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Mechanisms of Mammalian Sperm-Egg Interaction Leading to Fertilization

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Abstract

Mammalian fertilization, a species-specific event, is the net result of a highly orchestrated process that collectively results in the fusion of two radically different-looking haploid cells, sperm and egg, to form a diploid zygote, a cell with somatic chromosome numbers. Prior to the interaction of the opposite gametes, mammalian spermatozoa undergo many fascinating changes during development in the testis, maturation in the epididymis, and capacitation in the female genital tract. Only the capacitated spermatozoa interact with the extracellular coat, the zona pellucida, which surrounds the mammalian oocyte. The tight and irreversible binding of the opposite gametes in the mouse and many other mammals studied, including human, starts a Ca^{2+}- dependent signal transduction pathway that results in the exocytosis of acrosomal contents at the site of the sperm binding. The hydrolytic action of the acrosomal glycohydrolases and proteinases, released at the site of the sperm-egg binding, along with the enhanced thrust generated by the hyperactivated spermatozoon, are important factors that regulate the penetration of the zona pellucida and fusion of the opposite gametes. The purpose of this editorial is to highlight the well programmed molecular events that are necessary before sperm-egg adhesion. In addition, my intention is to discuss the increasing controversy about the mechanism(s) that regulate mammalian sperm-egg interactions.

Keywords: Mammalian fertilization; Sperm-egg interaction; Sperm capacitation; Signal transduction; Acrosome reaction

Editorial

For successful mammalian fertilization in mammals, two radically different-looking haploid cells, sperm and egg, interact and unite to form a zygote, the cell with somatic chromosome numbers [1,2]. In the mouse and many other mammalian species studied, including man, ejaculated spermatozoa cannot immediately bind to an egg and fertilize it. They require a certain period of residence in the female genital tract to become functionally competent cells [3]. As spermatozoa traverse through the female genital tract, they undergo physiological priming (multiple biochemical and physiological changes), collectively referred to as capacitation. Only capacitated spermatozoa interact with the extracellular glycocalyx egg coat, the Zona Pellucida (ZP). At coitus, millions of spermatozoa are deposited in the female reproductive tract [4-6]. However, a vast majority of the deposited male gametes are eliminated and only a small percentage of spermatozoa enter the highly folded mucus-filled cervix that has three important functions. First, it prevents the entry of seminal plasma into the uterus. Second, it prevents the entry of morphologically abnormal spermatozoa as well as potentially infectious microbes into uterus. Finally, the uterus stores viable spermatozoa. Once in the uterus, spermatozoa move by passive and active processes towards the distal uterus and oviduct, the likely site of an *in vivo* fertilization in many species [1].

Spermatozoa that enter the distal oviduct region (ampulla), the site of fertilization, meet the ovulated egg(s) surrounded by cumulus complex [1,3]. The cumulus is thought to be dispersed by hyaluronidase, an enzyme present in the sperm acrosome as well as the sperm surface allowing capacitated and hyperactive spermatozoa free passage through the dispersed cumulus complex [3]. The precise site of capacitation may be different in various species; however, the *in vivo* process in several species is most efficient when spermatozoa pass through the uterus and oviduct [1]. The oviduct secretions collected from the estrus females have been demonstrated to be most efficient in rendering functional changes in spermatozoa *in vitro* [1]. Mammalian spermatozoa can also be capacitated *in vitro* by incubating ejaculated or caudal spermatozoa in a chemically defined medium supplemented with energy substances, such as glucose and pyruvate, and Bovine Serum Albumin (BSA) or beta-cyclodextrins [2,5]. The protein (BSA), a major component in the female genital tract or beta-cyclodextrnes, is believed to facilitate capacitation by efflux of sterols, mainly cholesterol, fatty acids and phospholipids from the sperm Plasma Membrane (PM). The precise mechanism(s) as to how the loss of cholesterol/phospholipids regulates the physiological priming of spermatozoa is not yet fully understood; however, it is generally believed that the loss of sterol increases fluidity and permeability of the sperm PM, making it fusogenic [1]. Studies from our group provide evidence suggesting that capacitation involves physiological priming of sperm PM that exposes acrosomal contents on the sperm surface [6].

As capacitation proceeds, a number of internal and external changes occur on spermatozoa. The known changes include: i) efflux of sterols, mainly cholesterol; ii) increased adenylyl cyclase activity and increased levels of cAMP; iii) protein tyrosine phosphorylation of a subset of sperm components; iv) elevated intra-sperm pH; v) loss of sperm surface molecules; vi) Ca^{2+} influx; vii) modification/alteration of sperm PM; viii) changes in the lectin-binding patterns; ix) hyperactive sperm motility; and x) membrane priming. However, with the exception of cholesterol efflux [7] and Ca^{2+} influx [8,9], the sequence of other changes and their physiological significance in sperm capacitation remain evasive.

The fact that only the capacitated spermatozoa bind to the zona pellucida and undergo the Ca^{2+}-dependent Acrosome Reaction (AR) suggests that major changes occur on the anterior head (peri-acrosomal) region of spermatozoa, an area believed to be involved in the sperm-egg binding [10]. Since the acrosome acts in concert with the sperm PM overlying the organelle, a brief discussion on its organization will contribute to our understanding of the physiological priming of membranes in the capacitating/capacitated spermatozoa.

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Additional details on the formation and function of the acrosome can be found in a review article [11].

A well-developed acrosome is a sac-like structure with an Inner Acrosomal Membrane (IAM) and an Outer Acrosomal Membrane (OAM) covering the anterior portion of the nucleus. The size and shape of the acrosome varies from species to species and depends on the morphology of sperm head. The shape of the acrosome generally falls into two categories, a sickle-shaped in rodents or a skull-cap/paddle-shaped (spatulate) in several larger species, including man [11]. The sperm acrosome is a Golgi-derived secretory organelle that resembles the cellular lysosome in many ways. However, the organelle is considered analogous to a secretory granule. Its important features as reported by Burgess and Kelly [12] include: i) the secretory contents are stored over an extended period of time and are present in the concentrated form; ii) the contents form a dense structure and are stored for several weeks during sperm development in the testis, sperm maturation in the epididymis and capacitation in the female genital tract; and iii) the organelle undergoes secretion (the AR) as a result of an external stimulus following the tight and irreversible binding spermatozoa to the zona-intact egg [1,3].

The sperm acrosome contains a variety of acid glycohydrolases, proteinases, phosphatases, esterases and aryl sulfatases. These enzymes have been described in sufficient details in earlier review articles [3,4] and will not be repeated here. After several decades of investigation, researchers are beginning to understand the complex nature of the acrosome. Several biochemical and morphological studies have provided evidence for the involvement of cytoskeletal domains such as actin, calmodulin and α-spectrin-like antigens in the organization of the acrosome [11]. In addition, the organelle contains filamentus structures primarily associated with the OAM. However, the functional significance of the filamentous structures, if any, is not yet known.

As stated above, capacitated (acrosome-intact) spermatozoa interact with the zona-intact egg in a highly precise manner. Multiple studies in the mouse provide evidence suggesting that sperm-egg interaction is a two step process. First, the capacitated spermatozoa loosely and reversibly adhere to the zona-intact egg by means of the PM overlying the acrosome; the second step is a tight and irreversible binding [5]. Many sperm cells can adhere to the zona-intact egg; however, usually only one sperm will penetrate the ZP and fertilize the egg [2].

All mammalian eggs are surrounded by an extracellular coat, the ZP. The coat in various species studied is a relatively simple structure composed of three glycoproteins designated ZP1, ZP2 and ZP3; the pig and human ZP has a fourth form (ZP4) as well [2,5]. In the mouse, two of the three glycoproteins, ZP2 and ZP3, interact non-covalently to form long filaments which are interconnected by ZP1 forming a three diamentional network of cross-linked filaments that form the extracellular matrix [13]. Such a structure may explain the elasticity of the ZP and the relative ease of its penetration by the acrosome-reacted spermatozoon. The ZP mediates several events, including, relative species-specificity, sperm activation (i.e., induction of the AR), block to polyspermy, and protection of the embryo from fertilization to implantation [1,2,13].

Considerable progress has been made in the identification and characterization of complementary molecules on the ZP and sperm PM that are believed to be important for the gamete interaction [14]. In particular, the work on mouse ZP (mZP) has resulted in the identification of primary (mZP3) and secondary (mZP2) binding sites for homologus spermatozoa [13,14]. The mZP2 and mZP3 as

well as these molecules in several other species are glycoproteins which like most glycoproteins are complex molecules with extensive microheterogeneity [15]. Since both glycoproteins are sulfated, the microheterogeneity and the acidic nature of these glycoproteins are most likely due to N-and O-glycosylation of the polypeptide backbone and sulfation of the glycan chains.

Accumulated evidence listed in earlier review articles [2,14] strongly suggested that glycan units of mZP3 provide the primary ligand site(s) for the capacitated spermatozoa. Two of these evidences are highly specific and worth repeating here. First, sperm binding activity of the mZP3 is sensitive *in vitro* to the treatment with trifluoromethane sulfonic acid, an acid known to break glycosidic bonds between monosaccharide residues of N-linked and O-linked units without altering the protein backbone. Second, the ability of mZP3 to competitively inhibit sperm-zona binding *in vitro* is unaffected by the treatment with pronase, a protease that digests the protein backbone of the mZP3; however, the resulting N- and O-linked glycopeptides ranging in size from 1.5 to 6.0 kDa are still able to inhibit sperm-egg (zona) binding in a concentration-dependent manner [14,16]. Taken together, these data agree with the conclusion that the glycan units on mZP3 provide the binding site(s) for the capacitated spermatozoa.

Despite these advances, considerable controversy remains regarding the precise identity of the terminal sugar residue(s) on the glycan unit(s) responsible for the ligand activity of the mZP3. For instance, studies published in the 1980s provided evidence suggesting that the sperm–binding activity was associated with α-linked galactosyl residue(s) present at the nonreducing terminus of an O-linked oligosaccharide [17] or with N-acetylglucosamine residue(s) [18]. However, the experimental evidence from other investigators suggested the involvement of additional sugar residues including mannosyl [19,20], sialyl [21], and fucosyl [22]. A recent article presented evidence suggesting that oviduct-specific glycoprotein and heparin modulate sperm-zona interaction [23].

In the late 1990s, investigators used gene disruption approaches to address the potential role of galactosyl or N-acetylglucosamine residue(s) in sperm-zona (egg) interaction. The female α-1, 3-galacosyl transferase null (-/-) mice produced oocytes with the ZP that were devoid of Gal-epitopes. However, these mice were fully fertile, a result consistent with the investigators conclusion that Gal-epitopes are not involved in sperm-zona interaction leading to fertilization in the mouse [24]. Similarly, male mice devoid of sperm β-1, 4-galactosyltransferase (GT), an enzyme that recognizes N-acetylglucosamine residue(s) on mZP3, were still fertile [25]. Combined, these data provide evidence that neither galacosyl residue(s) on the mZP3 nor GT on the mouse spermatozoa are required for sperm-egg interaction. To the best of my knowledge, there are no genetically engineered mouse models to rule out the suggested role for mannosyl, sialyl or fucosyl residue(s) in sperm-egg interaction.

As stated above, mZP2 and mZP3 glycoproteins are highly glycosylated containing a variety of N-linked glycan chains including high mannose/hybrid-type, bi-, tri-, and tetra-antennary complex-type and poly-N- acetyllactosaminyl-type in addition to an O-linked trisaccharide [15,26,27]. We also presented evidence suggesting that the high mannose/hybrid-type glycane chains on mZP3 [27] may be a part of the recognition/binding site(s) for the sperm surface mannosidase [28,29]. Many details as to how the sperm PM enzyme could bind to these oligosaccharides units are described previously [29] and will not be repeated here.

Like mZP3, the porcine ZP glycoprotein 3 (pZP) has been

reported to possess receptor activity. The 55-kDa pZP3 is also highly glycosylated containing N-linked and O-linked glycan units as well as poly-N-acetyllactosaminyl glycans [30]. The pZP3 contains neutral and acidic N-linked glycan chains; however, only the neutral glycans were demonstrated to inhibit sperm-egg binding *in vitro*, a result consistent with the suggestion that only the neutral glycan chains have a role in the recognition and binding of the opposite gametes [31,32]. Several other sperm surface molecules in various species have been proposed to function as receptor molecules. These molecules have been described in sufficient details in an earlier article [2].

Several lines of evidence discussed in the above articles are consistent with the argument that a carbohydrate recognition mechanism is important in sperm-egg (zona) interaction. Despite the overwhelming evidence presented in support of the hypothesis that sugar residue(s) on the ZP are ligand(s) for the sperm surface receptors [2,3,5,14,16], a recent report by Professor Dean and associates presented evidence suggesting that the gamete interaction in the mouse depends on the cleavage status of the mZP2 protein [33]. In brief, a ZP2 cleavage model for the recognition of the opposite gametes requires an intact ZP2, where as a glycan release model postulates that the mZP3 glycan residue(s) is ligand for spermatozoa. The investigators tested these two models by replacing endogenous mZP2 with a mutant ZP2 that can not be cleaved and a glycan release model where the mZP3 lacks O-linked glycan(s). Spermatozoa bound to the two-cell ZP2 mutant embryos despite fertilization and cortical granule exocytosis. However, despite the absence of the O-linked glycan residues from the ZP3 mutant eggs, spermatozoa still fertilized them. These data, according to the investigators, demonstrate that sperm-egg binding depends on the cleavage status of ZP2 [33].

The investigators of the above article, however, did not address several important points: First, the structural similarities/dissimilarities between endogenous and mutant mZP2; second, has the replacement of endogenous mZP2 glycoprotein with mutant mZP2 protein altered the three-dimentional structure of the zona pellucida; and finally, the possible outcome if all N-linked glycan chains or some of the other sugar residues implicated in sperm-egg interaction were removed from the mZP3. Some similar concerns were raised in a recent article [34]. Unless these concerns have been satisfactorily addressed, it is reasonable to suggest that the mechanism(s) underlying sperm-egg interaction remains an unresolved issue.

The interaction of the opposite gametes triggers the signaling pathway that activates spermatozoa by opening of Ca^{2+} channels on the sperm PM. This event elevates intrasperm Ca^{2+} and other second messengers. The net result is the fusion of the OAM and the overlying sperm PM at multiple sites and the exocytosis of the acrosomal contents at the site of the sperm-egg binding. The hydrolytic action of the acrosomal enzymes released along with the hyperactivated beat pattern of the bound spermatozoa are important factors that direct the acrosome-reacted spermatozoon to penetrate the egg coat and fuse with the egg [3,35]. Although the molecules involved in sperm-egg fusion will be of interest to many readers of this editorial, they are beyond the scope of this editorial article. Interested readers are referreded to an earlier article [36].

In conclusion, the editorial covers many aspects of mammalian sperm-egg interaction that lead to fertilization. Extensive progress has been made in this field of reproductive biology and physiology. Although the identity of the molecule(s) that initiates sperm-egg interaction remains controversial, it is only a matter of time before this controversy is resolved. The identification and characterization of various molecules that are functionally significant in the process of fertilization will allow new strategies to regulate the fertilization event and alter sperm function and male fertility.

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