—Mini Review—

Molecular Interactions between Sperm and Oocytes in the Mouse

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Abstract: *Sperm and oocytes must undergo several steps for successful fertilization, including sperm capacitation, the acrosome reaction and completion of oocyte maturation. During these steps, it is believed that specific molecules interact with precise timing. Although an increasing body of information on the fertilization-related molecules of sperm or oocytes has been accumulated through biological and gene targeting analyses, the information on these molecular interactions remains limited. Nonetheless, the current molecular information is the basis for future advances in the understanding of the mechanisms underlying fertilization. In this review, we introduce molecules that are involved in sperm-oocyte interactions at the site of fertilization, and address the molecular events during the sperm-cumulus, sperm-zona, and sperm-oolemmal interactions. Although the information introduced in this review has been obtained primarily from mice, similar molecules are likely engaged in analogous processes in other speicies.*

Key words: *Fertilization, Sperm, Cumulus, Zona, Oolemma, Mouse*

Overview of Fertilization

Mammalian fertilization has been studied extensively over the past hundred years. The majority of studies to date have focused on the physiology of fertilization, however, the molecular mechanisms of fertilization are not well understood. Nonetheless, there have been significant recent advances in this field.

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Although the morphology of gametes, especially sperm, varies among species, the physiological features of mammalian fertilization are shared. These include the following steps: Sperm migration through the female reproductive tract; sperm passage through the cumulus oophorus; sperm penetration into the zona pellucida, sperm entry into the ooplasm; formation of pronuclei; and cleavage of the fertilized eggs. Both sperm and oocytes must be fertilization-competent before they can interact at the site of fertilization. Sperm are modified during passage through the female reproductive tract (capacitation, hyperactivation and acrosome reaction), while oocyte maturation in the ovary must be timed precisely.

The molecular mechanisms involved in each of these steps are believed to be highly sophisticated and species-specific. The numbers of candidate molecules involved in each step of fertilization are presented in Table 1, however, the information is still incomplete. In this review, we present an overview of recent findings concerning the molecules putatively involved in the sperm-oocyte interaction, during fertilization in the mouse.

Molecular Events in the Cumulus Oophorus

Sperm penetration of the cumulus oophorus

The cumulus cells surrounding the oocytes contribute significantly to the ability of oocytes to be fertilized. In particular, the structural integrity of cumulus cells (cumulus expansion) is vital. Hyaluronic acid (HA) is the major matrix component of the expanded cumulus oophorus, and sperm express hyaluronidase in order to penetrate the structure. Thus far, the only candidate for such activity, that is expressed on sperm, is Spam1

Table 1. Candidate molecules involved in sperm-oocyte interaction

Interaction sites	Candidate molecules in			Reference
	Sperm	Oocyte/cumulus	Others	
Cumulus	Spam1?	Hyaluronic acid		[1]
	Basigin	?		[7]
	?		Progesterone	[10]
	?		Prostaglandins	[11]
Zona	?	ZP1		[13]
	Proacrosin?	ZP2		[30]
	ZRK?	ZP3		[23]
	β-galactosyltransferase?	ZP3		[25]
	sp56?	ZP3		[28]
	SED-1	?		[39]
	Zonadhesin	?		[37]
	ADAM2	?		[42]
	ADAM3	?		[43]
Oolemma	SLLP1	?		[45]
	ADAMs2 and 3	Integrin $\alpha 6\beta 1$ and $\alpha 9\beta 1$?		[46, 47, 48, 49]
		?	DE	[51]
	Izumo	?		[54]
	Equatorin	?		[53]
	?	CD9		[57, 58, 59, 60]
	?	GPI anchored proteins		[55, 56]

(originally called PH-20) [1]. Spam1 is a glycerophosphoinositol (GPI)-anchored protein, comprising an N-terminal active hyaluronidase domain (responsible for HA digestion), and a C-terminal adhesion domain [2]. However, despite many reports detailing this interaction, it is still possible for Spam1-deficient sperm to penetrate the cumulus oophorus, and fertilize cumulus-enclosed oocytes [3]. This suggests that other molecules might compensate for the function of Spam1, in Spam1-deficient mice, or that functionally redundant molecules might exist.

Sperm must be prepared for binding to the zona pellucida before arriving at the zona. Angiotensin I converting enzyme (ACE), expressed on the entire region of the sperm head and midpiece, may play a role in this preparation. Targeted disruption of ACE resulted in male infertility [4]. Subsequent analyses revealed that germinal ACE possesses the novel activity of cleaving GPI-anchored proteins on sperm. In addition, treatment of ACE-deficient sperm with PI-PLC (a common GPI-anchored protein-cleaving enzyme) rescued sperm-zona binding, suggesting that release of GPI-anchored proteins rendered sperm available for binding to the zona. [5].

The sperm molecule, basigin, may also have a function in the passage through the cumulus oophorus. It is a transmembrane glycoprotein belonging to an immunoglobulin superfamily, expressed on sperm, cumulus cells, granulosa cells, and the endometrial epithelium [6, 7]. Targeted disruption of basigin results in defects in spermatogenesis, fertilization and implantation, implying multi-functional effects in different tissues and organs [8]. During fertilization, basigin may be involved in the sperm-cumulus interaction or in the sperm-zona interaction, since anti-basigin has been shown to inhibit *in vitro* fertilization of cumulus-enclosed, zona-intact oocytes [7]. However, the receptor for basigin, possibly expressed in the cumulus cells or the zona pellucida, remains to be identified.

Several molecules presented by cumulus cells have been reported to promote fertilization by facilitating penetration of the cumulus oophorus. Progesterone, originating in follicular fluid or cumulus cells, is one of these molecules [9]. The primary function of progesterone in the cumulus oophorus is to induce the acrosome reaction [10]. Prostaglandins are also trapped in the cumulus oophorus, and may render sperm competent for fertilization [11], although there is little evidence for this.

Molecular Events during Interaction between Sperm and Zonae

Structural features of the zona pellucidae

Following penetration of the cumulus oophorus,

sperm encounter a barrier, the zona pellucida. This consists of three major glycoproteins, ZP1, ZP2, and ZP3, with apparent molecular masses of 200, 120 and 83 kDa, respectively [12], although additional minor molecules, including HA, exist. The zona pellucida is believed to play an integral role in the recognition of capacitated sperm, and in the prevention of polyspermy.

Targeted disruption of ZP1 was found to produce an abnormal zona pellucida structure. ZP2 and ZP3 were expressed in ZP1-mutant ovaries and organized the abnormal zona pellucida. This resulted in morphological disorders, such as ectopic clusters of granulosa cells, fewer embryos obtained after mating, and a significant reduction in litter size [13]. More severe damage to the zona pellucida was observed in ZP2-mutant mice [14]. In these mice, the zona pellucida, comprising ZP1 and ZP3 in the early stage of folliculogenesis, was very fragile, and was not sustained in pre-ovulatory follicules. Zona-free oocytes in ZP2-mutants were fertilized, and progressed to the blastocyst stage *in vitro*. However, the resultant embryos were unable to develop to term, suggesting that the developmental potential of the mutant oocytes was never realized during oocyte maturation. The most severe effects of mutagenesis on the zona pellucida were seen in ZP3-null mice. In these mutants the zona pellucida structure was completely absent [15, 16], and the mice were sterile. These results strongly suggest that all of the zona glycoproteins are essential for the construction of the zona pellucida, as well as for oocyte fertilizability.

Sperm-zona interaction

In order to penetrate the zona pellucida, sperm must undergo the acrosome reaction. In this reaction the plasma membrane overlying the acrosomal cap fuses with the outer acrosomal membrane, to release the acrosome contents and prepare for the subsequent sperm-zona and sperm-oocyte interactions. The acrosome reaction is easily induced by calcium influx. One of the natural inducers of this reaction is progesterone sustained in the cumulus oophorus, as described above [9].

ZP3 has emerged as a primary sperm receptor as an inducer of the acrosome reaction. ZP3 recognizes sperm, triggers calcium influx through transient receptor potential (Trp) proteins via activation of trimeric G proteins and phospholipase C [17], and then induces the acrosome reaction [18]. The signal transduction pathway of the ZP3-induced acrosome reaction is different from that of the progesterone-induced

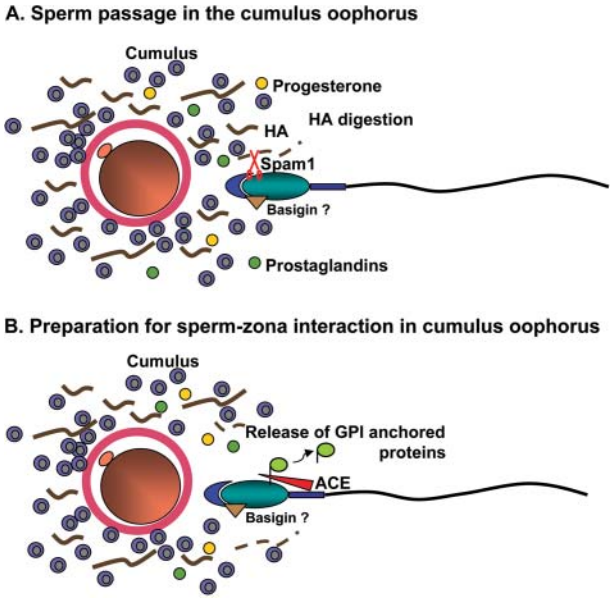

Fig. 1. Schematic diagrams of molecular interactions during sperm penetration of the cumulus oophorus. A) Sperm penetration in the cumulus oophorus. Sperm hyaluronidase, Spam1, may digest HA via direct interaction during passage. Other molecules may be involved in this process. B) Preparation of sperm for binding to the zona. ACE expressed on the sperm head and mid-piece may digest GPI-anchored proteins on sperm, including Spam1. This process may render sperm ready for binding to the zona.

acrosome reaction, because although PLCδ4-deficient sperm is unable to undergo the acrosome reaction induced by the zona pellucida, it is partially able to undergo the progesterone-induced reaction [19].

Several candidate oligosaccharide structures have been reported for the sperm recognition site. Although still a matter of debate, terminal O-linked trisaccharides, containing β-N-acetylglucosamine (GlcNAc) residues of ZP3, have been implicated in sperm binding [20]. Interestingly, human sperm are unable to bind to either the normal mouse zona pellucida, or to transgenic mouse zona pellucida carrying human ZP3. In constrast, mouse sperm is able to penetrate both these zonae [21]. This suggests that only mouse-specific carbohydrate moieties may be required for induction of the acrosome reaction, or that an unknown molecular mechanism of sperm-zona interaction exists.

Since ZP3 plays an integral role in sperm-zona binding, studies have been undertaken to identify ZP3-binding molecules in sperm. One of these identified molecules is ZRK (zona receptor kinase, p95) [22].

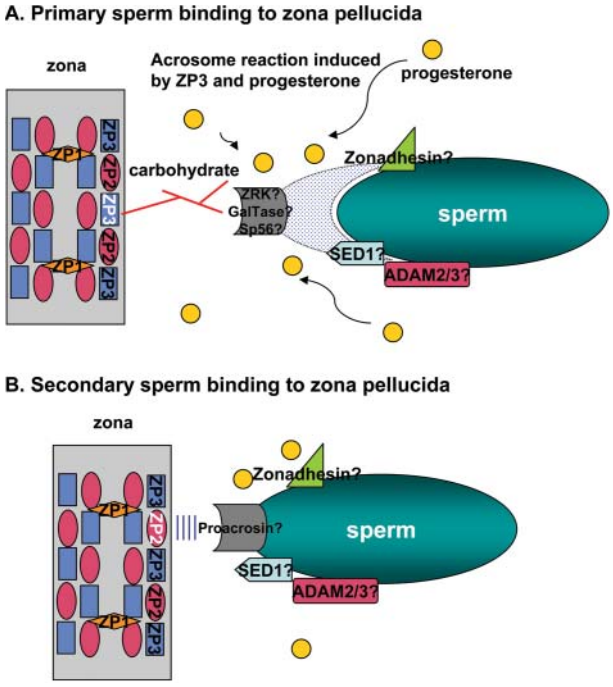

Fig. 2. Schematic diagrams of molecular interaction during sperm-zona interaction. A) Primary binding of sperm to the zona. Oligosaccharides on ZP3 recognize sperm proteins (p95, GalTase, sp56?), initiating the acrosome reaction. Progesterone trapped in the cumulus oophorus also triggers the acrosome reaction. B) Secondary binding of sperm to the zona. ZP2 is thought to bind to sperm proteins (proacrosin) to sustain the acrosome-reacted sperm on the zona. The other sperm proteins such as zonadhesin, SED1 and ADAM2/3 may participate in either primary or secondary binding.

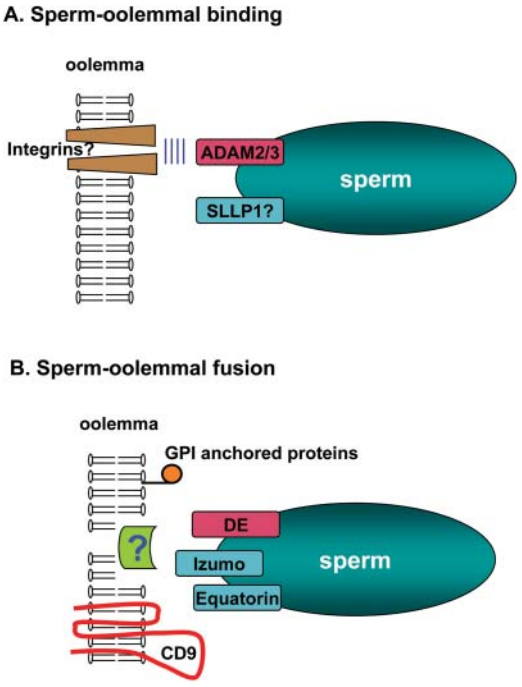

Fig. 3. Schematic diagrams of molecular interactions during the sperm-oolemmal interaction. A) Sperm-oolemmal binding. Sperm ADAMs2 and 3 may stabilize the sperm on the oolemma, and may be able to bind oocyte molecules (integrin α6, α9?). Additionally, SLLP1 acts as a paste to prevent the sperm detaching from the oolemma. B) Sperm-oolemmal fusion. DE originated from seminal plasma, Izumo and/or equatorin may recognize the oolemma and promote sperm-oolemmal fusion. Furthermore, GPI-anchored molecules and CD9 on oocytes support fusion, although the counter-receptors for the candidate molecules have not as yet been identified.

ZRK is a tyrosine-phosphorylated hexokinase, a monoclonal antibody against ZRK inhibited sperm-zona binding, and recombinant ZP3 was found to activate ZRK [23]. However, the nature of the ZP3-ZRK interaction is a matter of controversy, since the ZRK gene sequence exhibits close similarity to the proto-oncogene c-*mer* [24], which encodes a cytoplasmic protein. One possible candidate for a ZP3 receptor is β1,4-galactosyltransferase (β1,4-GalTase). Purified sperm β1,4-GalTase was shown to inhibit sperm-zona binding [25], and to recognize N-acetylglucosamine residues on ZP3 terminal oligosaccharides [20]. However, β1,4-GalTase null mutant sperm binds more strongly to the zona pellucida than does wild-type sperm [26]. In addition, there is no direct evidence that β1,4-GalTase interacts with ZP3. Another possible ZP3 receptor is sp56, a member of the protein receptor family that includes the α-subunits of the complement 4B-binding protein. sp56 has also been shown to interact directly with ZP3. Although sp56 is believed to interact with the terminal galactose residues of ZP3, this molecule may be intra-acrosomal [27], and not present on the surface membrane, as originally reported [28]. Although little evidence is available to date, α-fucosyltransferase (FucTase) may also serve as a ZP3 receptor, because the substrates for FucTase, but not for GalTase, inhibit sperm-zona binding [29].

Following the acrosome reaction, it is necessary for sperm to remain tightly bound to the zona pellucida. ZP2 is believed to be responsible for the secondary phase of the sperm-zona interaction, since anti-ZP2 inhibits binding of acrosome-reacted sperm to the zona pellucida, whereas anti-ZP3 does not [30]. However,

the possibility has been raised that a carbohydrate moiety, which is common to both ZP3 and ZP2, might be responsible for the secondary binding [31]. Although ZP2-interacting molecules are not well understood, proacrosin is one candidate molecule. Proacrosin is found within the acrosomal vesicle of all mammalian sperm, and is re-localized within the inner acrosomal membrane to mediate adhesion to the zona pellucida [32]. The binding is mediated by a strong ionic interaction between polysulphate groups on ZP2, and basic residues on an internal proacrosin peptide. This supports the hypothesis that ZP2-proacrosin interactions play a role in the retention of acrosome-reacted sperm on the egg surface [33]. However, under normal conditions, proacrosin-deficient sperm are still capable of zona penetration and fertilization [34], indicating the existence of redundant mechanisms. Hence, identification of sperm molecules that interact with each zona protein has yet to be established.

Other candidates for such molecules have been reported by several groups: α-D-mannosidase has been identified as a possible zona receptor, since mannose-containing oligosaccharide exhibits a marked reduction in sperm-zona binding, as well as strong inhibition of sperm mannosidase activity [35]. Zonadhesin, a sperm membrane protein, was also found to bind to the zona pellucida in pigs [36]. The mouse homologue of zonadhesin has been identified, and reported to be similar to von Willbrand factor. Binding to the zona was highly species-specific, and may bind to the carbohydrate moiety of zona proteins [37]. Additionally, another molecule, SED1, was found to adhere to immobilized zona pellucida glycoproteins in the pig [38]. Mouse SED1 is enriched on the plasma membrane overlying the acrosomal cap; mutational analysis revealed that a Notch-like EGF repeat, and a discoidin/C domain, participated in recognition of the zona pellucida through interaction with all of the zona proteins [39]. Other candidate molecules include sperm ADAM2 and ADAM3, belonging to the ADAM family containing pro, metalloprotease, disintegrin, cysteine-rich, EGF-repeat, transmembrane and cytoplasmic domains [40]. According to immunological and biological assays, these two proteins were thought to interact with oolemmal integrin α6 [41]. However, the observed infertility of ADAM2 or ADAM3 knockout mice was due mainly to low affinity of sperm to the zona pellucida [42, 43]. Thus, the mechanism by which ADAM2 and ADAM3 interact with the zona pellucida remains to be ascertained.

Molecular Events during Sperm-oolemmal Interaction

Physiological feature of sperm-oolemmal binding and fusion

Immediately following penetration of the zona pellucida, sperm undergo oolemmal binding and subsequent fusion, which occurs between the equatorial segment of the sperm and the microvillar region of the oocyte. Ultrastructural analysis has revealed that the entire sperm, bound to the oolemma, is enclosed by microvilli. The sperm plasma membrane around the equatorial segment begins to fuse with the plasma membrane of the oocytes [44]. Hence, the molecules localized in the equatorial segment of the sperm, and in the microvillar region of the oocyte, are believed to be of vital importance in sperm-oolemmal binding and fusion. However, there is little information on the molecules involved in this process.

Molecular aspects of sperm-oolemmal binding and fusion

Only a few sperm molecules have been reported as adhesion molecules participating in the sperm-oolemmal interaction. SLLP1 (a sperm lysozyme-like protein) was identified in the acrosome of human sperm, and has been cloned from both the human and mouse [45]. This molecule localizes around the equatorial segment of the sperm following the acrosome reaction. Definition of complementary SLLP1-binding sites on the oolemma supports the hypothesis that a c lysozyme-like protein is involved in the binding of spermatozoa to the oolemma. In addition, ADAM proteins (ADAM1/2/3), expressed on the sperm surface, may be involved in sperm-oolemmal adhesion (but not in sperm-oolemmal fusion), as described above. The counter-receptor for the ADAMs 2 and 3 expressed on the oolemma was thought to be the integrin group of molecules. Integrin $\alpha 6\beta 1$ was shown as a strong candidate, since both anti-ADAM2 or anti-ADAM3, and anti-integrin α6, inhibited sperm-oolemmal binding, and the ADAM2/3-integrin α6 interaction [46, 47]. More recently, integrin $\alpha 9\beta 1$ has been identified as another candidate, as recombinant ADAM2/3 was shown to adhere to integrin α9-expressing cells [48, 49]. However, oocyte-specific knockout of β1 integrins (including $\alpha 6\beta 1$) had no negative affect on fertility [50], indicating that either the interaction between the ADAM2/3 and β1 integrins is not essential, or that another class of integrins may compensate for the interaction.

The molecular processes involved in sperm-oolemmal fusion are mostly unknown. The sperm protein DE

(CRISP-1) is a candidate ligand for the oolemmal sperm receptor [51]. DE is synthesized in an androgen-dependent manner by the proximal segments of the epididymis and associates with the sperm surface during epididymal transit [52]. Originally localized on the dorsal region of the acrosome, DE migrates to the equatorial segment concomitantly with the occurrence of the acrosome reaction. Purified DE and anti-DE inhibited sperm-egg fusion, suggesting that DE might be essential for this process. Another candidate molecule is equatorin [53]. A monoclonal antibody (MN9) recognizes 38 and 48 kDa molecules (equatorin) localized around the equatorial segment of the sperm head. This antibody prevents fertilization without interfering with sperm motility, sperm-zona binding, or sperm penetration of the zona, indicating that equatorin may participate in sperm-egg fusion. More recently, a strong candidate as a ligand for the sperm receptor was reported, and has been cloned very recently [54]. This molecule, Izumo, is a novel immunoglobulin superfamily protein, the epitope of which appears on sperm after the acrosome reaction. Gene targeting of Izumo results in the loss of fusion ability, but not of binding ability, suggesting that Izumo is a fusion regulator.

Molecular information on oolemmal proteins involved in fusion is also limited. GPI-anchored proteins must function in sperm-egg fusion, since PI-PLC restricts the ability of oocytes to fuse with sperm [55]. Additionally, oocyte-specific knockout of GPI-anchored proteins (by targeting of a portion of the Pig-a gene, that encodes an enzyme involved in GPI anchor biosynthesis), resulted in severe loss of fusogenic activity of oocytes [56]. The specific GPI-anchored proteins involved have yet to be identified.

CD9 is a possible modulator of sperm-oolemmal fusion. This molecule belongs to a tetraspan superfamily that has four consensus membrane-spanning regions. CD9 is expressed in many tissues and organs and is abundant on oocytes, but not on sperm [57]. Targeted mutagenesis of CD9 resulted in the loss of the oocyte fusion ability [58–60]. Although the specific molecules that regulate sperm-oocyte fusion have yet to be identified, direct interaction with the soluble ligand PSG17 might modulate the activity of CD9 during sperm-egg fusion [61].

It is believed that specific interaction between a ligand on sperm, and a sperm receptor on the oocyte, triggers fusion. However, none of the reported molecules on sperm or oocytes have been shown to interact with other specific molecules, and further investigation is necessary.

Conclusions

The molecules introduced in this review are involved in the process of fertilization. However, there remain significant gaps in the knowledge, since not all of the molecular interactions between sperm and oocytes have been subjected to gene targeting analysis. Recently there have been significant advances in the understanding of the molecular mechanisms underlying fertilization, due to the application of gene targeting. However, it is important for us to bear in mind that gene targeting, resulting in irregular expression, may have secondary effects on other molecules. Some of these may be able to function as back-up molecules for those targeted by genetic manipulation, and others as promoting molecules. Therefore, care must be taken when considering the phenotypic consequences of such targeting studies.

In this review, we have presented recent findings from studies carried out in mice. Although different species may utilize different molecules for fertilization, the underlying mechanisms involved are likely to be similar. Consequently the molecular information obtained from mice contributes to the body of knowledge regarding the molecular mechanisms of fertilization, and may be of use to those investigating the molecular basis of infertility in human patients.

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