Editorial Article

Sperm-Egg Interactions and Mammalian Fertilization

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Mammalian fertilization, a species-specific event, is a highly programmed process by which two radically different looking haploid cells, sperm and egg, interact and fuse to form a diploid zygote, a cell with the somatic chromosome numbers [1]. The process is the net result of a complex sequence of events that prepare ejaculated spermatozoa to recognize and irreversibly bind the egg's extracellular coat, the zona pellucida, (ZP) [2-7]. The tight and irreversible binding of the opposite gametes in the mouse and many other mammals studied, including human, starts a Ca^{2+}-dependant signal transduction pathway that results in the exocytosis of acrosomal contents at the site of sperm binding. The hydrolytic action of the acrosomal enzymes (i.e., proteinases, glycohydrolases, sulphatases, esterases etc.), released at the site of sperm-egg binding, along with the hyper-activated spermatozoon, are important factors that regulate the penetration of the ZP and fusion of the opposite gametes [8,9]. This editorial focuses on the well programmed molecular events that are necessary before sperm-egg adhesion and fertilization. My intention is to discuss the increasing controversy about the sperm and egg molecules that are important in the process of fertilization.

In the mouse and many 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 where they undergo physiological priming, collectively referred to as capacitation, a multifaceted process that produces hyper-activated spermatozoa [10]. Only capacitated spermatozoa can recognize and bind to the zona-intact egg in a species- specific manner and fertilize it [1,7].

All mammalian eggs are surrounded by an extracellular coat, the ZP [11,12]. The coat in various species 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 [13]. In the mouse, two of the three glycoproteins, mZP2 and mZP3, interact non-covalently to form long filaments that are
inter-connected by ZP1 forming a three dimensional network of cross-linked filaments that form the insoluble extracellular matrix [11]. Such a structure may explain the elasticity of the ZP and the relative ease of its penetration by the acrosome-reacted spermatozoon [2,5,7]. The ZP mediates several events, including, relative species-specificity, sperm activation (i.e., induction of the acrosome reaction), block to polyspermy, and protection of the zygote from fertilization to implantation [1].

It should be noted that mZP2 and mZP3 as well as porcine ZP3 (pZP3) are glycosylated and like many mammalian glycoproteins contain a variety of N-linked oligosaccharides including poly-N-acetylglucosaminyl glycan chains [14,15]. The fact that the poly-N-acetulglucosaminyl chains contribute 23 kDa and 16kDa to the molecular mass of mZP2 and mZP3, respectively [14,15] suggests that the two ZP glycoproteins likely contain a variety of structurally variable N-linked polylactosaminyl units. My group has used multiple approaches to demonstrate that in addition to polylactosaminyl units,

mZP2 and mZP3 contain a variety of N-linked bi-, tri-, and tetra- antennary complex-type glycans as well as high mannase/hybrid-type glycans [15,16]. The fact that mZP2 and mZP3 contain sulphate/phosphate residues on glycan chains as well as several monosaccharide residues on the non-reducing terminus contributes to the extensive heterogencity in the structure of N-linked glycan chains [14-16].

In addition to multiple N-linked glycan chains, mZP3 glycoprotein also contains O-linked oligosaccharide of an apparent molecular mass of 3.9 kDa with a terminal α1, 3-galactosyl residue [17,18] and an O-linked trisaccharide with a terminal β-linked glucosaminyl residue [14]. The reported presence of a number of N- and O linked oligosaccharides on mZP3 makes it difficult to identify and chemically characterize the bioactive (functional) oligosaccharide(s). The efforts are further hampered by the small amount of ZP glycoproteins that can be purified and subjected to structure –function studies.

Despite numerous hurdles, researchers have made considerable progress in the last four decades. For instance, accumulated evidence mentioned in earlier review articles [2-4] strongly suggested that glycan units of mZP3 provide the primary ligand site(s) for the capacitated spermatozoa. For instance, studies published in the 1980's provided evidence suggesting that the sperm-binding activity was associated with α-linked galactosyl residue(s) present at the non-reducing terminus of an O-linked oligosaccharide [17,18] or with N-acetylglucosamine residue(s) [19]. However, the experimental evidence from other investigators suggested the involvement of additional sugar residues including mannosyl [20-22], sialyl [23], and fucosyl [24]. A recent article presented evidence suggesting that oviduct-specific glycoprotein and heparin modulate sperm-zona interaction [25].

In the late 1990's, investigators used gene disruption approaches to address the potential role of α-galactosyl [26] or N-acetylglucosominyl [27] residues in sperm-zona (egg) interaction. The α-1, 3-galactosyl transferase null (-/-) female mice produced oocytes with the ZP that was devoid of galactosyl-epitopes. However, these mice were fully fertile, a result consistent with the conclusion that galactosyl residues are not involved in sperm-zona interaction [26]. Similarly, male mice devoid of β1, 4-galactosyltransferase (GT), an enzyme that was postulated to recognize N-acelylglucosamine residue(s) on mZP3, were still fertile [27]. Combined, these data provided evidence that neither galactosyl residue(s) on the mZP3 nor GT on the mouse sperm are required for sperm-egg interaction. There are no genetically engineered animal models to rule out the suggested role for mannosyl [20-22], sialyl [23] or fucosyl [24] residue(s) in sperm-egg interaction.

Like mZP3, the procine ZP3 (ρZP3) has been reported to possess receptor activity. The 55- kDa ρZP3 is also highly glycosylated containing N- and O- linked glycan units as well as poly-N-acetylglucosaminylglycans [28]. The ρZP3 has been demonstrated to contain neutral and acidic N-linked glycan chains; however, only the neutral glycans were demonstrated to inhibit sperm-egg binding *invitro*, a result consistent with the suggestion that only neutral glycan chains have a role in the recognition and binding of the opposite gametes [29]. Several sperm surface macromolecules (proteins, glycoproteins, glycohydrolases, etc.) have been proposed to function as receptor molecules. Many details and a list of the putative receptor molecules in various species have been included in two previous articles [4,30].

In spite of the overwhelming evidence presented in support of the hypothesis that sperm-egg interaction is mediated by carbohydrates, a recent article by Professor Dean and associates reported that the interaction of opposite gametes depends on the cleavage status of the mZP2 glycoprotein [31]. In brief, a mZP2 cleavage model for the sperm-egg recognition in the mouse requires an intact mZP2, whereas a glycan release model postulates that the mZP3 glycan residue(s) is the ligand for sperm plasma membrane molecule(s). The investigators tested these two models by replacing endogenous mZP2 with

a mutant ZP2 that cannot be cleaved and a glycan release model where mZP3 lacks O-linked glycan units. Spermatozoa bound to the two-cell ZP2 mutant embryos despite the absence of O-linked glycan residue(s) from the ZP3 mutant eggs, spermatozoa still fertilized them [31]. These data, according to the investigators, support their suggestion that sperm-egg binding depends on the cleavage status of mZP2.

The investigators of the above article, however, have not addressed several important issues: First, the structural similarities/dissimilarities between endogenous and mutant mZP2; second, has the replacement of endogenous mZP2 glycoprotein with the mutant ZP2 altered the three-dimensional structure of the ZP; third, is the mZP3 of the ZP with the mutant ZP2 still involved in sperm-egg binding, a prerequisite event before the bound sperm undergoes the acrosome reaction, penetrates the ZP and fuses with the egg: and finally, the possible outcome if all N-linked glycan chains were removed from the mZP3. Some similar concerns have been raised in earlier articles [7,32]. Unless these concerns have been satisfactorily addressed, it is reasonable to conclude that the mechanism(s) underlying sperm-egg interaction leading to fertilization remains an unresolved issue.

The interaction of the opposite gametes triggers the signaling pathway that activates spermatozoon by opening the Ca^{2+} channels on the sperm plasma membrane [9,10]. This event elevates intrasperm Ca^{2+} and other second messengers. The net result is the fusion of the outer acrosomal membrane and the overlying plasma membrane at multiple sites and the exocytosis of the acrosomal contents at the site of sperm-egg binding [7-9]. The hydrolytic action of the acrosomal enzymes released, along with the hyperactivated beat pattern of the bound sperm are important factors that direct the acrosome-reacted spermatozoon to penetrate the egg coat and fertilize the egg [8]. Although the molecules involved in sperm-egg fusion will be of interest to many readers, they are beyond the scope of this editorial. Interested readers are referred to an earlier review [33].

In summary, the editorial covers many aspects of mammalian sperm-egg interaction leading to fertilization. Although the identity of the molecule(s) that initiates sperm-egg binding remains controversial, it is just a matter of time before the controversy is resolved. The identification and characterization of sperm surface molecules that are functionally significant in the process of fertilization will allow new strategies to regulate these events andcontrol sperm function and male fertility.

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