S. R. Hammes: Epidermal growth factor receptor signaling is required for normal ovarian steroidogenesis and oocyte maturation. *Proc Natl Acad Sci USA*, 102(45), 16257-16262 (2005)

131.M. Jamnongjit, A. Gill and S. Hammes: Epidermal growth factor receptor signaling is required for normal ovarian steroidogenesis and oocyte maturation. *Proc Natl Acad Sci USA*, 102(45), 16257-16262 (2005)

132.S. Yeh, M.-Y. Tsai, Q. Xu, X.-M. Mu, H. Lardy, K.-E. Huang, H. Lin, S.-D. Yeh, S. Altuwaijri, X. Zhou, L. Xing, B. F. Boyce, M.-C. Hung, S. Zhang, L. Gan and C. Chang: Generation and characterization of androgen receptor knockout (ARKO) mice: An *in vivo* model for the study of androgen functions in selective tissues. *Proc Natl Acad Sci USA*, 99(21), 13498-13503 (2002)

133.A. Sen and S. R. Hammes: Granulosa cell-specific androgen receptors are critical regulators of ovarian development and function. *Molecular endocrinology (Baltimore, Md)*, 24(7), 1393-403 (2010)

134.M. Li, J.-S. Ai, B.-Z. Xu, B. Xiong, S. Yin, S.-L. Lin, Y. Hou, D.-Y. Chen, H. Schatten and Q.-Y. Sun: Testosterone Potentially Triggers Meiotic Resumption by Activation of Intra-Oocyte SRC and MAPK in Porcine Oocytes. *Biol Reprod*, biolreprod.108.069245 (2008)

135.M. Shimada and T. Terada: FSH and LH induce progesterone production and progesterone receptor synthesis in cumulus cells: a requirement for meiotic resumption in porcine oocytes. *Mol. Hum. Reprod.*, 8(7), 612-618 (2002)

136.Y. Yamashita, M. Shimada, T. Okazaki, T. Maeda and T. Terada: Production of Progesterone from De Novo-Synthesized Cholesterol in Cumulus Cells and Its Physiological Role During Meiotic Resumption of Porcine Oocytes. *Biol Reprod*, 68(4), 1193-1198 (2003)

137.S. M. Borman, C. L. Chaffin, K. M. Schwinof, R. L. Stouffer and M. B. Zelinski-Wooten: Progesterone Promotes Oocyte Maturation, but Not Ovulation, in Nonhuman Primate Follicles Without a Gonadotropin Surge. *Biol Reprod*, 71(1), 366-373 (2004)

138.K. Evaul and S. R. Hammes: Cross-talk between G protein-coupled and epidermal growth factor receptors regulates gonadotropin-mediated steroidogenesis in Leydig cells. *The Journal of biological chemistry*, 283(41), 27525-33 (2008)

139.L. B. Lutz, L. M. Cole, M. K. Gupta, K. W. Kwist, R. J. Auchus and S. R. Hammes: Evidence that androgens are the primary steroids produced by Xenopus laevis ovaries and may signal through the classical androgen receptor to promote oocyte maturation. *Proc Natl Acad Sci USA*, 98(24), 13728-13733 (2001)

140.W.-H. Yang and S. R. Hammes: Xenopus laevis CYP17 regulates androgen biosynthesis independent of the cofactor cytochrome b5. *J Biol Chem*, 280(11), 10196-201 (2005)

141.M. E. Lieberman, A. Tsafriri, S. Bauminger, W. P. Collins, K. Ahren and H. R. Lindner: Oocytic meiosis in cultured rat follicles during inhibition of steroidogenesis. *Acta Endocrinol (Copenh)*, 83(1), 151-7 (1976)

142.A. Tsafriri, M. Popliker, R. Nahum and Y. Beyth: Effects of ketoconazole on ovulatory changes in the rat: implications on the role of a meiosis-activating sterol. *Mol Hum Reprod*, 4(5), 483-9 (1998)

143.M. L. Hibbert, R. L. Stouffer, D. P. Wolf and M. B. Zelinski-Wooten: Midcycle administration of a progesterone synthesis inhibitor prevents ovulation in primates. *Proc Natl Acad Sci U S A*, 93(5), 1897-901 (1996)

144.J. C. Osborn, R. M. Moor and I. M. Crosby: Effect of alterations in follicular steroidogenesis on the nuclear and cytoplasmic maturation of ovine oocytes. *J Embryol Exp Morphol*, 98, 187-208 (1986)

145.R. M. Moor, C. Polge and S. M. Willadsen: Effect of follicular steroids on the maturation and fertilization of mammalian oocytes. *J Embryol Exp Morphol*, 56, 319-35 (1980)

146.G. Ning, H. Ouyang, S. Wang, X. Chen, B. Xu, J. Yang, H. Zhang, M. Zhang and G. Xia: 3',5'-Cyclic Adenosine Monophosphate Response Element Binding Protein Up-Regulated Cytochrome P450 Lanosterol 14{alpha}-Demethylase Expression Involved in Follicle-Stimulating Hormone-Induced Mouse Oocyte Maturation. *Mol Endocrinol*, 22(7), 1682-1694 (2008)

147.S. M. Downs, B. Ruan and G. J. Schroepfer: Meiosis-activating sterol and the maturation of isolated mouse oocytes. *Biol Reprod*, 64(1), 80-9 (2001)

148.M. E. Lieberman, A. Tsafriri, S. Bauminger, W. P. Collins, K. Ahrén and H. R. Lindner: Oocytic meiosis in cultured rat follicles during inhibition of steroidogenesis. *Acta Endocrinol*, 83(1), 151-7 (1976)

149.Z. Lu, G. Xia, A. G. Byskov and C. Y. Andersen: Effects of amphotericin B and ketoconazole on mouse oocyte maturation: implications on the role of meiosis-activating sterol. *Mol Cell Endocrinol*, 164(1-2), 191-6 (2000)

150.R. P. Norris, M. Freudzon, L. M. Mehlmann, A. E. Cowan, A. M. Simon, D. L. Paul, P. D. Lampe and L. A. Jaffe: Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43 gap junctions in mouse ovarian follicles: one of two paths to meiotic resumption. *Development*, 135(19), 3229-38 (2008)

151.M. Sasseville', M.-C. Gagnon, C. Guillemette, R. Sullivan, R. B. Gilchrist and F. J. Richard: Regulation of gap junctions in porcine cumulus-oocyte complexes: contributions of granulosa cell contact, gonadotropins, and lipid rafts. *Mol endocrinol*, 23(5), 700-10 (2009)

152.M. Shimada, T. Maeda and T. Terada: Dynamic changes of connexin-43, gap junctional protein, in outer layers of cumulus cells are regulated by PKC and PI 3-kinase during meiotic resumption in porcine oocytes. *Biol Reprod*, 64, 1255-1263 (2001)

153.M. Shimada and T. Terada: Phosphatidylinositol 3-Kinase in Cumulus Cells and Oocytes Is Responsible for Activation of Oocyte Mitogen-Activated Protein Kinase During Meiotic Progression Beyond the Meiosis I Stage in Pigs. *Biol Reprod*, 64(4), 1106-1114 (2001)

154.M. Calder, A. Caveney, L. Smith and A. Watson: Responsiveness of bovine cumulus-oocyte-complexes (COC) to porcine and recombinant human FSH, and the effect of COC quality on gonadotropin receptor and Cx43 marker gene mRNAs during maturation *in vitro*. *Reproductive Biology and Endocrinology*, 1(1), 14 (2003)

155.A. M. Ratchford, C. R. Esguerra and K. H. Moley: Decreased oocyte-granulosa cell gap junction communication and connexin expression in a type 1 diabetic mouse model. *Mol endocrinol*, 22(12), 2643-54 (2008)

156.M. Nitta, K. Yogo, M. Ohashi, M. Akiyama, Y. Kunitomo, T. Ogawa, N. Ishida-Kitagawa, J. Miyoshi, E. Sato and T. Takeya: Identification and expression analysis of connexin-45 and connexin-60 as major connexins in porcine oocytes. *J Anim Sci*, 88(10), 3269-79 (2010)

157.T. Y. Li, D. Colley, K. J. Barr, S.-P. Yee and G. M. Kidder: Rescue of oogenesis in Cx37-null mutant mice by oocyte-specific replacement with Cx43. *J Cell Sci*, 120(Pt 23), 4117-25 (2007)

158.A. Gill and S. R. Hammes: G[beta][gamma] signaling reduces intracellular cAMP to promote meiotic progression in mouse oocytes. *Steroids*, 72(2), 117 (2007)

159.K. Shitsukawa, C. B. Andersen, F. J. Richard, A. K. Horner, A. Wiersma, M. van Duin and M. Conti: Cloning and Characterization of the Cyclic Guanosine Monophosphate-Inhibited Phosphodiesterase PDE3A Expressed in Mouse Oocyte. *Biol Reprod*, 65(1), 188-196 (2001)

160.A. Tsafriri, S.-Y. Chun, R. Zhang, A. J. W. Hsueh and M. Conti: Oocyte Maturation Involves Compartmentalization and Opposing Changes of cAMP Levels in Follicular Somatic and Germ Cells: Studies Using Selective Phosphodiesterase Inhibitors. *Developmental Biology*, 178(2), 393 (1996)

161.M. A. Mayes and M.-A. Sirard: Effect of Type 3 and Type 4 Phosphodiesterase Inhibitors on the Maintenance of Bovine Oocytes in Meiotic Arrest. *Biol Reprod*, 66(1), 180-184 (2002)

162.R. E. Thomas, D. T. Armstrong and R. B. Gilchrist: Differential Effects of Specific Phosphodiesterase Isoenzyme Inhibitors on Bovine Oocyte Meiotic Maturation. *Developmental Biology*, 244(2), 215 (2002)

163.É. P. L. G. F. Martin F. Laforest, ncedil and J. R. ois: Fundamental significance of specific phosphodiesterases in the control of spontaneous meiotic resumption in porcine oocytes. *Molecular Reproduction and Development*, 70(3), 361-372 (2005)

164.C. G. Grupen, M. Fung and D. T. Armstrong: Effects of milrinone and butyrolactone-I on porcine oocyte meiotic progression and developmental competence. *Reproduction, Fertility and Development*, 18(3), 309-317 (2006)

165.J. T. Jensen, K. M. Schwinof, M. B. Zelinski-Wooten, M. Conti, L. V. DePaolo and R. L. Stouffer: Phosphodiesterase 3 inhibitors selectively block the spontaneous resumption of meiosis by macaque oocytes *in vitro*. *Hum. Reprod.*, 17(8), 2079-2084 (2002)

166.D. Nogueira, C. Albano, T. Adriaenssens, R. Cortvrindt, C. Bourgain, P. Devroey and J. Smitz: Human Oocytes Reversibly Arrested in Prophase I by Phosphodiesterase Type 3 Inhibitor *In vitro*. *Biol Reprod*, 69(3), 1042-1052 (2003)

167.S. Masciarelli, K. Horner, C. Liu, S. H. Park, M. Hinckley, S. Hockman, T. Nedachi, C. Jin, M. Conti and V. Manganiello: Cyclic nucleotide phosphodiesterase 3A-deficient mice as a model of female infertility. *J. Clin. Invest.*, 114(2), 196-205 (2004)

168.S. J. Han, S. Vaccari, T. Nedachi, C. B. Andersen, K. S. Kovacina, R. A. Roth and M. Conti: Protein kinase B/Akt phosphorylation of PDE3A and its role in mammalian oocyte maturation. *EMBO J*, 25, 5716-5725 (2006)

169.M. Sasseville, N. Côté, C. Guillemette and F. Richard: New insight into the role of phosphodiesterase 3A in porcine oocyte maturation. *BMC Develpmental Biology*, 6, 47 (2006)

170.L. Vanhoutte, D. Nogueira, J. Gerris, M. Dhont and P. De Sutter: Effect of temporary nuclear arrest by phosphodiesterase 3-inhibitor on morphological and functional aspects of *in vitro* matured mouse oocytes. *Mol Reprod Dev*, 75(6), 1021-30 (2008)

171.J. T. Jensen, M. B. Zelinski, J. E. Stanley, J. W. Fanton and R. L. Stouffer: The phosphodiesterase 3 inhibitor ORG 9935 inhibits oocyte maturation in the naturally selected dominant follicle in rhesus macaques. *Contraception*, 77(4), 303-7 (2008)

172.Y.-m. Shu, H.-t. Zeng, Z. Ren, G.-l. Zhuang, X.-Y. Liang, H.-w. Shen, S.-z. Yao, P.-q. Ke and N.-n. Wang: Effects of cilostamide and forskolin on the meiotic resumption and embryonic development of immature human oocytes. *Hum Reprod*, 23(3), 504-13 (2008)

173.M. Sasseville, N. Côté, C. Vigneault, C. Guillemette and F. J. Richard: 3'5'-cyclic adenosine monophosphate-dependent up-regulation of phosphodiesterase type 3A in porcine cumulus cells. *Endocrinology*, 148(4), 1858-67 (2007)

174.R. P. Norris, W. J. Ratzan, M. Freudzon, L. M. Mehlmann, J. Krall, M. A. Movsesian, H. Wang, H. Ke, V. O. Nikolaev and L. A. Jaffe: Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. *Development*, 136(11), 1869-78 (2009)

175.S. Vaccari, J. L. Weeks, M. Hsieh, F. S. Menniti and M. Conti: Cyclic GMP signaling is involved in the luteinizing hormone-dependent meiotic maturation of mouse oocytes. *Biol Reprod*, 81(3), 595-604 (2009)

176.M. Sasseville, N. Côté, M.-C. Gagnon and F. J. Richard: Up-regulation of 3'5'-cyclic guanosine monophosphate-specific phosphodiesterase in the porcine cumulus-oocyte complex affects steroidogenesis during *in vitro* maturation. *Endocrinology*, 149(11), 5568-76 (2008)

177.V. Sriraman, M. D. Rudd, S. M. Lohmann, S. M. Mulders and J. S. Richards: Cyclic guanosine 5'-monophosphate-dependent protein kinase II is induced by luteinizing hormone and progesterone receptor-dependent mechanisms in granulosa cells and cumulus oocyte complexes of ovulating follicles. *Mol endocrinol*, 20(2), 348-61 (2006)

178.A. Schmitt and A. R. Nebreda: Signalling pathways in oocyte meiotic maturation. *J Cell Sci*, 115(12), 2457-2459 (2002)

179.C. G. Liang, Y. Q. Su, H. Y. Fan, H. Schatten and Q. Y. Sun: Mechanisms regulating oocyte meiotic resumption: roles of mitogen-activated protein kinase. *Mol Endocrinol*, doi:10.121/me.2006-0408 (2007)

180.A. R. Nebreda and I. Ferby: Regulation of the meiotic cell cycle in oocytes. *Current Opinion in Cell Biology*, 12(6), 666 (2000)

181.M. Rasar, D. B. DeFranco and S. R. Hammes: Paxillin Regulates Steroid-triggered Meiotic Resumption in Oocytes by Enhancing an All-or-None Positive Feedback Kinase Loop. *J. Biol. Chem.*, 281(51), 39455-39464 (2006)

182.P. Mueller, T. R. Coleman, A. Kumagai and W. G. Dunphy: Myt1: a membrane-associated inhibitory kinase that phosphorylates Cdc2 on both threonine-14 and tyrosine-15. *Science*, 270, 86-90 (1995)

183.J. E. Ferrell, Jr., M. Wu, J. C. Gerhart and G. S. Martin: Cell cycle tyrosine phosphorylation of p34cdc2 and a microtubule-associated protein kinase homolog in Xenopus oocytes and eggs. *Mol. Cell. Biol.*, 11(4), 1965-1971 (1991)

184.A. R. Nebreda, J. V. Gannon and T. Hunt: Newly synthesized protein(s) must associate with p34cdc2 to activate MAP kinase and MPF during progesterone-induced maturation of Xenopus oocytes. *EMBO J*, 14, 5597-5607 (1995)

185.M. Frank-Vaillant, C. Jessus, R. Ozon, J. L. Maller and O. Haccard: Two Distinct Mechanisms Control the Accumulation of Cyclin B1 and Mos in Xenopus Oocytes in Response to Progesterone. *Mol. Biol. Cell*, 10(10), 3279-3288 (1999)

186.P. A. Culp and T. J. Musci: c-mos and cdc2 Cooperate in the Translational Activation of Fibroblast Growth Factor Receptor-1 during Xenopus Oocyte Maturation. *Mol. Biol. Cell*, 10(11), 3567-3581 (1999)

187.R. Wang, S. Y. Jung, C. F. Wu, J. Qin, R. Kobayashi, G. E. Gallick and J. Kuang: Direct roles of the signaling kinase RSK2 in Cdc25C activation during Xenopus oocyte maturation. *Proc Natl Acad Sci USA*, 107(46), 19885-90 (2010)

188.H.-Y. Fan and Q.-Y. Sun: Involvement of Mitogen-Activated Protein Kinase Cascade During Oocyte Maturation and Fertilization in Mammals. *Biol Reprod*, 70(3), 535-547 (2004)

189.L. B.-Y. Josefsberg, D. Galiani, S. Lazar, O. Kaufman, R. Seger and N. Dekel: Maturation-Promoting Factor Governs Mitogen-Activated Protein Kinase Activation and Interphase Suppression During Meiosis of Rat Oocytes. *Biol Reprod*, 68(4), 1282-1290 (2003)

190.Y.-Q. Su, S. Rubinstein, A. Luria, Y. Lax and H. Breitbart: Involvement of MEK-Mitogen-Activated Protein Kinase Pathway in Follicle-Stimulating Hormone-Induced but Not Spontaneous Meiotic Resumption of Mouse Oocytes. *Biol Reprod*, 65(2), 358-365 (2001)

191.C. Tong, H. Y. Fan, D. Y. Chen, X. F. Song, H. Schatten and Q. Y. Sun: Effects of MEK inhibitor U0126 on meiotic progression in mouse oocytes: microtuble organization, asymmetric division and metaphase II arrest.. *Cell Research*, 13(5), 375-83 (2003)

192.W. H. Colledge, M. B. L. Carlton, G. B. Udy and M. J. Evans: Disruption of c-mos causes parthenogenetic development of unfertilized mouse eggs. *Nature*, 370(6484), 65 (1994)

193.N. Hashimoto, N. Watanabe, Y. Furuta, H. Tamemoto, N. Sagata, M. Yokoyama, K. Okazaki, M. Nagayoshi, N. Takedat, Y. Ikawatll and S. Aizawai: Parthenogenetic activation of oocytes in c-mos-deficient mice. *Nature*, 370(6484), 68 (1994)

194.T. Choi, K. Fukasawa, R. Zhou, L. Tessarollo, K. Borror, J. Resau and G. F. Vande Woude: The Mos/mitogen-activated protein kinase (MAPK) pathway regulates the size and degradation of the first polar body in maturing mouse oocytes. *Proc Natl Acad Sci USA*, 93(14), 7032-7035 (1996)

195.K. Araki, K. Naito, S. Haraguchi, R. Suzuki, M. Yokoyama, M. Inoue, S. Aizawa, Y. Toyoda and E. Sato: Meiotic abnormalities of c-mos knockout mouse oocytes: activation after first meiosis or entrance into third meiotic metaphase. *Biol Reprod*, 55(6), 1315-1324 (1996)

196.T. Dedieu, L. Gall, N. Crozet, C. Sevellec and S. Ruffini: Mitogen-activated protein kinase activity during goat oocyte maturation and the acquisition of meiotic competence. *Mol Reprod Dev*, 45, 351-358 (1996)

197.J. Motlik, A. Pavlok, M. Kubelka, J. Kalous and P. Kalab: Interplay between CDC2 kinase and MAP kinase pathway during maturation of mammalian oocytes. *Theriogenology*, 49(2), 461 (1998)

198.B. Meinecke, U. Janas, E. Podhajsky and S. Meinecke-Tillmann: Histone H1 and MAP Kinase Activities in Bovine Oocytes following Protein Synthesis Inhibition. *Reproduction in Domestic Animals*, 36(3-4), 183-188 (2001)

199.G. Goudet, F. Belin, J. Bezard and N. Gerard: Maturation-promoting factor (MPF) and mitogen activated protein kinase (MAPK) expression in relation to oocyte competence for in-vitro maturation in the mare. *Mol. Hum. Reprod.*, 4(6), 563-570 (1998)

200.A. C. Gordo, C. L. He, S. Smith and R. A. Fissore: Mitogen activated protein kinase plays a significant role in metaphase II arrest, spindle morphology, and maintenance of maturation promoting factor activity in bovine oocytes. *Mol Reprod Dev*(59), 106-114 (2001)

201.J. Lee, T. Miyano and R. Moor: Localisation of phosphorylated MAP kinase during the transition from meiosis I to meiosis II in pig oocytes. *Zygote*, 8(2), 119-125 (2000)

202.J. Lee, T. Miyano and R. M. Moor: Localisation of phosphorylated MAP kinase during the transition from meiosis I to meiosis II in pig oocytes. *Zygote*, 8(2), 119-25 (2000)

203.Q.-Y. Sun, L. Lai, K.-W. Park, B. Kuhholzer, R. S. Prather and H. Schatten: Dynamic Events Are Differently Mediated by Microfilaments, Microtubules, and Mitogen-Activated Protein Kinase During Porcine Oocyte Maturation and Fertilization *In vitro*. *Biol Reprod*, 64(3), 879-889 (2001)

204.S. Ohashi, K. Naito, K. Sugiura, N. Iwamori, S. Goto, H. Naruoka and H. Tojo: Analyses of Mitogen-Activated Protein Kinase Function in the Maturation of Porcine Oocytes. *Biol Reprod*, 68(2), 604-609 (2003)

205.H.-Y. Fan, C. Tong, L. Lian, S.-W. Li, W.-X. Gao, Y. Cheng, D.-Y. Chen, H. Schatten and Q.-Y. Sun: Characterization of Ribosomal S6 Protein Kinase p90rsk During Meiotic Maturation and Fertilization in Pig Oocytes: Mitogen-Activated Protein Kinase-Associated Activation and Localization. *Biol Reprod*, 68(3), 968-977 (2003)

206.W. Tomek, F. A. M. Sterza, M. Kubelka, K. Wollenhaupt, H. Torner, M. Anger and W. Kanitz: Regulation of Translation During *In vitro* Maturation of Bovine Oocytes: The Role of MAP Kinase, eIF4E (Cap Binding Protein) Phosphorylation, and eIF4E-BP1. *Biol Reprod*, 66(5), 1274-1282 (2002)

207.M. Shimada, W.-X. Zeng and T. Terada: Inhibition of Phosphatidylinositol 3-Kinase or Mitogen-Activated Protein Kinase Kinase Leads to Suppression of p34cdc2 Kinase Activity and Meiotic Progression Beyond the Meiosis I Stage in Porcine Oocytes Surrounded with Cumulus Cells. *Biol Reprod*, 65(2), 442-448 (2001)

208.B. Meinecke and C. Krischek: MAPK/ERK kinase (MEK) signaling is required for resumption of meiosis in cultured cumulus-enclosed pig oocytes. *Zygote*, 11 7-16 (2003)

209.M. Inoue, K. Naito, T. Nakayama and E. Sato: Mitogen-activated protein kinase translocates into the germinal vesicle and induces germinal vesicle breakdown in porcine oocytes. *Biol Reprod*, 58(1), 130-136 (1998)

210.P. Kalab, J. Z. Kubiak, M. H. Verlhac, W. H. Colledge and B. Maro: Activation of p90rsk during meiotic maturation and first mitosis in mouse oocytes and eggs: MAP kinase-independent and -dependent activation. *Development*, 122(6), 1957-1964 (1996)

211.X. Tan, D. Y. Chen, Z. Yang, Y. C. Wang, M. Li, H. Schatten and Q. Y. Sun: Phosphorylation of p90rsk during meiotic maturation and parthenogenetic activation of rat oocytes: correlation with MAP kinases. *Zygote*, 9(3), 269-276 (2001)

212.M. Shimada, T. Maeda and T. Terada: Dynamic Changes of Connexin-43, Gap Junctional Protein, in Outer Layers of Cumulus Cells Are Regulated by PKC and PI 3-Kinase During Meiotic Resumption in Porcine Oocytes. *Biol Reprod*, 64(4), 1255-1263 (2001)

213.L. B. Josefberg and N. Dekel: Translational and post-translational modifications in meiosis of the mammalian oocyte. *Mol Cell Endo*, 187, 161-171 (2002)

214.S. Lazar, E. Gershon and N. Dekel: Selective degradation of cyclin B1 mRNA in rat oocytes by RNA interference (RNAi). *J Mol Endocrinol*, 33(1), 73-85 (2004)

215.A. Ali and M.-A. Sirard: Protein kinases influence bovine oocyte competence during short-term treatment with recombinant human follicle stimulating hormone. *Reproduction*, 130(3), 303-310 (2005)

216.P. Solc, A. Saskova, V. Baran, M. Kubelka, R. M. Schultz and J. Motlik: CDC25A phosphatase controls meiosis I progression in mouse oocytes. *Dev Bioiology*, 317(1), 260-269 (2008)

217.J. Kalous, P. Solc, V. Baran, M. Kubelka, R. M. Schultz and J. Motlik: PKB/AKT is involved in resumption of meiosis in mouse oocytes. *Biol. Cell*, 98(2), 111-123 (2006)

218.S. Brunet and B. Maro: Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: integrating time and space. Reproduction, 130(6), 801-811 (2005)

219.K. T. Jones: Mammalian egg activation: from Ca2+ spiking to cell cycle progression. Reproduction, 130(6), 813-823 (2005)

220.S. Madgwick and K. Jones: How eggs arrest at metaphase II: MPF stabilisation plus APC/C inhibition equals Cytostatic Factor. Cell Division, 2(1), 4 (2007)

221.P. L. B.-S. J. A. C. O. E. X. Y. X. Cindy Tian: Association of MPF, MAPK, and nuclear progression dynamics during activation of young and aged bovine oocytes. Molecular Reproduction and Development, 62(1), 132-138 (2002)

222.L. B.-Y. Josefsberg, D. Galiani, A. Dantes, A. Amsterdam and N. Dekel: The Proteasome Is Involved in the First Metaphase-to-Anaphase Transition of Meiosis in Rat Oocytes. Biol Reprod, 62(5), 1270-1277 (2000)

223.C. M. Pfleger and M. W. Kirschner: The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. Genes Dev., 14(6), 655-665 (2000)

224.A. Zur and M. Brandeis: Timing of APC/C substrate degradation is determined by fzy/fzr specificity of destruction boxes. *EMBO J*, 21(17), 4500-10 (2002)

225.A. Reis, M. Levasseur, H. Chang, D. J. Elliott and K. T. Jones: The CRY box: a second APCcdh1-dependent degron in mammalian cdc20 *EMBO Reports*, 7(10), 1040-1045 (2006)

226.A. Reis, H. Y. Chang, M. Levasseur and K. T. Jones: APCcdh1 activity in mouse oocytes prevents entry into the first meiotic division. *Nature Cell Biology*, 8(5), 539-40 (2006)

227.T. Yamamuro, K. Kano and K. Naito: Functions of FZR1 and CDC20, Activators of the Anaphase-Promoting Complex, During Meiotic Maturation of Swine Oocytes. *Biol Reprod*, biolreprod.108.070326 (2008)

228.S. L. Jaspersen, J. F. Charles and D. O. Morgan: Inhibitory phosphorylation of the APC regulator Hct1 is

controlled by the kinase Cdc28 and the phosphatase Cdc14. *Current Biology*, 9(5), 227 (1999)

229.A. D. Rudner and A. W. Murray: Phosphorylation by Cdc28 Activates the Cdc20-dependent Activity of the Anaphase-promoting Complex. *J. Cell Biol.*, 149(7), 1377-1390 (2000)

230.C. Kraft, F. Herzog, C. Gieffers, K. Mechtler, A. Hagting, J. Pines and J. M. Peters: Mitotic regulation of the human anaphase-promoting complex by phosphorylation. *EMBO J*, 22(24), 6598-609 (2003)

231.A. Reis, S. Madgwick, H. Y. Chang, I. Nabti, M. Levasseur and K. T. Jones: Prometaphase APCcdh1 activity prevents non-disjunction in mammalian oocytes. *Nature Cell Biology*, 9(10), 1192-98 (2007)

232.F. Jin, M. Hamada, L. Malureanu, K. B. Jeganathan, W. Zhou, D. E. Morbeck and J. M. van Deursen: Cdc20 is critical for meiosis I and fertility of female mice. *PLoS Genet*, 6(9) (2010)

233.J. E. Holt, J. Weaver and K. T. Jones: Spatial regulation of APCCdh1-induced cyclin B1 degradation maintains G2 arrest in mouse oocytes. *Development*, 137(8), 1297-304 (2010)

234.E. Pennisi: CELL CYCLE: Cell Division Gatekeepers Identified. *Science*, 279(5350), 477-478 (1998)

235.R. Li and A. W. Murray: Feedback control of mitosis in budding yeast. *Cell*, 66(3), 519 (1991)

236.R.-H. Chen, A. Shevchenko, M. Mann and A. W. Murray: Spindle Checkpoint Protein Xmad1 Recruits Xmad2 to Unattached Kinetochores. *J. Cell Biol.*, 143(2), 283-295 (1998)

237.D.-Y. Jin, C. A. Kozak, F. Pangilinan, F. Spencer, E. D. Green and K.-T. Jeang: Mitotic Checkpoint LocusMAD1L1Maps to Human Chromosome 7p22 and Mouse Chromosome 5. *Genomics*, 55(3), 363 (1999)

238.M. S. Schwab, B. T. Roberts, S. D. Gross, B. J. Tunquist, F. E. Taieb, A. L. Lewellyn and J. L. Maller: Bub1 is activated by the protein kinase p90Rsk during Xenopus oocyte maturation. *Current Biology*, 11(3), 141 (2001)

239.D. Zhang, M. Li, W. Ma, Y. Hou, Y.-H. Li, S.-W. Li, Q.-Y. Sun and W.-H. Wang: Localization of Mitotic Arrest Deficient 1 (MAD1) in Mouse Oocytes During the First Meiosis and Its Functions as a Spindle Checkpoint Protein. *Biol Reprod*, 72(1), 58-68 (2005)

240.H. A. Homer, A. McDougall, M. Levasseur and M. Herbert: Restaging the spindle assembly checkpoint in female mammalian meiosis I. *Cell Cycle* 4(5), 650-653 (2005)

241.H. A. Homer, A. McDougall, M. Levasseur, A. P. Murdoch and M. Herbert: Mad2 is required for inhibiting securin and cyclin B degradation following spindle depolymerisation in meiosis I mouse oocytes. *Reproduction*, 130(6), 829-843 (2005)

242.N. Steuerwald, J. Cohen, R. J. Herrera, M. Sandalinas and C. A. Brenner: Association between spindle assembly checkpoint expression and maternal age in human oocytes. *Mol. Hum. Reprod.*, 7(1), 49-55 (2001)

243.C. Roghi, R. Giet, R. Uzbekov, N. Morin, I. Chartrain, R. Le Guellec, A. Couturier, M. Dorée, M. Philippe and C. Prigent: The Xenopus protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. *J Cell Sci*, 111 (Pt 5), 557-72 (1998)

244.L.-J. Yao, Z.-S. Zhong, L.-S. Zhang, D.-Y. Chen, H. Schatten and Q.-Y. Sun: Aurora-A Is a Critical Regulator of Microtubule Assembly and Nuclear Activity in Mouse Oocytes, Fertilized Eggs, and Early Embryos. *Biol Reprod*, 70(5), 1392-1399 (2004)

245.L. J. Yao and Q. Y. Sun: Characterization of aurora-a in porcine oocytes and early embryos implies its functional roles in the regulation of meiotic maturation, fertilization and cleavage. *Zygote*, 13(1), 23-30 (2005)

246.S. Uzbekova, Y. Arlot-Bonnemains, J. Dupont, R. Dalbies-Tran, P. Papillier, S. Pennetier, A. Thelie, C. Perreau, P. Mermillod, C. Prigent and R. Uzbekov: Spatio-Temporal Expression Patterns of Aurora Kinases A, B, and C and Cytoplasmic Polyadenylation-Element-Binding Protein in Bovine Oocytes During Meiotic Maturation. *Biol Reprod*, 78(2), 218-233 (2008)

247.T. Andrésson and J. V. Ruderman: The kinase Eg2 is a component of the Xenopus oocyte progesterone-activated signaling pathway. *EMBO J*, 17(19), 5627-37 (1998)

248.R. Mendez, L. E. Hake, T. Andresson, L. E. Littlepage, J. V. Ruderman and J. D. Richter: Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. *Nature*, 404(6775), 302-7 (2000)

249.C. Ma, C. Cummings and X. J. Liu: Biphasic activation of Aurora-A kinase during the meiosis I-meiosis II transition in Xenopus oocytes. *Mol Cell Biol*, 23(5), 1703-16 (2003)

250.A. Castro, E. Mandart, T. Lorca and S. Galas: Involvement of Aurora A kinase during meiosis I-II transition in Xenopus oocytes. *J Biol Chem*, 278(4), 2236-41 (2003)

251.A. Saskova, P. Solc, V. Baran, M. Kubelka, R. M. Schultz and J. Motlik: Aurora kinase A controls meiosis I progression in mouse oocytes. *Cell Cycle*, 7(15), 2368-73 (2008)

252.S. I. R. Lane, H.-Y. Chang, P. C. Jennings and K. T. Jones: The Aurora kinase inhibitor ZM447439 accelerates first meiosis in mouse oocytes by overriding the spindle assembly checkpoint. *Reproduction*, 140(4), 521-30 (2010)

253.S. Lazar, D. Galiani and N. Dekel: cAMP-dependent PKA negatively regulates polyadenylation of c-mos mRNA in rat oocytes. *Mol Endocrinol*, 16(2), 331-341 (2002)

254.M. H. Verlhac, C. Lefebvre, J. Z. Kubiak, M. Umbhauer, P. Rassinier, W. Colledge and B. Maro: Mos activates MAP kinase in mouse oocytes through two opposite pathways. *EMBO J*, 19(22), 6065-6074 (2000)

255.M.-H. Verlhac, C. Lefebvre, P. Guillaud, P. Rassinier and B. Maro: Asymmetric division in mouse oocytes: with or without Mos. *Current Biology*, 10(20), 1303 (2000)

256.L. Liu and X. Yang: Interplay of Maturation-Promoting Factor and Mitogen-Activated Protein Kinase Inactivation during Metaphase-to-Interphase Transition of Activated Bovine Oocytes. *Biol Reprod*, 61(1), 1-7 (1999)

257.W. Tomek and T. Smiljakovic: Activation of Akt (protein kinase B) stimulates metaphase I to metaphase II transition in bovine oocytes. *Reproduction*, 130(4), 423-430 (2005)

258.J. Tay, R. Hodgman and J. D. Richter: The Control of Cyclin B1 mRNA Translation during Mouse Oocyte Maturation. *Developmental Biology*, 221(1), 1 (2000)

259.S. Madgwick, V. L. Nixon, H.-Y. Chang, M. Herbert, M. Levasseur and K. T. Jones: Maintenance of sister chromatid attachment in mouse eggs through maturation-promoting factor activity. *Developmental Biology*, 275(1), 68 (2004)

260.V. L. Nixon, M. Levasseur, A. McDougall and K. T. Jones: Ca2+ Oscillations Promote APC/C-Dependent Cyclin B1 Degradation during Metaphase Arrest and Completion of Meiosis in Fertilizing Mouse Eggs. *Current Biology*, 12(9), 746 (2002)

261.B. J. Tunquist, P. A. Eyers, L. G. Chen, A. L. Lewellyn and J. L. Maller: Spindle checkpoint proteins Mad1 and Mad2 are required for cytostatic factor-mediated metaphase arrest. *J. Cell Biol.*, 163(6), 1231-1242 (2003)

262.J. Dumont, M. Umbhauer, P. Rassinier, A. Hanauer and M.-H. Verlhac: p90Rsk is not involved in cytostatic factor arrest in mouse oocytes. *J. Cell Biol.*, 169(2), 227-231 (2005)

263.C. Tsurumi, S. Hoffmann, S. Geley, R. Graeser and Z. Polanski: The spindle assembly checkpoint is not essential for CSF arrest of mouse oocytes. *J. Cell Biol.*, 167(6), 1037-1050 (2004)

264.M. P. Paronetto, E. Giorda, R. Carsetti, P. Rossi, R. Geremia and C. Sette: Functional interaction between p90Rsk2 and Emi1 contributes to the metaphase arrest of mouse oocytes. *EMBO J*, 23, 4649-4659 (2004)

265.N. Sagata, N. Watanabe, G. F. Vande Woude and Y. Ikawa: The c-mos proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature*, 342(6249), 512 (1989)

266.O. Haccard, B. Sarcevic, A. Lewellyn, R. Hartley, L. Roy, T. Izumi, E. Erikson and J. L. Maller: Induction of metaphase arrest in cleaving Xenopus embryos by MAP kinase. *Science*, 262, 1262-1265 (1993)

267.S. Gross, M. Schwab, A. Lewellyn and J. Maller: Induction of metaphase arrest in cleaving Xenopus embryos by the protein kinase p90Rsk. *Science*, 286, 1365-1367 (1999)

268.B. J. Tunquist and J. L. Maller: Under arrest: cytostatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. *Genes Dev.*, 17(6), 683-710 (2003)

269.P. Stein, P. Svoboda and R. M. Schultz: Transgenic RNAi in mouse oocytes: a simple and fast approach to study gene function. *Dev Biol*, 256(1), 188-94 (2003)

270.B. Wu, G. Ignotz, W. B. Currie and X. Yang: Expression of Mos proto-oncoprotein in bovine oocytes during maturation *in vitro*. *Biol Reprod*, 56(1), 260-265 (1997)

271.C. Lefebvre, M. E. Terret, A. Djiane, P. Rassinier, B. Maro and M.-H. Verlhac: Meiotic spindle stability depends on MAPK-interacting and spindle-stabilizing protein (MISS), a new MAPK substrate. *J. Cell Biol.*, 157(4), 603-613 (2002)

272.M. E. Terret, C. Lefebvre, A. Djiane, P. Rassinier, J. Moreau, B. Maro and M.-H. Verlhac: DOC1R: a MAP kinase substrate that control microtubule organization of metaphase II mouse oocytes. *Development*, 130(21), 5169-5177 (2003)

273.M.-H. Perrard, E. Chassaing, G. Montillet, O. Sabido and P. Durand: Cytostatic factor proteins are present in male meiotic cells and beta-nerve growth factor increases mos levels in rat late spermatocytes. *PLoS ONE*, 4(10), e7237 (2009)

274.K. Kikuchi, K. Naito, J. Noguchi, A. Shimada, H. Kaneko, M. Yamashita, F. Aoki, H. Tojo and Y. Toyoda: Maturation/M-Phase Promoting Factor: A Regulator of Aging in Porcine Oocytes. *Biol Reprod*, 63(3), 715-722 (2000)

275.K. Kikuchi, Y. Izaike, J. Noguchi, T. Furukawa, F. P. Daen, K. Naito and Y. Toyoda: Decrease of histone H1 kinase activity in relation to parthenogenetic activation of pig follicular oocytes matured and aged *in vitro*. *J Reprod Fertil*, 105, 325-330 (1995)

276.R. C. Chian, H. Nakahara, K. Niwa and H. Funahashi: Fertilization and early cleavage *in vitro* of ageing bovine oocytes after maturation in culture. *Theriogenology*, 37, 665-672 (992)

277.K. Kikuchi, K. Naito, J. Noguchi, A. Shimada, H. Kaneko, M. Yamashita, H. Tojo and Y. Toyoda: Inactivation of p34cdc2 kinase by the accumulation of its phosphorylated forms in porcine oocytes matured and aged *in vitro*. *Zygote*, 7, 173-179 (1999)

278.M. K. R. A. F. Christopher Malcuit: Calcium oscillations and mammalian egg activation. *Journal of Cellular Physiology*, 206(3), 565-573 (2006)

279.M. Whitaker: Calcium at Fertilization and in Early Development. *Physiol. Rev.*, 86(1), 25-88 (2006)

280.C. M. Saunders, M. G. Larman, J. Parrington, L. J. Cox, J. Royse, L. M. Blayney, K. Swann and F. A. Lai: PLC{zeta}: a sperm-specific trigger of Ca2+ oscillations in eggs and embryo development. *Development*, 129(15), 3533-3544 (2002)

281.C. Sette, M. P. Paronetto, M. Barchi, A. Bevilacqua, R. Geremia and P. Rossi: Tr-kit-induced resumption of the cell cycle in mouse eggs requires activation of a Src-like kinase. *EMBO J*, 21, 5386-5395 (2002)

282.Z. Kouchi, T. Shikano, Y. Nakamura, H. Shirakawa, K. Fukami and S. Miyazaki: The Role of EF-hand Domains and C2 Domain in Regulation of Enzymatic Activity of Phospholipase C{zeta}. *J. Biol. Chem.*, 280(22), 21015-21021 (2005)

283.M. Kurokawa, K.-i. Sato, H. Wu, C. He, C. Malcuit, S. J. Black, K. Fukami and R. A. Fissore: Functional, biochemical, and chromatographic characterization of the complete [Ca2+]i oscillation-inducing activity of porcine sperm. *Developmental Biology*, 285(2), 376 (2005)

284.L. M. Mehlmann, A. Chattopadhyay, G. Carpenter and L. A. Jaffe: Evidence That Phospholipase C from the Sperm Is Not Responsible for Initiating Ca2+ Release at Fertilization in Mouse Eggs. *Developmental Biology*, 236(2), 492 (2001)

285.C. Malcuit, M. Kurokawa and R. A. Fissore: Calcium oscillations and mammalian egg activation. *Journal of Cellular Physiology*, 206(3), 565-573 (2006)

286.J. Parrington, S. Brind, H. De Smedt, R. Gangeswaran, F. Anthony Lai, R. Wojcikiewicz and J. Carroll: Expression of Inositol 1,4,5-Trisphosphate Receptors in Mouse Oocytes and Early Embryos: The Type I Isoform Is Upregulated in Oocytes and Downregulated after Fertilization. *Developmental Biology*, 203(2), 451 (1998)

287.R. A. Fissore, F. J. Longo, E. Anderson, J. B. Parys and T. Ducibella: Differential Distribution of Inositol Trisphosphate Receptor Isoforms in Mouse Oocytes. *Biol Reprod*, 60(1), 49-57 (1999)

288.C. L. He, P. Damiani, T. Ducibella, M. Takahashi, K. Tanzawa, J. B. Parys and R. A. Fissore: Isoforms of the Inositol 1,4,5-Trisphosphate Receptor Are Expressed in Bovine Oocytes and Ovaries: The Type-1 Isoform Is Down-Regulated by Fertilizationand by Injection of Adenophostin A. *Biol Reprod*, 61(4), 935-943 (1999)

289.C. Malcuit, J. G. Knott, C. He, T. Wainwright, J. B. Parys, J. M. Robl and R. A. Fissore: Fertilization and Inositol 1,4,5-Trisphosphate (IP3)-Induced Calcium Release in Type-1 Inositol 1,4,5-Trisphosphate Receptor Down-Regulated Bovine Eggs. *Biol Reprod*, 73(1), 2-13 (2005)

290.K. Shiraishi, A. Okada, H. Shirakawa, S. Nakanishi, K. Mikoshiba and S. Miyazaki: Developmental Changes in the Distribution of the Endoplasmic Reticulum and Inositol 1,4,5-Trisphosphate Receptors and the Spatial Pattern of Ca2+ Release during Maturation of Hamster Oocytes. *Developmental Biology*, 170(2), 594 (1995)

291.L. M. Mehlmann, K. Mikoshiba and D. Kline: Redistribution and Increase in Cortical Inositol 1,4,5-Trisphosphate Receptors after Meiotic Maturation of the Mouse Oocyte. *Developmental Biology*, 180(2), 489 (1996)

292.C. L. He, P. Damiani, J. B. Parys and R. A. Fissore: Calcium, calcium release receptors, and meiotic resumption in bovine oocytes. *Biol Reprod*, 57(5), 1245-1255 (1997)

293.S. Miyazaki, M. Yuzaki, K. Nakada, H. Shirakawa, S. Nakanishi, S. Nakade and K. Mikoshiba: Block of Ca2+ wave and Ca2+ oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science*, 257, 251-255 (1992)

294.S. Miyazaki, H. Shirakawa, K. Nakada and Y. Honda: Essential Role of the Inositol 1,4,5-Trisphosphate Receptor/Ca2+ Release Channel in Ca2+ Waves and Ca2+ Oscillations at Fertilization of Mammalian Eggs. *Developmental Biology*, 158(1), 62 (1993)

295.R. A. Fissore, C. Pinto-Correia and J. M. Robl: Inositol trisphosphate-induced calcium release in the generation of calcium oscillations in bovine eggs. *Biol Reprod*, 53(4), 766-774 (1995)

296.C. Yue, K. L. White, W. A. Reed and T. D. Bunch: The existence of inositol 1,4,5-trisphosphate and ryanodine receptors in mature bovine oocytes. *Development*, 121(8), 2645-2654 (1995)

297.J. Petr, D. Urbankova, M. Tomanek, J. Rozinek and F. Jilek: Activation of *in vitro* matured pig oocytes using activators of inositol triphosphate or ryanodine receptors. *Animal Reproduction Science*, 70(3-4), 235 (2002)

298.L. Wang, K. L. White, W. A. Reed and K. D. Campbell: Dynamic changes to the inositol 1,4,5-trisphosphate and ryanodine receptors during maturation of bovine oocytes. *Cloning Stem Cells*, 7(4), 306-20 (2005)

299.S. Markoulaki, S. Matson, A. L. Abbott and T. Ducibella: Oscillatory CaMKII activity in mouse egg activation. *Developmental Biology*, 258(2), 464 (2003)

300.S. Markoulaki, M. Kurokawa, S.-Y. Yoon, S. Matson, T. Ducibella and R. Fissore: Comparison of Ca2+ and CaMKII responses in IVF and ICSI in the mouse. *Mol. Hum. Reprod.*, 13(4), 265-272 (2007)

301.H.-Y. Fan, C. Tong, M.-Y. Li, L. Lian, D.-Y. Chen, H. Schatten and Q.-Y. Sun: Translocation of the Classic Protein

Kinase C Isoforms in Porcine Oocytes: Implications of Protein Kinase C Involvement in the Regulation of Nuclear Activity and Cortical Granule Exocytosis. *Experimental Cell Research*, 277(2), 183 (2002)

302.A. Luria, T. Tennenbaum, Q. Y. Sun, S. Rubinstein and H. Breitbart: Differential Localization of Conventional Protein Kinase C Isoforms During Mouse Oocyte Development. *Biol Reprod*, 62(6), 1564-1570 (2000)

303.C. Tatone, S. Delle Monache, R. Iorio, D. Caserta, M. Di Cola and R. Colonna: Possible role for Ca2+ calmodulin-dependent protein kinase II as an effector of the fertilization Ca2+ signal in mouse oocyte activation. *Mol. Hum. Reprod.*, 8(8), 750-757 (2002)

304.S. Markoulaki, S. Matson and T. Ducibella: Fertilization stimulates long-lasting oscillations of CaMKII activity in mouse eggs. *Dev Biol*, 272, 15-25 (2004)

305.G. Halet, R. Tunwell, S. J. Parkinson and J. Carroll: Conventional PKCs regulate the temporal pattern of Ca2+ oscillations at fertilization in mouse eggs. *J. Cell Biol.*, 164(7), 1033-1044 (2004)

306.S. Madgwick, M. Levasseur and K. T. Jones: Calmodulin-dependent protein kinase II, and not protein kinase C, is sufficient for triggering cell-cycle resumption in mammalian eggs. *J Cell Sci*, 118(17), 3849-3859 (2005)

307.H.-Y. Chang, K. Minahan, J. A. Merriman and K. T. Jones: Calmodulin-dependent protein kinase gamma 3 (CamKIIgamma3) mediates the cell cycle resumption of metaphase II eggs in mouse. *Development*, 136(24), 4077-81 (2009)

308.M. Kalive, J. J. Faust, B. A. Koeneman and D. G. Capco: Involvement of the PKC family in regulation of early development. *Mol Reprod Dev*, 77(2), 95-104 (2010)

309.C. M. Combelles, R. A. Fissore, D. F. Albertini and C. Racowsky: *In vitro* maturation of human oocytes and cumulus cells using a co-culture three-dimensional collagen gel system. *Hum Reprod*, 20(5), 1349-58 (2005)

310.R. G. Edwards: Maturation *in vitro* of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature*, 208(5008), 349-51 (1965)

311.M. W. Jurema and D. Nogueira: *In vitro* maturation of human oocytes for assisted reproduction. *Fertil Steril*, 86(5), 1277-91 (2006)

312.J. Tornell and T. Hillensjo: Effect of cyclic AMP on the isolated human oocyte--cumulus complex. *Hum Reprod*, 8(5), 737-9 (1993)

Abbreviations: AC: adenylyl cyclase, AR: androgen receptor, AREG: amphiregulin, COC: cumulus-oocyte complex, CSF: cytostatic factor, Cx: connexin, EGF: epidermal growth factor, EREG: epiregulin, GV: germinal vescicle, GVBD: germinal vescicle breakdown, IP3R: inositol 3-phosphate receptor, IVF: *in vitro* fertilization, IVM: *in vitro* maturation, LH: luteinizing hormone, MPF: maturation-promoting factor, PDE: phosphodiestherase, PGC: primordial germ cells, RA: retinoic acid, RyR: ryanodine receptor. CYP51: Cytochrome P450 lanosterol 14α-demethylase, AURKA: Aurora Kinase A, PTX: pertusis toxin, KO: Knockout, GPR: G protein-coupled receptors, PI: phosphoinositide, PIP2: phosphatidyl inositol 4,5-bisphosphate, IP3: inositol 3-phosphate, DAG: diacyl glycerol, POF: Premature Ovarian Failure, AKAP: A Kinase Anchoring Protein, NRG: neuregulin, BTC: beta-cellulin, GDF9: Growth Differentiation Factor 9, BMP: Bone morphogenetic protein, MMP: matrix metalloproteinase, StAR: steroidogenic acute regulatory protein, PR: Progesterone receptor, CBX: carbenoxolone, HAS-2: hyaluron synthase 2, TSG-6: tumor necrosis factors-α-induced protein 6, rsk: ribosomal protein kinase, MKP: MAP kinase phosphatase, BUB: benzimidazole, SAC: Spindle Assembly Checkpoint Proteins, MAD: mitotic arrest deficient, MISS: MAPK-interacting and spindle stabilizing protein, ER: endoplasmic reticulum, PKC: Protein kinase C, PKA: Protein kinase A

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