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Minireview

Mammalian Fertilization: A Carbohydrate-Mediated Event[1]

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BACKGROUND

An essential step in the process of mammalian fertilization is the recognition and binding between spermatozoa and the egg's extracellular coat, the zona pellucida (ZP). Sperm-zona binding, at least in the mouse, is believed to take place in two stages. In the first stage, the capacitated spermatozoa loosely and reversibly attach to the surface of ZP; in a second stage, irreversible binding occurs [1, 2]. Most researchers agree that complementary molecules present on the surface of opposite gametes are involved in sperm-egg interaction. Although the chemical nature of the complementary molecules is poorly understood, growing evidence suggests that several steps in the fertilization process are mediated by carbohydrates. There is a vast literature on the functional significance of the glycoproteins of the reproductive system. The glycan portion of several glycocomponents is believed to modulate cell-cell adhesion including sperm-egg recognition and binding [2], sperm-oviductal adhesion [3], and implantation of the embryo [4, 5]. Several types of glycans, including high-mannose/hybrid-type [6], sialylated [7], glucosaminylated [8], fucosylated [9], and galactosylated [10] glycoproteins, have been implicated in various events during fertilization. Thus depending on the reproductive event under study, modification of the bioactive glycan will be expected to influence the fertilization event.

Since fertilization takes place in a complex microenvironment within the oviduct [1], one would expect that specific glycocomponents (glycoproteins/complex carbohydrates), or the enzymes of oviductal lumen that can modify these glycocomponents, would have an effect on the regulation of events leading to fertilization. Many excellent reviews on mammalian fertilization have appeared in the last decade [11–16]. However, most of these reviews do not discuss the functional significance of female reproductive tract secretions, especially the oviductal secretions, in the regulation of fertilization events. The present review will discuss their potential significance in the modification of existing glycocomponents of sperm plasma membrane and/or ZP.

There has been a longstanding interest in the basic biology of the fertilization processes. The success of in vitro fertilization in domestic animals and humans is a result of our knowledge of the events in animal models. These events are best understood in the mouse, although there is some information in other species, including the pig, guinea pig, and hamster [1, 2]. Successful fertilization in the mouse involves several sequential steps. These are 1) sperm capacitation in the female genital tract, 2) binding of the capacitated sperm to the ZP, 3) induction of sperm acrosome reaction, 4) penetration of ZP, and 5) fusion of the spermatozoon with the egg vitelline membrane. Because of space limitations, this minireview will focus primarily on the importance of carbohydrate residues in the first two steps of fertilization.

1. SPERM CAPACITATION

The sperm plasma membrane, a vital component during the early events in fertilization, undergoes extensive biochemical changes as spermatozoa transit from the proximal to the distal region of epididymis [1, 17–20]. In addition to maturation in the epididymis, mammalian spermatozoa must undergo functional changes between the events of mating and fertilization. The ejaculated spermatozoa cannot immediately fertilize an oocyte. During residence in the female genital tract they undergo a series of biochemical and functional modifications collectively referred to as capacitation [1]. These functional changes, including removal of seminal plasma proteins adsorbed to sperm plasma membrane and modification and/or reorganization of sperm surface molecules, are necessary before spermatozoa can bind to the ZP and penetrate it.

Much of the current knowledge on capacitation has been provided by the pioneering studies by Yanagimachi and Chang [21, 22]. These investigators were the first to report successful fertilization of hamster eggs in vitro by the use of spermatozoa recovered from the female reproductive tract after mating or the use of epididymal spermatozoa preincubated with oviductal secretions. These studies suggested the presence of one or more factors in the female genital tract responsible for the induction of capacitation. The precise site of capacitation may be different in different species; however, several studies suggest that capacitation is most efficient when the spermatozoa pass through the uterus and oviduct. The secretions collected from the oviduct of estrous females have been demonstrated to be most efficient in rendering the functional changes in spermatozoa.

Although the importance of capacitation has been known for several decades, the molecular mechanisms underlying these changes are not fully understood. Most researchers

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agree that capacitation results from multiple molecular changes in sperm plasma membrane proteins/glycoproteins and lipid components that likely modify ion channels in the plasmalemma of spermatozoa [20, 23]. These modifications allow the transmembrane flux of ions that are believed to be important in initiating the events of capacitation, hyperactivation, and acrosome reaction [1, 23]. The former two events take place before sperm-zona (oocyte) interaction, and the latter event, at least in the mouse, is initiated after sperm-egg interaction [2]. It is therefore reasonable to assume that these events are independent and likely to involve region-specific changes in sperm plasma membrane.

In most mammalian species studied, spermatozoa become hyperactive in the isthmus region of the oviduct, and the hyperactivated spermatozoa move to the ampullary environment [24, 25], the site of in vivo fertilization. A possible functional significance of hyperactivation could be that the hyperactivated beat pattern of a spermatozoon will definitely enhance its thrust at the site of its binding to ZP [26]. The enhanced thrust, in part, is likely to be important in order for the spermatozoon to penetrate ZP and fuse with the egg plasma membrane. Interestingly, a recent study showed that capacitated spermatozoa penetrated ovarian eggs incubated in the presence of an ovarian-specific glycoprotein (OGP) at a rate 3-fold higher than in eggs incubated in the absence of the OGP [27]. This and other [28] studies imply that the OGP has an important role in sperm capacitation and hyperactivation.

All mammalian spermatozoa studied thus far undergo capacitation after residing in the female genital tract for a certain period of time. Similarly, spermatozoa recovered from the female tract after mating, or cauda epididymal spermatozoa following incubation with the oocyte-cumulus masses and oviductal secretions, are able to fertilize an egg in vitro [28]. Mammalian sperm can also be capacitated in vitro in a chemically defined medium containing BSA and energy substrates, such as glucose and pyruvate, as well as components used in Krebs-Ringer bicarbonate medium [29]. It is important to point out that albumin is the major protein in the female genital tract secretions and is an important component during in vitro and in vivo capacitation [30]. The protein is believed to facilitate capacitation by altering (removing) fatty acids and/or cholesterol from sperm plasma membrane.

As capacitation proceeds, a number of biochemical changes occur on spermatozoa. The known changes include 1) increased adenylate cyclase and cAMP [31, 32], 2) increase in intracellular pH [33], 3) calcium influx [34], 4) loss of surface components [35], 5) modification/alteration of sperm plasma membrane [36], and 6) changes in the lectin-binding pattern of spermatozoa [37–39]. It is important to mention that the reported changes in the lectin-binding properties are indicative that the terminally exposed carbohydrate moieties on sperm surface glycoproteins are altered during capacitation. A possible explanation for these changes could be the association of an OGP secreted from epithelial cells of oviduct in several species [40–45]. Interestingly, investigators recently reported the binding of the glycoprotein to the posterial region of the sperm head, midpiece, and flagellum [46]. Alternatively, the existing sperm plasma membrane glycoproteins could be modified by glycosyltransferases present in the uterus and oviduct secretions. The synthetic enzymes show a temporal surge in their activities in the genital tract during the estrous cycle [47]. The potential modification of the endogenous sperm plasma membrane glycoproteins through addition of sugar residues to the terminally exposed glycans will be expected to alter their lectin-binding properties.

Since in vivo sperm capacitation takes place in the female genital tract, the OGP could have a direct role in sperm capacitation. Indeed, recent observations suggested that twice as many bovine sperm were capacitated in the presence of bovine OGP as in its absence [28]. In addition, spermatozoa capacitated in the presence of OGP showed increased ability to fertilize homologous oocytes, a result consistent with the suggestion that the oviduct and its secretions play an important role in sperm function [46].

Finally, it is of interest that several studies have appeared in the last decade implicating proteoglycans and glycosaminoglycans in the induction of capacitation [48]. Heparin, the most effective glycosaminoglycan, binds to spermatozoa from several species, including man [48]. The binding is said to be dependent on pH, ionic strength, and temperature, and thus has characteristics resembling those of a typical receptor-ligand interaction. However, the molecular mechanisms responsible for these interactions are not known.

From the preceding discussion, it is obvious that capacitation reflects multiple changes in the plasma membrane of mature spermatozoa. Although molecular details of these changes vary among species, the net result is the development of hyperactivated motility and responsiveness of spermatozoa to undergo acrosome reaction.

2. SPERM-EGG INTERACTION

As stated above, the capacitated spermatozoon interacts with the ZP in a highly precise manner. Extensive studies in the mouse seem to suggest that binding of capacitated spermatozoa to the ZP is a two-step process. First, spermatozoa loosely and reversibly adhere to the egg's extracellular coat by means of the plasma membrane overlying the acrosome. The second stage is a strong and irreversible binding. Many sperm can bind to the egg surface (Fig. 1A). However, usually only one sperm will penetrate the ZP and fuse with the egg plasma membrane. The fertilized oocyte usually has no bound sperm (Fig. 1B).

The ZP in the mouse [49], rat [50], and several other species is composed of three glycoproteins designated ZP1, ZP2, and ZP3, and the pig has a fourth form as well (Table 1; [50–68]). The three glycoproteins, at least in the mouse, are synthesized and secreted by the growing oocytes during oogenesis [51]. During the period of oocyte growth, the secreted zona glycoproteins interact noncovalently to form a three-dimensional network of cross-linked filaments forming extracellular matrix. Such a structure may explain the elasticity of ZP and the relative ease of its penetration by the acrosome-reacted spermatozoa. Many events are mediated by this extracellular matrix, including 1) relative species-specific sperm-egg recognition and binding of capacitated sperm, 2) sperm activation (induction of acrosome reaction), 3) block to polyspermy, and 4) protection of the growing embryo from fertilization to implantation [1].

In recent years, considerable progress has been made in the identification and characterization of complementary molecules on ZP and sperm plasma membrane that are believed to be responsible for gamete interaction. In particular, work on mouse ZP (mZP) has resulted in the identification of primary (mZP3) and secondary (mZP2) binding sites for homologous spermatozoa [2]. Targeted disruption of the mZP3 gene resulted in the production of oocytes lacking ZP and of infertile female mice [69]. Both mZP2

and mZP3 as well as these two glycoproteins in hamsters, pigs, and rats are highly glycosylated and, like other glycoconjugates, show extensive microheterogeneity. Evidence presented by Shimizu et al. [52] suggests that mZP2 and mZP3 glycoproteins are sulfated. Thus it appears that the microheterogeneity and acidic nature of these glycoproteins are due to differential glycosylation of the polypeptide backbone and sulfation of glycan chains.

Inclusion of relatively low concentrations of mZP3 (but not mZP1 or mZP2) in an in vitro sperm-egg binding assay decreased the number of sperm bound per egg in a dose-dependent manner [2]. The observed inhibition is apparently caused by competition of the added mZP3 for complementary binding site(s) on the plasma membrane overlying the sperm head. Moreover, when the radioiodinated mZP2 or mZP3 was incubated with the mouse spermatozoa, the latter glycoprotein showed higher binding to the sperm head. Combined, these data are consistent with the proposed ligand-like role for mZP3.

Several lines of evidence strongly suggest that the glycan portion of ZP is responsible for the ligand activity: 1) exposure of the purified mZP3 to denaturants or high temperatures (which would alter the polypeptide backbone) does not abolish their ability to inhibit sperm-egg binding [2]; 2) various lectins, a class of proteins that bind to carbohydrate moieties with high affinity and specificity, inhibit or abolish binding of sperm to zona-intact eggs [70]; 3) inclusion of specific monosaccharides, disaccharides, oligosaccharides, and glycoproteins in an in vitro sperm-egg binding assay mixture inhibits or prevents binding of the gametes [6, 7, 71–73]; 4) treatment of zona-intact eggs with exoglycosidases inhibits or prevents sperm-egg binding [8]; 5) after digestion of mZP3 with pronase, the resulting glycopeptides (ranging in size from 1.5–6.0 kDa) retain sperm-binding activity [2]; and 6) sperm-binding activity is sensitive to trifluoromethane sulfonic acid treatment, an acid treatment known to break the glycosidic bonds between the monosaccharide residues of *N*-linked and *O*-linked oligosaccharides [2]. Taken together, these studies provide strong evidence that the glycan portion of the glycoproteins is the ligand for spermatozoa.

Despite numerous advances, considerable controversy remains regarding the precise identity of the sugar residue(s) responsible for the ligand activity of mZP3. For instance, Florman and Wassarman [11, 74] have reported that sperm-binding activity is associated with an *O*-linked oligosaccharide chain of an apparent molecular mass of 3.9 kDa, and more precisely with an α-linked galactosyl residue(s) at the nonreducing terminus of the *O*-linked oligosaccharide unit. Indeed, the gene encoding murine α1,3-galactosyltransferase, an enzyme that adds nonreducing terminal α-galactosyl residues to glycoproteins, is expressed in female (but not male) germ cells [75].

Three recent studies suggest that α-linked galactosyl residue(s) may not be important for sperm adhesion to the ZP. First, Shur and coworkers [8] have presented evidence suggesting that *N*-acetylglucosaminyl (rather than α-galactosyl) residues on mZP3 are recognized by galactosyltransferase (GalTase) present on mouse spermatozoa. The sperm (enzyme)-zona (substrate) complex remains stable until the next event in fertilization is triggered. Several lines of evidence listed in a review article are consistent with this conclusion [76]. Second, our recent studies suggest that mZP3 contains 2–3 kDa of *O*-linked glycans [54]. Treatment of de-*N*-glycosylated mZP3 with mild alkali in the presence of 1 M NaB^3H4 released a radiolabeled glycan,

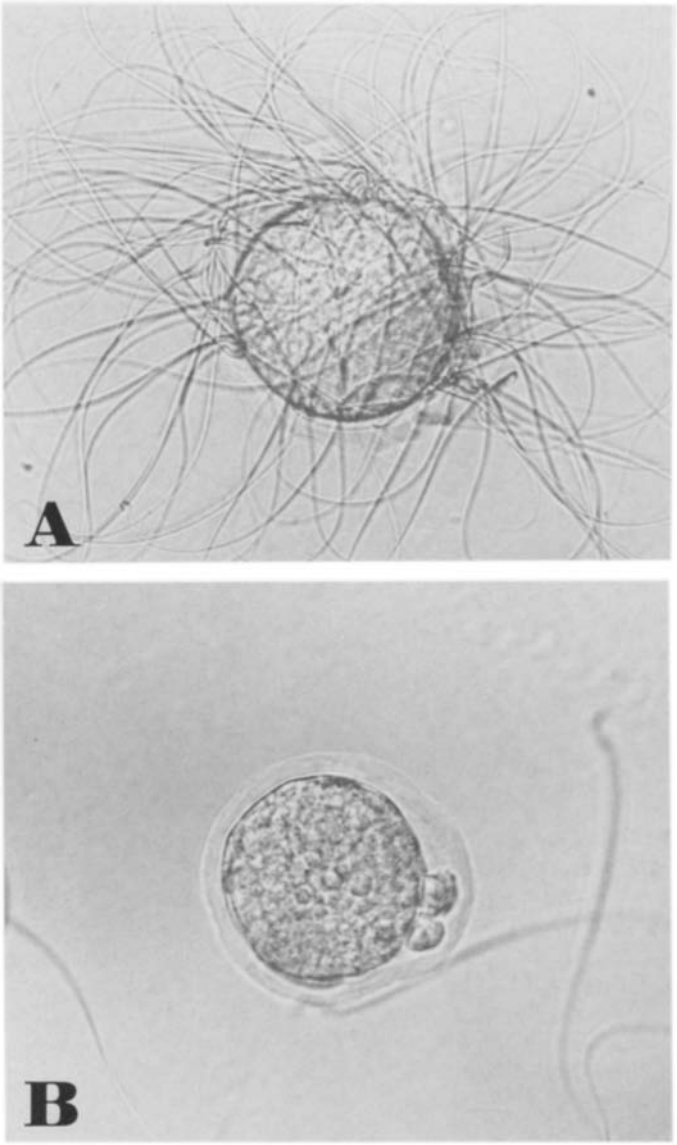

FIG. 1. Capacitated rat sperm bound to the ZP of rat oocyte. Many sperm bind to the zona-intact unfertilized egg in vitro (**A**); sperm do not bind to the fertilized egg (**B**). The photographs were taken following in vitro sperm-egg binding assay and washings to remove loosely bound spermatozoa.

identified as a trisaccharide with the structure GlcNAc-Galβ1,3GalNAcol [54]. The deduced *O*-linked oligosaccharide has a molecular mass of 586, suggesting that mZP3 contains 4–5 *O*-linked sugar chains. Interestingly, a recent study showed the presence of five *O*-glycosylation sites (serine sites) on mZP3 [77]. If the terminal *N*-acetylglucosaminyl residues of the *O*-linked trisaccharides are recognized by the mouse sperm GalTase, the trisaccharide could be a ligand (substrate) for the sperm enzyme [8]. Third, Thall et al. [78] used a gene disruption approach to address the role of Galα1,3Gal containing oligosaccharides in fertilization in the mouse. The authors generated mice that were deficient in the gene (α1,3GT) encoding the UDP-Gal;β-D-Galα1,3Gal-galactosyltransferase enzyme responsible for Galα1,3Gal synthesis and expression. Female α1,3 GT (−/−) mice yielded oocytes that were devoid of Galα1,3Gal epitopes. However, these mice were fully fertile, a result consistent with the investigators' conclusion

TABLE 1. Characteristics of mammalian ZP components.

Species	Zona components	Molecular mass (kDa) [Ref]	Isoelectric point (pI) [Ref]	No. of *N*-linked oligosaccharides	No. of *N*-glycosylation consensus sequence [Ref]	Molecular mass of *O*-linked oligosaccharides (kDa) [Ref]
Mouse	ZP1	200 [51] 185 [52]	4.1	+	6 [51]	
	ZP2	120 [51] 140 [52]	5.2 (4.9–5.6)	6	7 [53]	+ [54]
	ZP3	83 [51] 83 [52]	4.7 (4.2–5.2)	3/4	6 [55]	2–3 [54]
Rat	ZP1	205 [50]			4[a]	
	ZP2	119 [50]			8[a]	0 [50]
	ZP3	115 [50]			6 [56]	2 [50]
Hamster	ZP1	103 (dimer) [57]				
	ZP2	208 [58]				
	ZP3	56 [58]			4 [57]	
Human	ZP1	90–110 [58]	4.4–4.5 [58]			
	ZP2	64–78 [58]	4.5–4.6 [58]		6 [59]	
	ZP3	57–73 [58]	4.2–4.4 [58]		4 [60]	
Rabbit	rc85 [61]					
	rc75 [61]				7 [62]	
	rc55 [61]				6 [61]	
Porcine	ZP1	82 [63] 90 [64]	5.3 (4.3–6.4) [64]		7 [65]	
	ZP2	61 [63] 65 [64]	5.4 (4.3–6.6) [64]			
	ZP3	55 (α, β) [63] 55 (α, β) [65]	4.7 [64] 5.2 (3.8–6.7) [64]		ZP3α 5 [66] ZP3β 5 [67]	
	ZP4	21 [63] 25 [64]	5.6 (3.8–6.5) [64]		2 [68]	

[a] Akatsuka K, Yoshida-Komiya H, Tulsiani DRP, Orgebin-Crist M-C, Hiroi M, Araki Y. Molecular characterization of the rat zona pellucida glycoproteins ZP1 and ZP2 (unpublished results).

that Galα1,3Gal epitopes are not required for fertilization in the mouse.

Moreover, in an earlier study from another laboratory, treatment of zona-intact mouse eggs with almond glycopeptidase, an endoenzyme that hydrolyzes β-aspartyl-glucosamine of all classes of *N*-linked glycans, greatly reduced sperm-egg binding [79]. This last study implies that *N*-linked oligosaccharide units may have a role in sperm-egg binding. We recently reported the presence of *N*-linked high-mannose/hybrid oligosaccharide chains on mZP2 and mZP3 [80], and we have presented evidence suggesting that these oligosaccharide units may be a part of the recognition/binding site(s) for mannosidase present on sperm plasma membranes [6, 16, 81, 82]. This enzyme is a glycosidase and belongs to a class of enzymes responsible for hydrolytic cleavage of glycosidic bonds. It is generally accepted that the catalytic mechanism of action of these enzymes follows the model advanced for lysozyme [83]. In such a model, there are two important carboxylic acid moieties in the active site: one ionized and the other protonated (see scheme 18 in Ichikawa et al. [83]). The former moiety stabilizes the resulting oxocarbonium ion, either by ion pair interaction or by covalent bonding, whereas the latter moiety facilitates departure of the cleaving group. Thus all hydrolytic enzymes have a common catalytic mechanism. The common feature of this mechanism is the formation of an enzyme:substrate (sugar) intermediate before cleavage of sugar residues. Because of this interaction between the enzyme and substrate, it has been proposed that the cell surface glycosidases have a role in cell-cell adhesion [84]. Since purified sperm surface mannosidase cleaves negligible amounts of [^{3}H]mannosyl residues from [^{3}H]mannose-containing glycoproteins after 4 h of incubation [85], it is surmised that an intermediate complex of sperm enzyme: zona substrate is formed that leads to the next step in fertilization before a significant amount of mannosyl residues is cleaved.

In addition, we have demonstrated the occurrence of asparagine-linked (*N*-linked) poly-*N*-acetyllactosaminyl glycans on mZP2 and mZP3 [54]. A recent report has confirmed the existence of these glycans on mZP3 [77]. In other cells, *N*-linked polylactosaminyl chains have a variety of terminal sequences including Galα1,3Galβ1,4GlcNAcβ1,3Gal-R. This α-linked galactosyl terminal sequence has been reported in a number of well-defined *N*-linked glycoconjugates, including cell-surface components. If this sequence is also present on mZP3, it may have a role in sperm-egg binding. Furthermore, several studies from other laboratories have implicated other sugar residues in sperm-egg interactions. These include sialyl [7] and mannosyl [6] in addition to α-galactosyl [10] and *N*-acetylglucosaminyl [8] residues.

It is interesting that like mZP3, the porcine ZP glycoprotein (pZP3) has been found to contain sperm receptor activity. The pZP3 (M_r 55 000) is also highly glycosylated, containing *N*-linked and *O*-linked oligosaccharides, and poly-*N*-acetyllactosaminyl glycans [63]. A recent study presented a structural analysis of *N*-linked glycan chains of pZP3 released and labeled following hydrazinolysis [86]. The labeled oligosaccharide chains were separated into neutral (28%) and acidic (72%) chains by anion-exchange HPLC. Evidence that the mixture of neutral *N*-linked glycans caused an inhibition of sperm-egg binding in vitro is consistent with the authors suggestion that *N*-linked glycans of pZP3 have a significant role in sperm recognition and binding [87].

It is important to point out that the mZP3 and pZP3, as

TABLE 2. Potential receptors for ZP that have been suggested to have a role in sperm-egg interaction.

Species	Proposed Receptors	References
Mouse	β-1, 4-galactocyltransferase	Shur [76]
	Fucosyltransferase	Ram et al. [90]
	56-kDa galactose-binding protein (sp56)	Cheng et al. [92]
	95-kDa protein	Leyton and Saling [93]
	α-D-mannosidase	Cornwall et al. [6]
	Protease-sensitive site	Benau and Storey [91]
	Sulfoglycolipid immobilizing protein	Tanphaichitr et al. [96]
Rat	α-D-mannosidase	Tulsiani et al. [81, 85]
Hamster	Fucose-binding protein	Huang et al. [9]
Human	PH-20	Lin et al. [97]
	α-D-mannosidase	Tulsiani et al. [82]
	Mannose-binding protein	Benoff et al. [98]
	FA-1	Naz et al. [99]
	Selectin-like molecule	Dell et al. [100]
Macaque	PH-20	Cherr et al. [101]
Porcine	Fucose-binding protein	Topfer-Petersen et al. [102]
	Proacrosin	Jones et al. [103]
	Sperm protein 38 (sp38)	Mori et al. [104]
	150-kDa protein (zonadhesin)	Hardy and Garbers [105]
	APz	Peterson and Hunt [106]
Rabbit	RSA	O'Rand et al. [107]
	Sperm protein 17 (sp17)	Richardson et al. [108]
Guinea pig	PH-20	Primakoff et al. [109]

well as the glycans used for the in vitro sperm-egg binding inhibition (see above), were purified from ovarian ZP rather than from eggs collected from the oviduct. Although it has been reported that mZP3 purified from ovarian and oviductal oocytes are chemically and functionally comparable [88], other reports suggest structural changes on the surface of eggs exposed to the oviductal environment [89]. The reported structural changes could be explained by the binding of an OGP or of other glycoproteins such as glycosyltransferases discussed above. It is also possible that the glycosyltransferase activities present in the oviductal fluid are involved in modification of the terminally exposed glycan chains on the surface of the ZP. Any alteration in the sugar residues of the ZP glycoproteins in the oviduct will be expected to have an effect on the sperm receptor's ability to recognize and bind to these altered glycan units. These studies point out the possibility that the glycan and its complementary receptor in the microenvironment of the oviduct may be different from those identified by in vitro sperm-egg binding studies.

Several sperm surface proteins from various species have been proposed to function as receptor molecules on spermatozoa (Table 2). Extensive studies in the mouse have resulted in the identification of several putative receptors. Among the antigens that investigators have suggested may serve as receptors on mouse spermatozoa are sperm surface galactosyltransferase [76], fucosyltransferase [90], a trypsin inhibitor-sensitive site [91], a ZP3-binding 56-kDa sperm protein [92], a 95-kDa sperm plasma membrane protein [93] that has been suggested to be a unique hexokinase [94], and a 115-kDa α-D-mannosidase present on the plasma membrane of spermatozoa from several species [16]. A trypsin-like serine protease (acrosin/proacrosin) present in the sperm acrosome as well as on the surface of spermatozoa has long been believed to have a receptor-like role in the mouse and pig. However, a recent study showed that sperm from mice carrying a targeted mutation of the acrosin gene can bind to and penetrate mouse oocytes [95]. This result suggests that acrosin/proacrosin is not essential for fertilization in the mouse.

Human spermatozoa are known to possess α-D-mannosidase [82] and a mannose-binding protein [98]. It has been suggested that each of these macromolecules has a receptor-like role in binding to mannose-containing oligosaccharide on ZP. Interestingly, mZP2 and mZP3 have been shown to contain high-mannose/hybrid-type oligosaccharides [80]. It has also been suggested that a human sperm antigen designated FA-1 [99] and a selectin-like molecule [100] bind to homologous ZP.

Although the pZP has been the subject of numerous studies, very few studies have been directed toward identifying pig sperm proteins that interact with the zona-intact egg in a species-specific manner [102–104]. Using immobilized pZP as an affinity medium, Hardy and Garbers [105] have recently reported the presence of an antigen, named zonadhesin, on porcine spermatozoa. The purified sperm protein has a molecular mass of 150 kDa and consists of two nonidentical subunits of 105 and 45 kDa. Zonadhesin, expressed by the haploid spermatids, is homologous to both von Willebrand factor and mucins. Receptor-like roles have been proposed for other porcine sperm plasma membrane proteins, including a fucose-binding protein [102], a 53-kDa fucosylated protein that interacted with the carbohydrate moiety of ZP glycoprotein [103], sperm protein 38 [104], and adhesion protein (APz) [106].

An integral membrane protein, PH-20, with a molecular mass of 64 kDa, has been reported on both the plasma membrane and inner acrosomal membrane of guinea pig [109] and macaque [101] sperm. The antigen, localized on the posterior head region of sperm, is thought to be essential in sperm adhesion to ZP. However, its complementary molecule on ZP has not yet been identified, and it is not known whether the guinea pig and macaque sperm adhesion to ZP is a carbohydrate-mediated event.

This brief summary of the research of numerous investigators strongly suggests that a carbohydrate recognition

mechanism is involved in sperm-zona binding. The mechanism underlying the interaction of the opposite gametes remains one of the principal unresolved issues in reproductive biology. Most investigators agree that complementary molecules initiate sperm-egg recognition by a receptor-ligand mechanism. However, the identity and chemical nature of the receptor (on sperm) and ligand (on ZP) are not yet known. Also not known are 1) whether sperm-egg interaction is a net result of a single or of multiple receptor-ligand interactions and 2) whether the complementary molecules identified based on in vitro sperm-egg binding assay will be the same in the microenvironment of the oviduct.

CONCLUSIONS

This minireview clearly supports a role for carbohydrate on the surface of ZP, and complementary carbohydrate-binding enzymes or lectin-like molecules on sperm plasma membranes in sperm-egg interaction. Although the sequence of events during fertilization varies among species, the mechanisms underlying sperm capacitation, sperm-egg interaction, and induction of the acrosome reaction show many similarities. Despite its enormous importance, the detailed mechanism regulating these events during the fertilization process continues to be one of the principal unresolved issues in reproductive biology. In most cases, the identity and chemical nature of the receptor (on sperm) and ligand (on zona) remain uncertain. The evidence for the presence of a large diversity in the structure of glycans on ZP suggests that perhaps several ligand (glycan)-receptor interactions are involved before gamete interaction and successful fertilization. A recent report [110] strongly suggests that the initial molecular interaction between sperm and ZP is a complex binding event and that it may reflect multiple sperm proteins with multivalent ZP3. Understanding the mechanisms underlying capacitation, sperm-egg interaction, and the acrosome reaction in fertilization awaits new approaches and development of new and more sensitive methods for the characterization of carbohydrate residues and their complementary molecules.

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