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—Review—

Molecular Mechanisms Underlying Pig Oocyte Maturation and Fertilization

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Abstract. Since the pig is not only an important farm animal, but also a model animal for biomedical applications, the development of reproductive technologies in this species has been very important. *In vitro* oocyte maturation and fertilization (IVM-IVF) are basic techniques for a number of oocyte- or embryo-related technologies. The practical aspects for pig oocyte IVM-IVF have been reviewed, while the molecular mechanisms underlying oocyte meiotic maturation and fertilization have not been well summarized, although accumulating data have been obtained in recent one decade. This review will focus on what is known about the molecular mechanisms of porcine oocyte maturation and fertilization such as first meiosis resumption, meiotic spindle assembly, second meiosis metaphase (MII) arrest during oocyte maturation, sperm-egg recognition and fusion, sperm acrosome reaction, second meiosis resumption, sperm chromatin decondensation, and pronucleus formation during fertilization, as well as the establishment of polyspermy block.
Key words: Pig, Oocyte, Meiosis, Fertilization, Polyspermy

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Animal reproductive technology has been very important in the last two decades, and the development of efficient procedures for assisted reproduction in livestock has generated multiple opportunities for commercialization [1]. The development of efficient reproductive biotechnology has been increasingly required in pigs, since it is not only an important farm animal, but also a model animal for biomedical applications, such as human disease processes, drug development, and human organ xenotransplantation [2]. Recently, somatic cell cloned pigs have been produced [3–5], and the production of gene knock out pig has been achieved by nuclear transfer of donor cells with the modification of the α (1,3)-galactosyltrnasferase (GalT) gene [6]. However, the efficiency is still extremely low. Although several systems have been established to generate embryos *in vitro*, the quality of embryos produced *in vitro* is inferior to those produced *in vivo*. The major problems include improper oocyte maturation, both nucleus and cytoplasmic, and polyspermy. Despite the progress, the quality of oocytes matured *in vitro*, defined as the potential of that oocyte to develop into a viable offspring, is still not satisfactory, limiting the improvement of other reproductive techniques in pigs [7]. Thus, the importance of the development and identification of defined *in vitro* conditions for oocyte maturation and fertilization should not be underestimated, since most reproductive technologies rely on these basic techniques. The technology of pig oocyte *in vitro* maturation, fertilization and embryo development and related problems have been well reviewed [8–

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10].

Looking into the basic aspects of oocyte meiotic maturation and fertilization, fully-grown follicular oocytes of most mammals are arrested at G2 phase of the first meiosis, and resume meiosis *in vivo* in response to specific signals, often hormones, or *in vitro* after being liberated from their follicular environment. During and following the first meiosis resumption, chromatin starts to condense, germinal vesicle breakdown (GVBD) is initiated, the metaphase I spindle is organized, and the first polar body is extruded. Immediately thereafter, the oocytes enter meiosis II and are then arrested again at the metaphase II (MII) stage. Upon stimulation by sperm or parthenogenetic stimuli, oocytes resume meiosis II and complete maturation, emitting the second polar body. After sperm penetration, oocyte develops a mechanism to block further penetration of surplus spermatozoa (polyspermy). Sperm chromatin and maternal chromosomes are decondensed and transformed into male and female pronuclei, respectively, and the two pronuclei get apposed to make maternal and paternal genetic materials mixed (syngamy), starting mitosis of embryogenesis.

In recent one decade, the molecular mechanisms by which mammalian oocyte meiotic maturation and fertilization are controlled have been extensively studied. On the other hand, oocyte is an ideal model to do basic researches on cell cycle regulation, since it has a long period of maturation and a big cytoplasm, and it is possible to use relatively a small number of cells to analyze the regulators. In addition, the meiosis of oocytes has been proved useful for understanding cell cycle regulation, since mitotic and meiotic cell cycle share many regulators, although there are important differences between the two processes. On the other hand, the potential applications of reproductive biotechnology stimulate active researches in this field. The pioneer work in this field has been conducted in lower animals such as *Caenorhabditis elegans* and *Xenopus laevis*. In mammals, the majority of documentation on the regulation of oocyte meiotic maturation and fertilization is from mice, while much work has been done also in pigs due to its special importance in biomedicine and agriculture, convenient availability of oocytes, and its unique characteristics of oocyte meiosis and fertilization. This review will focus on the molecular mechanisms underlying pig oocyte meiotic maturation and fertilization.

Molecular Aspects of Oocyte Meiotic Maturation

Maturation of the oocytes includes two aspects: nuclear maturation and cytoplasmic maturation. Generally, an oocyte is regarded to be matured when the first polar is extruded (nuclear maturation) and the oocyte is arrested at the MII stage. Although nuclear matured oocytes can be fertilized, they may be developmentally incompetent due to the deficiency in some cytoplasmic factors needed for full cytoplasmic maturation. Thus, the development of full developmental potential requires synchronous nuclear maturation and cytoplasmic maturation.

Nuclear maturation

1. Acquisition of meiotic competence

Growing pig oocytes (diameter ≤ 90 μm) are unable to resume meiosis *in vitro*. The inability of growing oocytes to resume meiosis is not resulted from a lack of maturation promoting factor (MPF) and/or mitogen-activated protein (MAP) kinase, two important signal molecules that control meiosis resumption. The amount of the two subunits of MPF, $p34^{cdc2}$ and cyclin B, in growing oocytes are comparable to that in fully-grown oocytes. However, growing oocytes contain phosphorylated $p34^{cdc2}$ and they are incapable of activating MPF until growth is completed [11]. Meiotically incompetent oocytes also contain the same amount of $p44^{ERK1}$and $p42^{ERK2}$, two isoforms of MAP kinase, as in fully-grown oocytes, but MAP kinase is not activated after culture *in vitro* of such oocytes [12]. Treatment of growing oocytes or meiotically incompetent oocytes with okadaic acid, an inhibitor of protein phosphatases 1 and 2A, stimulates the activation of both MPF and MAP kinases by an as yet unidentified pathway and facilitates the meiosis resumption of growing oocytes [11, 13]. Thus, acquisition of meiotic competence in growing pig oocytes correlates with their ability to activate MPF and MAP kinase [14].

2. Resumption of first meiosis

In the pig, full meiotic competence of the oocyte is reached in ovarian follicles in a diameter of 3 mm or more [15]. Meiosis resumption occurs when

oocytes are released from follicle environment and cultured *in vitro* for 20–24 h. Several protein kinases have been proved to regulate meiosis resumption.

1) MPF: It has been well established that meiosis resumption is facilitated by MPF in pig oocytes. During oocyte maturation *in vitro*, MPF activation begins at the time of GVBD, sharply rises at the MI stage, declines at anaphase-telophase I transition, and reaches a high level again at the MII stage [16]. When a GV stage oocyte is fused with an oocyte at the MI or MII stage containing a high level of MPF, GVBD is induced instantly [16]. Microinjection of heterologous (mouse) maturing cytoplasm into immature pig oocytes also results in GVBD after 8 h of culture [17]. On another hand, inhibition of MPF activation blocks GVBD [18, 19]. Translational arrest of cdc25, a protein phosphatase upstream to MPF, inhibits plasma membrane disassembly and spindle formation [20].

Activation of MPF is usually induced by tyrosine dephosphorylation of $p34^{cdc2}$ and synthesis of cyclin B. In fully-grown pig oocytes, synthesized cyclin B and $p34^{cdc2}$ are already present in an associated complex [21], however, the increase in active MPF up to the effective concentration requires active protein synthesis [17, 22]. The process of GVBD is totally blocked by cycloheximide, while the condensation of chromatin is not influenced, suggesting that GVBD and chromosome condensation could be regulated independently [23].

2) MEK/MAP kinase/$p90^{rsk}$: MAP kinase exists in an inactive form in the GV stage pig oocytes, and it is activated around the time of GVBD [12, 24–26]. Since it is difficult to judge the nuclear status before kinase assay because of the accumulation of dark lipid droplets in pig oocyte cytoplasm and since the oocyte cell cycle progresses asynchronously, it is difficult to tell the relationship between MAP kinase activation and meiosis resumption. Until recently, the requirement of MAP kinase activity for meiotic resumption is still controversial in pigs. It is found that at the G2/M transition stage, a part of the cytoplasmic MAP kinase moves to the germinal vesicle and exists in an active form before GVBD. Injection of active MAP kinase into the germinal vesicle markedly accelerates GVBD in a small part of the oocytes [27]. However, injection of antisense RNA of pig c-mos protein, an upstream kinase of MAP kinase, into pig oocytes does not affect GVBD, although MAP kinase phosphorylation (activation) is completely inhibited. These results contradict to the above finding and indicate that MAP kinase activation is not required for GVBD induction in denuded oocytes [28]. Other recent reports show that the presence of MEK inhibitor, PD98059 or U0126, in the maturation medium blocks both MAP kinase activation in both cumulus-enclosed and denuded oocytes, but prevents GVBD only in cumulus-enclosed oocytes [19, 29], suggesting that MAP kinase activation is required for meiosis resumption in cumulus-enclosed oocytes. $p90^{srk}$ has been shown to be the downstream mediator of MAP kinase function in pig oocytes [29]. MAP kinase/$p90^{rsk}$ is also activated in cumulus cells during *in vitro* culture, and their activation is blocked by U0126. It is highly possible MEK/MAP kinase/$p90^{rsk}$ activity is not required for the spontaneous resumption in denuded oocytes, but activation of this cascade in cumulus cells is indispensable for the gonadotropin-induced meiotic resumption of cumulus-enclosed pig oocytes [29].

3) PKC: Several isoforms of classical PKC (α, βI, and γ) have been revealed in pig oocytes, and they are concentrated in the germinal vesicles [30]. Although it is clear that PKC activation inhibits GVBD in denuded mouse oocytes [31], its role in meiotic resumption of cumulus-enclosed pig oocytes is not well defined. There are reports indicate that pharmacological and physiological PKC activators inhibit or delay the meiotic resumption [32, 33], while another report shows that PKC activator enhances the spontaneous maturation rate [34]. In addition, PKC inhibitors block meiotic resumption induced by FSH in a culture system containing hypoxanthine [35]. A recent report suggests that meiotic resumption of pig oocytes is associated with the PKC pathway in cumulus cells [36]. The exact role of PKC in meiotic resumption needs further clarification.

4) cAMP: The concentration of cAMP in oocytes reaches a peak at 8 h of culture, but it is dramatically decreased after 12 h culture and decreases further at 28 h [37]. Transported into oocytes from the cumulus cells via gap junctions, cAMP plays an important role in meiotic resumption [37]. Addition of dbcAMP or elevation of cAMP by treatment with forskolin or IBMX inhibits meiosis resumption in a dose-dependent

manner [33, 38, 39]. Inhibition of protein kinase A, a cAMP-dependent protein kinase, also inhibits GVBD [32]. It is possible that cAMP inhibits GVBD by down-regulation of MAP kinase activation [33].

5) Connexin-43 and PI 3-kinase: Recently, it was reported that the meiotic resumption of pig oocytes was associated with the reduction of gap junctional protein connexin (GJC)-43 in the outer layers of cumulus cells via the PKC and/or phosphatidylinositol (PI) 3-kinase pathway. PI 3-kinase activation in cumulus cells can phosphorylate connexin-43 and subsequently close GJC. PI 3-kinase activation in cumulus cells also contributes to the activation of MAP kinase and MPF in pig oocytes [36, 40].

3. Assembly of meiotic apparatus

1) MAP kinase/p90^{rsk}: Phosphorylated MAP kinase and p90^{rsk} are always associated with areas where there is microtubule assembly. After GVBD, phosphorylated MAP kinase and p90^{rsk} distribute in the area around the condensed chromosomes, in the meiotic spindle at the MI stage, in the polar regions at the early anaphase I stage, in the midzone of the elongated spindle at anaphase I to telophase I transition, and in the spindle at the MII stage [25, 29, 41]. Phosphorylated MAP kinase is localized to the area where there is extensive microtubule assembly in taxol-treated oocytes [42]. In addition to the close correlation of microtubule assembly and MAP kinase/p90^{rsk} distribution, MAP kinase and p90^{rsk} keep highly phosphorylated from the MI to MII stages, a period when microtubules are assembled in the spindle [24, 26, 29], while MPF activity decreases during anaphase I to telophase I. Inhibition of MAP kinase activation during MI-to-MII transition results in the failure of the first polar body emission and MII spindle formation [25]. Although signals of cyclin B1 are detected on spindle poles [41], no evidence shows the importance of MPF in microtubule assembly in pig oocytes. Therefore, it is concluded that activation of MAP kinase/p90^{rsk} plays important functional roles in the regulation of the microtubule organization.

2) NuMA and γ-tubulin: After GVBD, nuclear mitotic apparatus protein (NuMA) is aggregated to the vicinity of the chromosomes, and γ-tubulin is also localized to the area surrounding the chromosomes. At the MI stage, NuMA is observed at both poles of MI spindle, while γ-tubulin is localized along the spindle microtubules. NuMA and γ-tubulin become relocalized to the spindle midzone during anaphase I and telophase I. These data suggest that both NuMA and γ-tubulin are involved in microtubule assembly and that pig meiotic spindle poles are probably formed by the bundling of microtubules at the minus ends by NuMA [42, 43].

3) Polo-like kinase 1: Polo-like kinase 1 (Plk1) is accumulated in the GVs, localized to the spindle poles at the MI stage, and then translocated to the middle region of the spindle at anaphase-telophase I. Plk1 is also localized in MII spindle poles and on the spindle fibers and on the middle region of anaphase-telophase II spindles, while it accumulates as several dots in the cytoplasm after taxol treatment. These results suggest that Plk1 is a pivotal regulator of microtubule organization and cytokinesis in pig oocytes [44].

4) CENP-E: CENP-E, a kinetochore motor protein, is involved in meiotic apparatus organization. It is localized on meiotic chromosomes from diakinesis stage to anaphase I, at the spindle midzone or on the kinetochores of segregated chromosomes at telophase I. CENP-E level remains high during meiosis I/meiosis II (MI/MII) transition [45].

4. Metaphase II arrest

It has been well accepted that the high level of MPF and MAP kinase activity in MII oocytes works as a cytostatic factor (CSF) and causes the MII arrest in vertebrate oocytes, including mammals. MPF is considered to be an important component of CSF involved in MII arrest in pig oocytes. In MII-arrested oocytes, MPF activity reaches the highest level, and the enhanced ability for oocyte activation is related to decreased activity of MPF [46, 47]. In addition, inactivation of MPF and degradation of cyclin B correspond to the transition of the oocytes from MII arrest to anaphaseII/telophase II [48].

Recent evidence suggests that MAP kinase and p90^{rsk} may also play an important role in arresting the pig oocytes at the MII stage [28, 29]. When MAP kinase/p90^{rsk} is inactivated by MAP kinase kinase (MEK) inhibitor U0126, MII block is released and oocytes enter interphase [29, 49]. When c-mos antisense RNA is injected into GV stage oocytes, some are transferred into interphase from MI directly without arriving at MII, and most of the other oocytes, even those that are transferred into second meiosis, do not arrest at MII but are activated spontaneously [28]. p90^{rsk} acts

downstream to MAP kinase in pig oocytes [29], and it may mediate CSF function, as in *Xenopus oocytes* [50].

Cytoplasmic maturation

Many oocytes matured *in vitro* seem to be deficient in some, as yet unidentified, cytoplasmic factors, and therefore, developmentally incompetent. *In vitro* maturation conditions may cause incomplete movement of mitochondria to the inner cytoplasm and thus affect cytoplasmic maturation [51]. Addition of cysteine, cysteamine, glutamine, gonodotropins, epidermal growth factor (FGF), β-mercaptoethanol, cysteamine or follicular fluid to the medium or existence of cumulus cells to some extent improve cytoplasmic maturation as indicated by the high developmental competence [7, 52–58]. Intracellular glutathione (GSH) level and the ability of cytoplasm to decondense sperm nucleus or to induce male prunucleus formation have been used as indexes for the evaluation of cytoplasmic maturation, and the addition of above elements during maturation may increase GSH concentration or male pronucleus formation [53, 55, 59, 60]. Gap junctional communication (GJC) between cumulus cells and oocyte is important for regulating the cytoplasmic factors responsible for cytoplasmic maturation. If the GJC is blocked, GSH concentration is decreased [61].

Molecular Basis Underlying Sperm-Egg Recognition and Fusion

The initial stages of fertilization, species-specific gamete recognition and adhesion, are thought to involve complementary recognition molecules on sperm and eggs. Sperm associated proteins interact with zona pellucida of oocytes, resulting in acrosome reaction and subsequent sperm penetration. Although the most of related works have been conducted in mice, preliminary understanding of pig sperm-egg interaction has also been achieved.

Two dimensional gel electrophoresis of pig oocyte zona pellucida, an extracellular coat, resolves four major acidic, charge-heterogenous glycoproteins with molecular weights of 82kd (pZP1), 61kd (pZP2), 55kd (pZP3), and 21kd (pZP4) [62]. In the mouse it has been well established that ZP3 is the primary sperm receptor while ZP2 has secondary sperm receptor properties. In the pig, preincubation of boar sperm with ZP3 or O-linked glycans inhibits sperm attachment to zona pellucida, and ligand competition bioassays suggest that O-glycans mediates, at least in part, the sperm adhesive properties of ZP3 [63]. In pZP3α and pZP3β, the homologues of rabbit rec55 and mouse ZP3, respectively, O-linked oligosaccharides are confined within delaminated domains rather than widely dispersed on the polypeptide backbone [64]. However, the role of pZP3 in primary recognition is still not defined [65]. pZP1 is a homologue of mouse ZP2, and it can bind to acrosome-reacted sperm, and to 55kd proacrosin and a 44kd protein of the boar sperm. pZP1 has been shown to have sperm receptor activity similar to that observed in the rabbit and nonhuman primates [66]. It is considered acting as a secondary sperm-binding receptor on the zona pellucida [67].

Several carbohydrate-binding sperm proteins have been suggested to play a pivotal role as counter receptors for certain oligosaccharides attached to the zona pellucida. In 1987, Jones and Brown [68] separated a protein of approximately molecular weight 53kd from boar spermatozoa, and found that it has affinity for glycoproteins from zona pellucida. This protein was identified as proacrosin that plays important roles during the initial stages of sperm-egg interaction [69]. Procarosin may serve as both primary sperm receptor and maintaining the sperm on the zona pellucida once acrosome reaction has occurred [70]. Spermadhesin, including AQN-1, AQN-3, and AWN (AWN1, AWN2), is a family of carbohydrating-binding and zona pelludida-binding proteins that are involved in species-specific sperm-egg recognition and are also candidates for the primary gamete recognition [71–73]. Spermadhesin AWN1 has special affinity for certain O-linked oligosaccharide structures of pig oocyte zona pellucida [74]. Zonaadhesin, a mosaic protein in sperm membrane fractions, binds directly and in a species-specific manner to the zona pellucidae of pig oocytes. It is composed of two covalently associated peptide chains, p105 (105 kd) and p45 (45 kd) [75]. The binding sites of zonadhesin are distributed over the entire zona pellucida of oocyte [76]. Another potential sperm-egg interaction molecule is P-selectin on sperm,

which binds to the oocyte P-selectin ligand [77]. Other candidates for primary sperm-egg binding have also been suggested, such as 17 kd ACR.3 protein [78] and β-galatosyltransferase (GalTase) [79], but their functions need further clarification. A report indicates that although GalTase is able to bind to porcine zona proteins, its function in porcine sperm-zona binding is not necessary or sufficient [80].

Molecules involved in sperm-egg fusion has been extensively studied in mice and guinea pigs, and it has been well established that fertilin (previously called as PH-30) on sperm plasma membrane interacts with integrins on egg plasma membrane (PM), leading to the fusion [81]. In pigs, however, the molecular basis underlying gamete fusion is poorly understood. It was reported that pig oocyte integrin $\alpha v\beta 1$ interacts with a ligand on the sperm plasma membrane during fertilization [82]. Sperm plasma membrane proteins have been demonstrated to inhibit IVF in concentration-dependent manner [83]. Four sperm plasma membrane proteins, 62, 39, 27 and 7 kd, respectively, are proved to be the predominant binders of egg PM, and the amount of 62 kd protein was significantly correlated with the ability of sperm to penetrate zona-free hamster ova [84].

Sperm Acrosome Reaction

Acrosome reaction (AR) is an exocytotic process that makes available the enzymatic machinery needed for sperm penetration through the zona pellucida. In pigs, acrosome-intact boar sperm exhibit concentrated zona protein binding over the plasma membrane of acrosomal ridge [85]. Although there is a report that interaction between laminin, entrapped in the expanded cumuli, and specific integrins present on the sperm membrane can initiate AR [86], most evidence suggests that progesterone and zona pellucida initiate sperm acrosome reaction *in vitro* [87–90]. As to the mechanisms, it is suggested that increasing Ca^{2+} caused by entry of Ca^{2+} through sperm plasma membrane Ca^{2+} channels triggers the acrosome reaction during fertilization [91, 92]. In addition to Ca^{2+}, sperm also requires bicarbonate for acrosome reaction [93]. L-arginine induces nitric oxide synthesis and stimulates capacitation and acrosome reaction of boar spermatozoa only when active sperm anion transport is present as a result of bicarbonate supplementation [94]. A glycine receptor/Cl^- channel is also involved in the zona-initiated sperm acrosome reaction [90].

Molecular Aspects of Egg Activation

During pig *in vitro* fertilization, sperm penetration begins at 3 h post-insemination. Sperm penetration quickly induces the resumption of meiosis and cortical reaction that blocks polyspermy. By 5 h, decondensing sperm heads and anaphase II plate are observed in half the eggs, and by 8h, both female and male pronuclei have formed [95].

Resumption of the second meiosis

1. Two models

Although the mechanisms by which the spermatozoa trigger the oocyte to resume meiosis have been extensively studied, conclusions about this process is still elusive in pigs [96]. Two models, plasma membrane receptor-mediated activation model and soluble sperm factor(s) model, have been suggested to explain the resumption of the second meiosis after sperm penetration. By microinjection of guanosine-5′-O-(3′-thiotriphosphate), a hydrolysis-resistant analog of GTP, into mature eggs [97] and by microinjection of mRNA encoding a G-protein-coupled receptor into maturing oocytes [98], it was shown that G-protein-coupled signal transduction system exists in pig oocytes, and this pathway can signal a series of intracellular changes that lead to meiosis resumption. Another study shows that pertossis toxin-insensitive G-protein activation may result in Ca^{2+} transients and meiotic resumption [99]. Although G-protein-coupled receptor results in full complement of oocyte activation events, whether this pathway transduces the activating signal at sperm-induced oocyte activation requires further clarification.

There is also evidence supporting the sperm factor model. Injection of crude extract of pig sperm induces meiotic resumption, suggesting that sperm might activate oocytes by introducing a soluble factor into the oocytes [100].

2. Ca^{2+}

In the pig, sperm penetration into the egg induces a series of Ca^{2+} spikes which continue for at

least 3 h [101]. Sperm extract injection also induces an immediate rise in Ca^{2+}, followed by repetitive Ca^{2+} transients [100]. Limited knowledge is gained as to how the fertilizing sperm elicits the release of Ca^{2+} from the oocyte's intracellular stores. It is believed that calcium increase after egg activation needs extracellular influx [102]. Recently, it is demonstrated that the calcium released from IP3-sensitive stores triggers the calcium release from ryanodine-sensitive intracellular stores, which is necessary for oocyte activation [103]. Furthermore, the calmodulin inhibitors reduce the activation of oocytes induced by stimulation of ryanodine receptors or IP3 receptors [103]. A sperm-specific phospholipase C (PLC)-ζ was recently shown to trigger Ca^{2+} oscillations in mouse eggs indistinguishable from those at fertilization, and PLC-ζ from a single sperm was sufficient to produce Ca^{2+} oscillations as well as embryo development to blastocyst [104]. Ca^{2+} may interact with calmodulin to cause the activation of calmodulin-dependent protein kinase II, which inactivates maturation prompting factor (MPF) and meiosis resumption (our unpublished data).

3. pH

An intracellular pH increase at fertilization is necessary for activation of the sea urchin eggs. Recently, intracellular pH has also been shown to increase during parthenogenetic activation of pig oocytes, and such an increase does not need the increase in external and intracellular free calcium, but may be dependent upon a sodium and/or bicarbonate flux into the oocytes [102, 105, 106]. However, no pH increase is detected when eggs are fertilized by subzonal sperm injection [107]. Whether pH increase plays roles in pig activation under physiological conditions needs further clarification.

4. Protein kinase inhibition

During fertilization and parthenogenetic activation, PKC is translocated to the plasma membrane soon after sperm penetration or stimulation [30]. However, unlike in mouse oocytes, PKC activation might not be correlated with meiosis reinitiation, since PKC activators do not stimulate meiosis resumption [108]. On the contrary, protein kinase inhibition promotes release of MII block in pig eggs. Not only broad-spectrum inhibitors [109], but also selective inhibitors for myosin light chain kinase, protein kinase A or protein kinase C induce meiosis resumption and subsequent pronuclear formation [110, 111]. In the mouse, repetitive Ca^{2+} transients ensure the destruction of cyclin B, a subunit of MPF, during a protracted exit from meiosis [112]. In pigs, the histone H1 kinase activity sharply decreases upon egg activation [113] and the spontaneous parthenogenetic activation may be attributable in part to the gradual decrease in MPF activity during prolonged culture [114].

Pronucleus formation

As mentioned above, inhibition of protein phosphorylation causes pig oocyte activation and pronucleus formation. However, a direct factor that causes pronucleus formation has not been well defined. After fertilization, MAP kinase is kept highly active while the second meiosis resumes and the second polar body extrudes. MAP kinase dephosphorylation is detected when pronucleus is formed [26, 48]. On the other hand, treatment of fertilized eggs with okadaic acid, a selective protein phoaphatase inhibitor, results in prompt phosphorylation of MAP kinase and, at the same time, the breakdown of pronuclear membrane [13]. MPK kinase inactivation is also reported to induce metaphase-to-interphase transition following parthenogenetic activation [49]. It is possible that inactivation of MAP kinase may be required for pronucleus formation. Another report suggests that post-fertilization disappearance of a 25kd polypeptide and the concomitant appearance of a dominant 22 kd polypeptide have a close temporal relationship with the formation with pronuclei [95]. Conventional PKCs migrate to the pronuclei in fertilized eggs [30], but its functional importance is unclear.

Sperm chromatin decondensation

It is reported that the existence of follicle cells in maturation culture enhances male pronucleus (mPN) formation [115] and that the presence of cumulus cells at fertilization increases the rate of mPN formation when compared to cumulus-free oocytes [116]. However, the exact mechanism by which somatic cells affect mPN formation is not known. Other report indicates that increased intracellular content of GSH promotes the mPN formation frequency after fertilization [10, 59, 117]. It is highly possible that GSH mediates pig sperm head decondensation and thus increases mPN formation, as in other species.

Molecular Mechanisms of Polyspermy Prevention

Polyspermy is generally considered as a pathological phenomenon in mammals. However, exceptionally high incidence of polyspermic fertilization has been revealed in pig fertilization. Incidence of polyspermy in porcine eggs *in vivo* can reach 30–40%, and polyspermy rate in the *in vitro* fertilized eggs can be as high as 65% [118]. Polyspermy is considered being the most difficult problem to overcome in pig IVF. The reasons for the occurrence of polyspermy in pig oocytes are not clear, and relatively little is known about the exact mechanisms for preventing polyspermy in this species.

Like in other animals, pig oocytes contain cortical granules (CGs) that are enriched with galactosyl-rich glycoconjugates [119]. The CGs undergo centrifugal migration to form a monolayer next to the plasma membrane during oocyte maturation [120]. Following penetration by sperm, cortical reaction occurs and cortical contents are extruded into the perivitelline space, preventing polyspermic penetration by hardening the zona pellucia [121]. Defense mechanism against polyspermy in pig oocytes is classically viewed as zona reaction, since A23187-induced cortical reaction can prevent sperm penetration of ZP-intact oocytes, but not ZP-free oocytes [122]. However, perivitelline spermatozoa are usually observed, and there may be a secondary block at the vitelline surface [123]. *In vitro*-matured and *in vivo*-matured pig oocytes possess equal ability to release CGs upon sperm penetration [122], and high polyspermy of pig oocytes inseminated *in vitro* is not due to delayed or incomplete CG exocytosis but more likely to a delayed zona reaction and/or simultaneous sperm penetration [101]. A sperm-oviduct cell co-culture followed by IVF on oviduct cells selectively reduced the rate of polyspermy by 40% and 50% [124]. Furthermore it was suggested that oviductal secretions contain some factor(s) that interact with oocytes and/or spermatozoa to prevent or reduce multiple sperm entry [125].

As to the molecular basis of cortical reaction, preliminary studies have shown that G-protein, Ca^{2+} rise and protein kinase C (PKC) participate in this exocytotic process. Pig oocytes contain a G-protein-coupled signal transduction system that can signal a series of intracellular changes, leading to activation events associated with fertilization including cortical reaction [97, 98]. Fertilization induces Ca^{2+} oscillations in pig oocytes [101] and one of the earliest calcium-dependent events is the exocytosis of CGs. PKC stimulators induce 90% of the pig eggs completely or partially release their CG, and this effect can be overcome by PKC inhibition. When the intracellular free Ca^{2+} is chelated with acetoxymethal ester form of 1,2-bis(O-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM), and then treated with PKC activator in Ca^{2+}-free medium, the proportions of eggs with CG release are not changed, suggesting that PKC acts downstream to Ca^{2+} in CG exocytosis induction [108]. Further work shows that PKCα, -βI, and -γ are expressed in pig oocytes. Confocal microscopy and specific antibody microinjection prove that activation of α-isotype PKC induces CG exocytosis in porcine eggs [30].

In summary, pig oocytes are most extensively used for basic researches among large domestic animals in recent years because of its availability, unique characteristics and importance in biomedical research. Studies have revealed some aspects of the molecular mechanisms underlying oocyte maturation and fertilization in this species as well as several unique characteristics compared to other mammals. Especially, much is known about the regulation of oocyte meiotic resumption, spindle assembly, MII arrest, sperm-oocyte recognition and oocyte activation as well as cortical reaction at the molecular level. Further understanding of oocyte cytoplasmic maturation and the occurrence of polyspermy is required.

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References

1. **Faber DC, Molina JA, Ohlrichs CL, Vander Zwaag DF, Ferre, LB**. Commercialization of animal biotechnology. *Theriogenology* 2003; 59: 125–138.
2. **Prather RS, Hawley RJ, Carter Db, Lai L, Greenstein JL**. Transgenic swine for biomedicine and agriculture. *Theriogenology* 2003; 59: 115–123.
3. **Betthauser J, Forsberg E, Augenstein M, Childs L, Eilertsen K, Enos J, Forsythe T, Golueke P, Jurgella G, Koppang R, Lesmeister T, Mallon K, Mell G, Misica P, Pace M, Pfister-Genskow M, Strelchenko N, Voelker G, Watt S, Thompson S, Bishop M**. Production of cloned pigs from *in vitro* systems. *Nat Biotechnol* 2000; 18: 1055–1059.
4. **Onishi A, Iwamoto M, Akita T, Mikawa S, Takeda K, Awata T, Hanada H, Perry AC**. Pig cloning by microinjection of fetal fibroblast nuclei. *Science* 2000; 289: 1188–1190.
5. **Polejaeva IA, Chen SH, Vaught TD, Page RL, Mullins J, Ball S, Dai Y, Boone J, Walker S, Ayares DL, Colman A, Campbell KH**. Cloned pigs produced by nuclear transfer from adult somatic cells. *Nature* 2000; 407: 86–90.
6. **Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL, Im GS, Samuel M, Bonk A, Rieke A, Day BN, Murphy CN, Carter DB, Hawley RJ, Prather RS**. Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. *Science* 2002; 295: 1089–1092.
7. **Hunter RH**. Oviduct function in pigs, with particular reference to the pathological condition of polyspermy. *Mol Reprod Dev* 1991; 29: 385–391.
8. **Prather RS, Day BN**. Practical considerations for the *in vitro* production of pig embryos. *Theriogenology* 1998; 149: 23–32.
9. **Abeydeera LR**. *In vitro* fertilization and embryo development in pigs. *Reprod Suppl* 2001; 58: 159–173.
10. **Nagai T**. The improvement of *in vitro* maturation systems for bovine and porcine oocytes. *Theriogenology* 2001; 55: 1291–1301.
11. **Christmann L, Jung T, Moor RM**. MPF components and meiotic competence in growing pig oocytes. *Mol Reprod Dev* 1994; 38: 85–90.
12. **Sun QY, Lai L, Bonk A, Prather RS, Schatten H**. Cytoplasmic changes in relation to nuclear maturation and early embryo developmental potential of porcine oocytes: effects of gonadotropins, cumulus cells, folliclular size and protein synthesis inhibition. *Mol Reprod Dev* 2001; 59: 131–137.
13. **Sun QY, Wu GM, Lai L, Bonk A, Cabot R, Park KW, Day BN, Prather RS, Schatten H**. Regulation of mitogen-activated protein kinase phosphorylation, microtubule organization, chromatin behavior, and cell cycle progression by protein phosphatases during pig oocyte maturation and fertilization. *Biol Reprod* 2002; 66: 580–588.
14. **Kanayama N, Miyano T, Lee J**. Acquisition of meiotic competence in growing pig oocytes correlates with their ability to activate Cdc2 kinase and MAP kinase. *Zygote* 2002; 10: 261–270.
15. **Marchal R, Vigneron C, Perreau C, Bali-Papp A, Mermillod P**. Effect of follicular size on meiotic and developmental competence of porcine oocytes. *Theriogenology* 2002; 57: 1523–1532.
16. **Mattioli M, Galeati G, Bacci ML, Barboni B**. Changes in maturation-promoting activity in the cytoplasm of pig oocytes throughout maturation. *Mol Reprod Dev* 1991; 30: 119–125.
17. **Prochazka R, Motlik J, Fulka J**. Activity of maturation promoting factor in pig oocytes after microinjection and serial transfer of maturing cytoplasm. *Cell Differ Dev* 1989; 27: 175–181.
18. **Wu GM, Sun QY, Mao JD, Lai L, McCauley TC, Park KW, Prather RS, Didion BA, Day BN**. High developmental competence of pig oocytes after meiotic inhibition with a specific M-phase promoting factor kinase inhibitor, butyrolactone I. *Biol Reprod* 2002; 67: 170–177.
19. **Meinecke B, Krischek C**. MAPK/ERK kinase (MEK) signaling is required for resumption of meiosis in cultured cumulus-enclosed pig oocytes. *Zygote* 2003; 11: 7–16.
20. **Dai Y, Lee C, Hutchings A, Sun Y, Moor R**. Selective requirement for Cdc25C protein synthesis during meiotic progression in porcine oocytes. *Biol Reprod* 2000; 62: 519–532.
21. **Chen WY, Yang JG, Li PS**. Effect of dexamethasone on the expression of p34cdc2 and cyclin B1 in pig oocytes in vitro. *Mol Reprod Dev* 2000; 56: 74–79.
22. **Motlik J, Kubelka M**. Cell-cycle aspects of growth and maturation of mammalian oocytes. *Mol Reprod Dev* 1990; 27: 366–375.
23. **Kubelka M, Rimkevicova Z, Guerrier P, Motlik J**. Inhibition of protein synthesis affects histone H1 kinase, but not chromosome condensation activity, during the first meiotic division of pig oocytes. *Mol Reprod Dev* 1995; 41: 63–69.
24. **Inoue M, Naito K, Aoki F, Toyoda Y, Sato E**. Activation of mitogen-activated protein kinase during meiotic maturation in porcine oocytes. *Zygote* 1995; 3: 265–271.
25. **Lee J, Miyano T, Moor RM**. Localization of phosphorylated MAP kinase during the transition from meiosis I to meiosis II in pig oocytes. *Zygote* 2000; 8: 119–125.
26. **Sun QY, Lai L, Park KW, Kuhholzer B, Prather RS, Schatten H**. Dynamic events are differently mediated by microfilaments, microtubules, and mitogen-activated protein kinase during porcine oocyte maturation and fertilization *in vitro*. *Biol*

Reprod 2001; 64: 879–889.

27. **Inoue M, Naito K, Nakayama T, Sato E**. Mitogen-activated protein kinase translocates into the germinal vesicle and induces germinal vesicle breakdown in porcine oocytes. *Biol Reprod* 1998; 58: 130–136.
28. **Ohashi S, Naito K, Sugiiura K, Iwamori N, Goto S, Naruoka H, Tojo H**. Mitogen-activated protein kinase function in the maturation of porcine oocytes. *Biol Reprod* 2003; 68: 604–609.
29. **Fan HY, Tong C, Lian L, Li SW, Gao WX, Cheng Y, Chen DY, Schatten H, Sun QY**. 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* 2003; 68: 968–977.
30. **Fan HY, Tong C, Li MY, Lian L, Chen DY, Schatten H, Sun QY**. 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. *Exp Cell Res* 2002; 277: 183–191.
31. **Sun QY, Rubinstein S, Breitbart H**. MAP kinase activity is downregulated by phorbol ester during mouse oocyte maturation and egg fertilization. *Mol Reprod Dev* 1999; 52: 380–386.
32. **Jung T, Lee C, Moor RM**. Effects of protein kinase inhibitors on pig oocyte maturation in vitro. *Reprod Nutr Dev* 1992; 32: 461–473.
33. **Fan HY, Li MY, Tong C, Chen DY, Xia GL, Song XF, Schatten H, Sun QY**. Inhibitory effects of cAMP and protein kinase C on meiotic maturation and MAP kinase phosphorylation in porcine oocytes. *Mol Repord Dev* 2002; 63: 480–487.
34. **Coskun S, Lin YC**. Mechanism of action of epidermal growth factor-induced porcine oocyte maturation. *Mol Reprod Dev* 1995; 42: 311–317.
35. **Su YQ, Xia GL, Byskov AG, Fu GD, Yang CR**. Protein kinase C and intracellular calcium are involved in follicle-stimulating hormone-mediated meiotic resumption of cumulus cell-enclosed porcine oocytes in hypoxanthine-supplemented medium. *Mol Reprod Dev* 1999; 53: 51–58.
36. **Shimada M, Maeda T, Terada T**. 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* 2001; 64: 1255–1263.
37. **Shimada M, Terada T**. Roles of cAMP in regulation of both MAP kinase and p34cdc2 kinase activity during meiotic progression, especially beyond the MI stage. *Mol Reprod Dev* 2002; 62: 124–131.
38. **Kim NH, Menino AR Jr**. Effects of stimulators of protein kinases A and C and modulators of phosphorylation on plasminogen activator activity in porcine oocyte-cumulus cell complexes during in vitro maturation. *Mol Reprod Dev* 1995; 40: 364–370.
39. **Funahashi H, Cantley TC, Day BN**. Synchronization of meiosis in porcine oocytes by exposure to dibutyryl cyclic adenosine monophosphate improves developmental competence following in vitro fertilization. *Biol Reprod* 1997; 57: 49–53.
40. **Shimada M, Terada T**. 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* 2001; 64: 1106–1114.
41. **Goto S, Naito K, Ohashi S, Sugiura K, Naruoka H, Iwamori N, Tojo H**. Effects of spindle removal on MPF and MAP kinase activities in porcine matured oocytes. *Mol Reprod Dev* 2002; 63: 388–393.
42. **Sun QY, Lai L, Wu GM, Park KW, Day BN, Prather RS, Schatten H**. Microtubule assembly after treatment of pig oocytes with taxol: correlation with chromosomes, gamma-tubulin, and MAP kinase. *Mol Reprod Dev* 2001; 60: 481–490.
43. **Lee J, Miyano T, Moor RM**. Spindle formation and dynamics of gamma-tubulin and nuclear mitotic apparatus protein distribution during meiosis in pig and mouse oocytes. *Biol Reprod* 2000; 62: 1184–1192.
44. **Yao LJ, Fan HY, Tong C, Chen DY, Schatten H, Sun QY**. Polo-like kinase-1 in porcine oocyte meiotic maturation, fertilization and early embryonic mitosis. *Cell Mol Biol* 2003 (In press).
45. **Lee J, Miyano T, Dai Y, Wooding P, Yen TJ, Moor RM**. Specific regulation of CENP-E and kinetochores during meiosis I/meiosis II transition in pig oocytes. *Mol Reprod Dev* 2000; 56: 51–62.
46. **Kikuchi K, Izaike Y, Noguchi J, Furukawa T, Daen FP, Naito K, Toyoda Y**. Decrease of histone H1 kinase activity in relation to parthenogenetic activation of pig follicular oocytes matured and aged in vitro. *J Reprod Fertil* 1995; 105: 325–330.
47. **Kikuchi K, Naito K, Noguchi J, Kaneko H, Tojo H**. Maturation/M-phase promoting factor regulates aging of porcine oocytes matured in vitro. *Clon Stem Cell* 2002; 4: 211–222.
48. **Miyano T, Dai Y, Lee J, Kano K, Moor RM**. Degradation of pig cyclin B1 molecules preceeds MAP kinase dephosphorylation during fertilization of the oocytes. *Zygote* 2000; 8: 153–158.
49. **Tatemoto H, Muto N**. Mitogen-activated protein kinase regulates normal transition from metaphase to interphase following parthenogenetic activation in porcine oocytes. *Zygote* 2001; 9: 15–23.
50. **Gross SD, Schwab MS, Lewellyn AL, Maller JL**. Induction of metaphase arrest in cleaving Xenopus embryos by the protein kinase p90. *Science* 1999; 286: 1365–1367.
51. **Sun QY, Wu GM, Lai L, Park KW, Cabot R, Cheong HT, Day BN, Prather RS, Schatten H**. Translocation of active mitochondria during pig

oocyte maturation, fertilization and early embryo development in vitro. *Reproduction* 2001; 122: 155–163.
52. **Ding J, Foxcroft GR**. Epidermal growth factor enhances oocyte maturation in pigs. *Mol Reprod Dev* 1994; 39: 30–40.
53. **Wang WH, Niwa K**. Synergetic effects of epidermal growth factor and gonadotropins on the cytoplasmic maturation of pig oocytes in a serum-free medium. *Zygote* 1995; 3: 345–350.
54. **Singh B, Meng L, Rutledge JM, Armstrong DT**. Effects of epidermal growth factor and follicle-stimulating hormone during in vitro maturation on cytoplasmic maturation of porcine oocytes. *Mol Reprod Dev* 1997; 46: 401–407.
55. **Abeydeera LR, Wang WH, Cantley TC, Prather RS, Day BN**. Presence of beta-mercaptoethanol can increase the glutathione content of pig oocytes matured in vitro and the rate blastocyst development after in vitro fertilization. *Theriogenology* 1998; 50: 747–756.
56. **Bing YZ, Rodriguez-Martinez H, Nagai T**. Effects of cysteamine, FSH, and estradiol-17β on in vitro maturation of porcine oocytes. *Theriogenology* 2001; 55: 867–876.
57. **Jeong BS, Yang X**. Cysteine, glutathione, and Percoll treatments improve porcine oocyte maturation and fertilization in vitro. *Mol Reprod Dev* 2001; 59: 330–335.
58. **Grupen CG, Nagashima H, Nottle MB**. Cysteamine enhances in vitro development of porcine oocytes matured and fertilized in vitro. *Biol Reprod* 1995; 53: 173–178.
59. **Yoshida M, Ishigaki K, Nagai T, Chikyu M, Pursel VG**. Glutathione concentration during maturation and after fertilization in pig oocytes: relevance to the ability of oocytes to form male pronucleus. *Biol Reprod* 1993; 49: 89–94.
60. **Abeydeera LR, Wang WH, Cantley TC, Rieke A, Murphy CN, Prather RS, Day BN**. Development and viability of pig oocytes matured in a protein-free medium containing epidermal growth factor. *Theriogenology* 2000; 54: 787–797.
61. **Mori T, Amano T, Shimizu H**. Roles of gap junctional communication of cumulus cells in cytoplasmic maturation of porcine oocytes cultured in vitro. *Boil Reprod* 2000; 62: 913–919.
62. **Yurewicz EC, Sacco AG, Subrananian MG**. Pathways to immunocontraception: biochemical and immunological properties of glycoprotein antigens of the porcine zona pellucida. *Adv Exp Med Boil* 1986; 207: 407–427.
63. **Yurewicz EC, Pack BA, Sacco AG**. Isolation, composition, and biological activity of sugar chains of porcine oocyte zona pellucida 55K glycoproteins. *Mol Reprod Dev* 1991; 30: 126–134.
64. **Yurewicz EC, Pack BA, Sacco AG**. Porcine oocyte zona pellucida M(r) 55,000 glycoproteins: identification of O-glycosylated domains. *Mol Reprod Dev* 1992; 33: 182–188.
65. **Miller DJ, Burkin HR**. Gamete adhesion molecules. *Reprod Suppl* 2001; 58: 147–158.
66. **Prasad SV, Skinner SM, Carino C, Wang N, Cartwright J, Dunbar BS**. Structure and function of the proteins of the mammalian Zona pellucida. *Cells Tissues Organs* 2000; 166: 148–164.
67. **Tsubamoto H, Hasegawa A, Inoue M, Yamasaki N, Koyama K**. Binding of recombinant pig zona pellucida 1 (ZP1) to acrosome-reacted spermatozoa. *J Reprod Fertil Suppl* 1996; 50: 63–67.
68. **Jones R, Brown CR**. Identification of a zona-binding protein from boar spermatozoa as proacrosin. *Exp Cell Res* 1987; 171: 503–508.
69. **Williams RM, Jones R**. Specificity of binding of zona pellucida glycoproteins to sperm proacrosin and related proteins. *J Exp Zool* 1993; 266: 65–73.
70. **Urch UA, Patel H**. The interaction of boar sperm proacrosin with its natural substrate, the zona pellucida, and with polysulfated polysaccharides. *Development* 1991; 111: 1165–1172.
71. **Sanz L, Calvete JJ, Mann K, Schafer W, Schmid ER, Topfer-Petersen E**. The complete primary structure of the boar spermadhesin AQN-1, a carbohydraye–binding protein involved in fertilization. *Eur J Biochem* 1992; 205: 645–652.
72. **Sanz L, Calvete JJ, Mann K, Schafer W, Schmid ER, Amselgruber W, Sinowatz F, Ehrhard M, Topfer-Petersen E**. The complete primary structure of the spermadhesin AWN, a zona pellucida-binding protein isolated from boar spermatozoa. *FEBS Lett* 1992; 300: 213–218.
73. **Topfer-Petersen E, Calvete JJ**. Sperm-associated protein candidates for primary zona pellucida-binding molecules: structure-function correlations of boar spermadhesins. *J Reprod Fertil Suppl* 1996; 50: 55–61.
74. **Dostalova Z, Calvete JJ, Sanz L, Topfer-Petersen E**. Boar spermadhesin AWN-1 oligosaccharide and zona pellucida binding characteristics. *Eur J Biochem* 1995; 230: 329–336.
75. **Hardy DM, Garbers DL**. Species-specific binding of sperm proteins to the extracellular matrix (zona pellucida) of the egg. *J Biol Chem* 1994; 269: 19000–19004.
76. **Hickox JR, Bi M, Hardy DM**. Heterogenous processing and zona pellucida binding activity of pig zonadhesin. *J Biol Chem* 2001; 276: 41502–41509.
77. **Geng JG, Raub TJ, Baker CA, Sawada GA, Ma L, Elhammer AP**. Expression of a P-selectin ligand in zona pellucida of porcine oocytes and P-selectin on acrosomal membrane of porcine sperm cells. Potential implications for their involvement in sperm-egg interactions. *J Cell Biol* 1997; 137: 743–754.
78. **Capkova J, Peknicova J**. Binding of boar

spermatozoa to porcine oocytes: effect of low molecular weight 17-kDa protein. *Mol Reprod Dev* 1997; 46: 168–175.
79. **Larson JL, Miller DJ**. Sperm from a variety of mammalian species express beta 1,4-galactosyltransferase on their surface. *Biol Reprod* 1997; 57: 442–453.
80. **Rebeiz M, Miller DJ**. Porcine sperm surface beta1,4-galactosyltransferase binds to the zona pellucida but is not necessary or sufficient to mediate sperm-zona pellucida binding. *Mol Reprod Dev* 1999; 54379–54387.
81. **Evans JP**. Sperm disintegrins, egg integrins, and other cell adhesion molecules of mammalian gamete plasma membrane interactions. *Front Biosci* 1999; 4: D114–131.
82. **Linfor J, Berger T**. Potential role of alphav and beta1 integrins as oocyte adhesion molecules during fertilization in pigs. *J Reprod Fertil* 2000; 120: 65–72.
83. **Betancourt M, Ducolomb Y, Jimenez I, Casas E, Bonilla E, Berger T**. Sperm plasma membrane receptors for the porcine oocyte plasma membrane. *Zygote* 1998; 6: 155–158.
84. **Ashe K, Berger T, Horner CM, Calvert CC**. Identification of porcine sperm plasma membrane proteins that may play a role in sperm-egg fusion. *Zygote* 1995; 3: 163–170.
85. **Burkin HR, Miller DJ**. Zona pellucida protein binding ability of porcine sperm during epididymal maturation and the acrosome reaction. *Dev Biol* 1994; 222: 99–109.
86. **Mattioli M, Lucidi P, Barboni B**. Expanded cumuli induce acrosome reaction in boar sperm. *Mol Reprod Dev* 1998; 51: 445–453.
87. **Berger T, Turner KO, Meizel S, Hedrick JL**. Zona pellucida-induced acrosome reaction in boar sperm. *Biol Reprod* 1989; 40: 525–530.
88. **Melendrez CS, Meizel S, Berger T**. Comparison of the ability of progesterone and heat solubilized porcine zona pellucida to initiate the porcine sperm acrosome reaction in vitro. *Mol Reprod Dev* 1994; 39: 433–438.
89. **Barboni B, Mattioli M, Seren E**. Influence of progesterone on boar sperm capacitation. *J Endocrinol* 1995; 144: 13–18.
90. **Melendrez CS, Meizel S**. Studies of porcine and human sperm suggesting a role for a sperm glycine receptor/Cl- channel in the zona pellucida-initiated acrosome reaction. *Biol Reprod* 1995; 53: 676–683.
91. **Okamura N, Tanba M, Fukuda A, Sugita Y, Nagai T**. Forskolin stimulates porcine sperm capacitation by increasing calcium uptake. *FEBS Lett* 1993; 316: 283–286.
92. **Tiwari-Woodruff SK, Cox TC**. Boar sperm plasma membrane Ca^{2+}-selective channels in planar lipid bilayers. *Am J Physiol* 1995; 268(5 Pt 1): C1284–12994.
93. **Tardif S, Dube C, Bailey JL**. Porcine sperm capacitation and tyrosine kinase activity are dependent on bicarbonate and calcium but protein tyrosine phosphorylation is only associated with calcium. *Biol Reprod* 2003; 68: 207–213.
94. **Funahashi H**. Induction of capacitation and the acrosome reaction of boar sperm by L-arginine and nitric oxide synthesis associated with the anion transport system. *Reproduction* 2002; 124: 857–864.
95. **Ding J, Clarke N, Nagai T, Moor RM**. Protein and nuclear changes in pigs at fertilization. *Mol Reprod Dev* 1992; 31: 287–296.
96. **Prather RS**. Basic mechanisms of fertilization and parthenogenesis in pigs. *Reprod Suppl* 2001; 58: 105–112.
97. **Machaty Z, Mayes MA, Prather RS**. Parthenogenetic activation of porcine oocytes with guanosine-5′-O-(3′-thiotriphosphate). *Biol Reprod* 1995; 52: 753–758.
98. **Kim JH, Machaty Z, Cabot RA, Han YM, Do HJ, Prather RS**. Development of pig oocytes activated by stimulation of an exogenous G protein-coupled receptor. *Biol Reprod* 1998; 59: 655–660.
99. **Machaty Z, Mayes MA, Kovacs LG, Balatti PA, Kim JH, Prather RS**. Activation of porcine oocytes via an exogenously introduced rat muscarinic M1 receptor. *Biol Reprod* 1997; 57: 85–91.
100. **Machaty Z, Bonk AJ, Kuhholzer B, Prather RS**. Porcine oocyte activation induced by a cytosolic sperm factor. *Mol Reprod Dev* 2000; 57: 290–295.
101. **Sun FZ, Hoyland J, Huang X, Mason W, Moor RM**. A comparison of intracellular changes in porcine eggs after fertilization and electroactivation. *Development* 1992; 115: 947–956.
102. **Wang WH, Machaty Z, Ruddock N, Abeydeera LR, Boquest AC, Prather RS, Day BN**. Activation of porcine oocytes with calcium ionophore: effects of extracellular calcium. *Mol Reprod Dev* 1999; 53: 99–107.
103. **Petr J, Urbankova D, Tomanek M, Rozinek J, Jilek F**. Activation of in vitro matured pig oocytes using activators of inositol triphosphate or ryanodine receptors. *Anim Reprod Sci* 2002; 70: 235–249.
104. **Saunders CM, Larman MG, Parrongton J, Cox LJ, Blayney LM, Swann K, Lai FA**. PLC zeta: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development* 2002; 129: 3533–3544.
105. **Ruddock NT, Machaty Z, Prather RS**. Intracellular pH increase accompanies parthenogenetic activation of porcine, bovine and murine oocytes. *Reprod Fertil Dev* 2002; 12: 201–207.
106. **Ruddock NT, Machaty Z, Milanick M, Prather RS**. Mechanism of intracellular pH increase during parthenogenetic activation of in vitro matured porcine oocytes. *Biol Reprod* 2002; 63: 488–492.
107. **Ruddock NT, Machaty Z, Cabot RA, Prather RS**. Porcine oocyte activation: differing roles of calcium

and pH. *Mol Reprod Dev* 2001; 59: 227–234.
108. **Sun QY, Wang WH, Hosoe M, Taniguchi T, Chen DY, Shioya Y**. Activation of protein kinase C induces cortical granule exocytosis in a Ca^{2+}-independent manner, but not the resumption of cell cycle in porcine eggs. *Dev Growth Differ* 1997; 39: 523–529.
109. **Mayes MA, Stogsdill PL, Prather RS**. Parthenogenetic activation of pig oocytes by protein kinase inhibition. *Biol Reprod* 1995; 53: 270–275.
110. **Wang WH, Sun QY, Hosoe M, Shioya Y**. Calcium- and meiotic-spindle-independent activation of pig oocytes by the inhibition of staurosporine-sensitive protein kinases. *Zygote* 1996; 5: 75–82.
111. **Green KM, Kim JH, Wang WH, Day BN, Prather RS**. Effect of myosin light chain kinase, protein kinase A, and protein kinase C inhibition on porcine oocyte activation. *Biol Reprod* 1999; 61: 111–119.
112. **Nixon VL, Levasseur M, McDougall A, Jones KT**. Ca^{2+} oscillations promote APC/C-dependent cyclin B1 degradation during metaphase arrest and completion of meiosis in fertilizing mouse eggs. *Curr Biol* 2002; 12: 746–750.
113. **Motlik J, Pavlok A, Kubelka M, Kalous J, Kalab P**. Interplay between CDC2 kinase and MAP kinase pathway during maturation of mammalian oocytes. *Theriogenology* 1998; 49: 461–469.
114. **Kikuchi K, Naito K, Noguchi J, Shimada A, Kaneko H, Yamashita M, Aoki F, Tojo H, Toyoda Y**. Maturation/M-phase promoting factor: a regulator of aging in porcine oocytes. *Biol Reprod* 2000; 63: 715–722.
115. **Nagai T, Ding J, Moor RM**. Effect of follicle cells and steriodogenesis on maturation and fertilization in vitro of pig oocytes. *J Exp Zool* 1993; 266: 146–151.
116. **Ka HH, Sawai K, Wang WH, Im KS, Niwa K**. Amino acids in maturation medium and the presence of cumulus cells at fertilization promote male pronuclear formation in porcine oocytes matured and penetrated in vitro. *Biol Reprod* 1997; 57: 1478–1483.
117. **Fanahashi H, Cantley TC, Stumpf TT, Terlouw SL, Day BN**. Use of low-salt culture medium for in vitro maturation of porcine oocytes is associated with elevated oocyte glutathione levels and enhanced male pronuclear formation after in vitro fertilization. *Biol Reprod* 1994; 51: 633–639.
118. **Xia P, Wang Z, Yang Z, Tan J, Qin P**. Ultrastructural study of polyspermy during early embryo development in pigs, observed by scanning electron microscope and transmission electron microscope. *Cell Tissue Res* 2001; 303: 271–275.
119. **Yoshida M, Cran DG, Pursel VG**. Confocal and fluorescence microscopic study using lectins of the distribution of cortical granules during the maturation and fertilization of pig oocytes. *Mol Reprod Dev* 1993; 36: 462–468.
120. **Wang WH, Sun QY, Hosoe M, Shioya Y, Day BN**. Quantified analysis of cortical granule distribution and exocytosis of porcine oocytes during meiotic maturation and activation. *Biol Reprod* 1997; 56: 1376–1382.
121. **Hatanaka Y, Nagai T, Tobita T, Nakano M**. Changes in the properties and composition of zona pellucida of pigs during fertilization in vitro. *J Reprod Fertil* 1992; 95: 431–440.
122. **Wang WH, Abeydeera LR, Prather RS, Day BN**. Morphologic comparison of ovulated and in vitro-matured porcine oocytes, with particular reference to polyspermy after in vitro fertilization. *Mol Reprod Dev* 1998; 49: 308–316.
123. **Hunter MG**. Oocyte maturation and ovum quality in pigs. *Rev Reprod* 2000; 5: 122–130.
124. **Nagai T, Moor RM**. Effect of oviduct cells on the incidence of polyspermy in pig eggs fertilized in vitro. *Mol Reprod Dev* 1990; 26: 377–382.
125. **Abeydeera LR**. In vitro production of embryos in swine. *Theriogenology* 2002; 57: 256–273.