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Invited Review

The role of carbohydrate residues in mammalian fertilization

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Summary. The fertilization process in mammals involves binding and fusion of free-swimming sperm and ovulated eggs. This review focuses on the role of carbohydrate residues in the process of sperm-egg interaction in mammals. The zona pellucida (ZP), the acellular glycoprotein coat surrounding the egg is highly glycosylated and posssess both Asn- (N-) linked and Ser/Thr- (O-)linked oligosaccharides, with an extreme structural heterogeneity between the different species. Different carbohydrates on ZP3, such as Galactose in α-linkage, N-acetylglucosamine in ß-linkage, were suggested as the complementary sperm receptors, mediating the primary binding between the spermatozoon and the ZP. Several suggested complementary ZP3 binding proteins on the sperm are sp56, ß-1,4-galactosyltransferase and p95. Some carbohydrate residues of the ZP undergo post-fertilization modifications that might alter the sperm receptor, thus assisting in the establishment of the block to polyspermy. The studies summarized in this review imply a main role for the carbohydrate residues in the process of sperm egg interaction.

Key words: Glycoproteins, Zona pellucida, Sperm egg interaction

Introduction

The fertilization process in mammals involves binding and fusion of free-swimming sperm and ovulated eggs. In recent years accessible information is accumulating regarding the role and importance of carbohydrate residues during sperm egg interaction. This process is species specific and consists of defined events that occur in compulsory order (Fig. 1): *Attachment*: A loose, nonspecific association between the spermatozoa and the zona pellucida (ZP), which is an acellular, glycoprotein coat surrounding the egg; *Primary binding*: A firm binding between spermatozoa and the ZP, that follows attachment. The binding is species specific and is mediated by complementary receptors on the ZP (sperm receptors) and the sperm head plasma membrane (ZP receptors); *Acrosome reaction*: The bound spermatozoa undergoes the acrosome reaction (AR), an exocytotic process that involves fusion and vesiculation of the sperm plasma and outer acrosomal membranes. This process results in release of the enzymatic acrosomal content and disclosure of the inner acrosomal membrane, thus exposing the ZP to the lytic enzymes of the acrosome that assists in ZP penetration. *Secondary binding*: Acrosome-reacted sperm remains bound to the ZP via secondary binding. This binding involves components of the sperm inner acrosomal membrane and secondary sperm receptors on the ZP; *Penetration*: The spermatozoon penetrates through the ZP, probably by combination of mechanical force created by the vigorous tail beatings, and enzymatic digestion of the ZP glycoproteins by proteinases associated with the acrosomal content (Fig. 2); *Fusion*: The sperm reaches the perivitelline space (PVS) and fuses with the egg plasma membrane, thereby fertilizing the egg. Fusion is followed by the cortical reaction (CR), an exocytotic process in which the cortical granules release their enzymatic content into the PVS. Cortical reaction results in modification of the ZP glycoproteins, zona hardening and establishment of the block to polyspermy. (Reviewed by Wassarman, 1988, 1990; Sidhu and Guraya, 1991; Yanagimachi, 1994; Snell and White, 1996).

This review will focus on the role of carbohydrate residues in the process of sperm-egg interaction in mammals.

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Zona pellucida structure and function

The ZP is a porous, extracellular matrix composed of long, interconnected filaments. The ZP is morphologically segregated and there is an asymmetry between the inner or outer surface, as was shown in the hamster (Phillips and Shalgi, 1980; Ahuja and Bolwell, 1983), and in the house musk shrew (Suprasert et al., 1989).

For most mammalian species studied, the ZP appears

to comprise only three glycoproteins: ZP1, ZP2, ZP3 (mouse: Bleil and Wassarman 1980; human: Shabanowitz and O'Rand 1988; hamster: Moller et al., 1990; rat: Araki et al., 1992). The glycoproteins are synthesized and secreted to form the ZP during the process of oocyte growth (Epifano et al., 1995a; Tong et al., 1995). Female mice homozygous for the null mutation (mZP3 -/-) showed complete absence of a ZP on growing oocytes (Liu et al., 1996). The genes encoding for ZP1, ZP2 and ZP3 in several mammalian species were cloned, and a relatively-high degree of conservation of genomic sequence and primary polypeptide structure between different mammalian species was found (mouse: Kinloch et al., 1988; Ringuette et al., 1988; hamster: Kinloch et al., 1990; human: Chamberlin and Dean, 1990; Liang and Dean, 1993; porcine: Taya et al., 1995). Recently it was reported that mouse ZP1 encodes a ZP protein that is partially conserved in mouse ZP2 and in fish, thus indicating to a conservation of the egg-envelope protein in species diverged 650 million years ago (Epifano et al., 1995b).

The ZP1, ZP2 and ZP3 glycoproteins in the mouse are 200 kD, 120 kD and 83 kD respectively (Bleil and Wassarman, 1980). High glycosylation of the ZP glycoproteins contributes to the diversity in molecular mass between the glycoproteins from different species. Numerous studies reveal the different role of each glycoprotein during the fertilization process. While ZP3 contains the primary sperm receptor and induces the acrosome reaction (Bleil and Wassarman, 1983; Wassarman, 1990; Yanagimachi, 1994), ZP2 is probably responsible for the secondary binding of the acrosome-reacted sperm (Bleil et al., 1988).

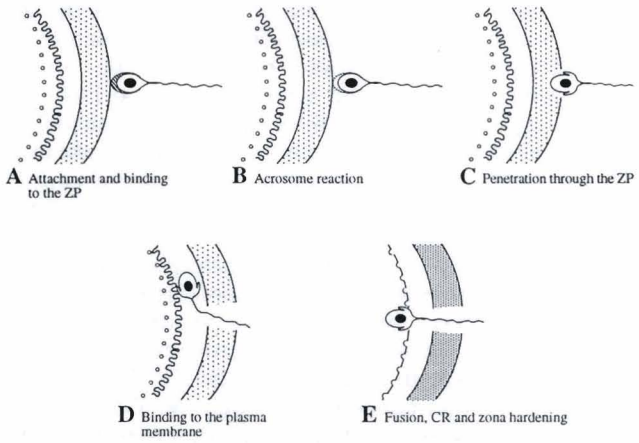

Fig. 1. Steps characterizing mammalian sperm-egg interaction.

Carbohydrate composition of the ZP

All ZP glycoproteins studied so far are highly glycosylated and posssess both Asn- (N-)linked and Ser/Thr- (O-)linked oligosaccharides (OS) (Wassarman, 1988; Noguchi and Nakano, 1993; Hokke et al., 1993, 1994; Nagdas et al., 1994). These OS largely contribute to the extensive heterogeneity of the ZP glycoproteins observed between the different species.

The structure of ZP linked carbohydrate chains have been studied in numerous species and revealed an extreme structural heterogeneity. Galactosyl (Gal) in α linkage was found on the O-linked OS chains of mouse ZP3 (Florman and Wassarman, 1985; Bleil and Wassarman, 1988), as well as N-acetylglucosaminyl (GlcNAc) residues (Miller et al., 1992). Mouse ZP2 and ZP3 were shown to possess N-linked high mannose/

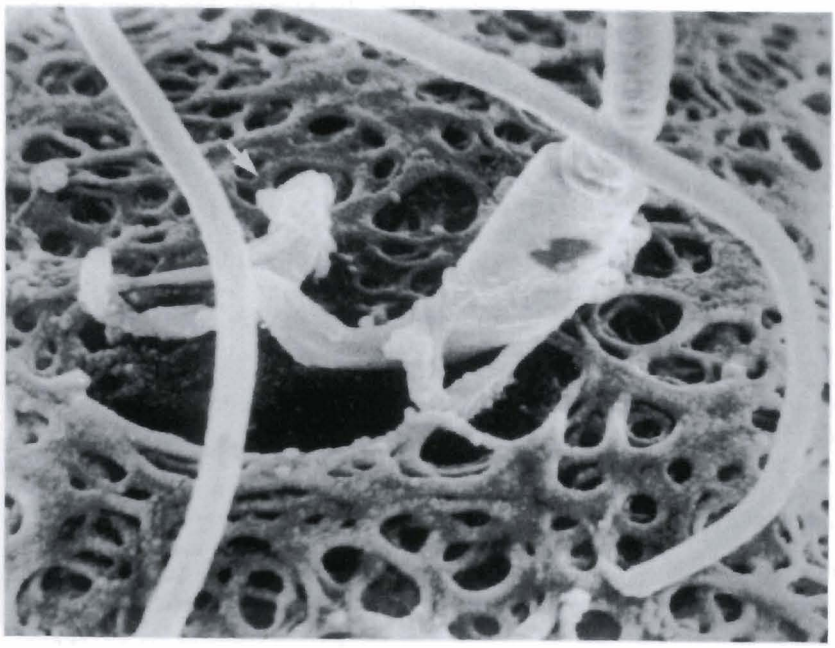

Fig. 2. A scanning electron micrograph of a golden hamster spermatozoon that had undergone the AR and is about to enter the ZP. The acrosomal ghost in seen on the ZP (arrow). x 5,000. (Shalgi and Phillips, unpublished)

hybrid OS chains (Tulsiani et al., 1992), N-linked polylactosaminyl glycans and O-linked trisaccharide with the structure GlcNAc-Galß1,3GalNAcol (Nagdas et al., 1994). In addition, it was shown that N-linked OS of mouse ZP2 and ZP3 are mainly fucosylated with tri- and tetra-antennary complex-type chains that have essentially similar structures (Noguchi and Nakano, 1993).

A qualitative characterization of the rat ZP OS indicated that O-linked OS units in the rat ZP3 are only 20-25% of those reported in the mouse ZP3 (Florman and Wassarman 1985; Araki et al., 1992). N-linked OS of high mannose/hybrid are present, as well as tri- and tetra-antennary complex chains on both ZP2 and ZP3. A significant amount of those mannosylated OS unites may be sulfated (Araki et al., 1992).

A number of histochemical studies examined the carbohydrate distribution on the ZP of different mammalian species. Various studies used lectins as sugar-specific probes. Lectins are sugar-binding proteins or glycoproteins of non-immune origin which agglutinate cells and/or precipitate glycoconjugates having saccharides of appropriate complementarity (Goldstein et al., 1980). Analysis of lectin binding to ovarian ZP of several species revealed that some sugar moieties, such as mannose (Man), ß-Gal and GlcNAc, are commonly present in most mammalian ZP, while others, such as disaccharide ß-galactose-D-N-acetyl-galactosamine (ß-Gal-D-GalNAc) are common only in rodents (Skutelsky et al., 1994). Rat ZP from ovarian and ovulated eggs were characterized by the presence of Man, Glc, α- and ß-Gal, GalNAc, fucose (Fuc) (Shalgi et al., 1991, Raz et al., 1996), and by the presence of sulfated groups (Aviles et al., 1994). These results support previous studies where ZP of mouse, rat and hamster eggs were shown to bind different lectins, with species-specific differences in the lectin-binding intensity and pattern (Nicolson et al., 1975; Oikawa et al., 1975; Yanagimachi and Nicolson, 1976; Wu et al., 1984). However, phylogenetically-close species, such as the rodents and rabbits, demonstrate high similarity in ZP saccharides, expressed by binding of some lectins (Skutelsky et al., 1994). The human ZP is characterized by Man, GlcNAc, ß-Gal and masked ß-Gal-(1-3)GalNAc that was exposed only after removing terminal sialic acid residues (Maymon et al., 1994).

Some of the lectins used exhibited an uneven distribution of their receptor sites (Nicolson et al., 1975; Shalgi et al., 1991; Aviles et al., 1994; Skutelsky et al., 1994; Raz et al., 1996), implying to an uneven distribution of carbohydrate residues on the ZP of these species.

Carbohydrate residues on the ZP as the sperm receptor

Several forms of evidence refer to different carbohydrate residues present on the ZP as key molecules in the process of sperm-egg interaction. Various studies in the mouse concluded that the sperm binding site is part of O-linked OS chains (Florman and Wassarman, 1985; Wassarman and Litscher, 1995), while in other species the involvement of O-linked or N-linked OS in sperm binding is still controversial (Yurewicz et al., 1991; Yonezawa et al., 1995).

In the mouse, egg ZP3 has been shown to serve in species-specific binding (Wassarman and Litscher, 1995). Different carbohydrates on ZP3 were suggested as the complementary sperm receptors, mediating the primary binding between the spermatozoon and the ZP (Fig. 3).

a. Galactose in α-linkage

In the mouse α-Gal at the non-reducing terminus of the O-linked OS chain of ZP3 was shown to have a crucial role in sperm binding. Removal of O-linked oligosaccharides, and specially galactose residues from O-linked OS of mouse ZP3, destroyed its sperm receptor activity (Florman and Wassarman 1985; Bleil and Wassarman, 1988). It was recently demonstrated that synthetic O-linked-related OS constructs possessing Gal at their non-reducing terminus were effective inhibitors of mouse gamete interaction in vitro. This inhibition was dependent on the size and branching pattern of the OS (Litscher et al., 1995).

The enzyme α1,3-galactosyltransferase (α1,3-GT) adds non-reducing terminal α-galactosyl residues to glycans and is responsible for Galα1-3Gal synthesis and expression. The gene encoding this enzyme in murine was shown to be expressed in female germ cells, but not

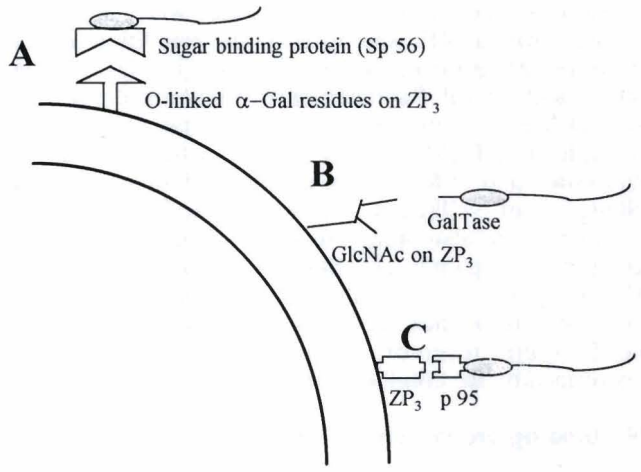

Fig. 3. Mammalian sperm binding to the ZP. The three main hypothesizes for ZP-sperm binding, established in the mouse system are presented. **A.** O-linked oligosacharide on ZP3 terminating in α-Galactose, binds to sp56, which is a peripheral membrane sugar binding protein on the sperm head. **B.** The carbohydrate residue N-acetylglucosamine (GlcNAc) on ZP3, binds to a plasma membrane form of the enzyme ß-1,4-Galactosyltransferase (GalTase) which is located on the sperm head. **C.** ZP3 binds to a sperm tyrosine-phosphorylated protein p95.

in spermatocytes or spermatides (Johnston et al., 1995). This finding is consistent with the model of sperm-egg binding in which a non-reducing terminal α-galactosyl residue of ZP3 is required. The biological significance for the suppression of α1,3-GT expression in male germ cells was suggested to prevent sperm-sperm aggregation (Johnston et al., 1995). In this context, mutant female mice deficient of the gene α1,3GT (-/-) yielded oocytes that are devoid of the Galα1-3Gal epitope, but were fully fertile (Thall et al., 1995). These results imply that the epitope Galα1,3Gal is neither required, nor is it the only requirement for mouse fertilization.

b. N-acetylglucosamine in ß-linkage

A number of studies have reported that in the mouse sperm initially bind to the ZP by an interaction between sperm ß-1,4-galactosyltransferase (GalTase) and terminal N-acetylglucosamine (GlcNAc) residues on ZP3 (Miller et al., 1992; Shur, 1993, Gong et al., 1995). It was shown that sperm receptor activity is lost after GlcNAc residues are masked or removed by N-acetyl-glucosaminidase (Miller et al., 1992). GalTase was found as the predominant glycosidase in mouse eggs cortical granules, which are released at egg activation into the PVS. There, it has been proposed that the enzyme acts on ZP3 to remove terminal GlcNAc residues, thereby inactivating ZP3 sperm receptor activity and establishing the block to polyspermy (Miller et al., 1993).

c. Other carbohydrate residues

Various other carbohydrate residues were suggested to have a role as ZP3 sperm receptors. Inhibition assays of sperm-ZP binding revealed a role for D-Man on human and rat ZP. Pretreatment of human spermatozoa with D-Man completely inhibited sperm penetration through the ZP (Mori et al., 1989), while α-methyl-mannoside and D-Man were potent inhibitors in the rat (Shalgi et al., 1986). L-Fuc and an L-Fuc polymer, fucoidin, were also shown to have a role in sperm-ZP recognition in guinea pig, hamster rat and human eggs (Huang et al., 1982; Shalgi et al., 1986), implying a common role of these residues as a general element in the ZP sperm receptor, whereas species specificity is determined by the combination of other carbohydrates.

ZP3 binding protein on the sperm

The mammalian sperm acquire the ability to fertilize the egg during a period of several hours post-ejaculation. This process, in which the spermatozoon acquires fertilization potential, is termed capacitation. During capacitation, there are marked changes in the distribution of carbohydrates and carbohydrate-related proteins (glycoproteins, lectins) on the sperm surface. Redistribution of surface glycoproteins during capacitation was shown in human, rabbit, boar and rat sperm (Villarroya and Scholler, 1987; O'Rand, 1979; Shalgi et al., 1990; Topfer-Petersen et al., 1990). Redistribution of surface sugar residues was shown by changes in lectin binding between capacitated and noncapacitated sperm of human, golden hamster, rabbit, guinea pig and mouse (Gordon et al., 1975; Kinsey and Koehler, 1978; Schwarz and Koehler, 1979; Cross and Overstreet, 1987; Benoff et al., 1993). Accordingly, a temporal surge in glycosyltransferase activity in oviductal fluid at the onset of ovulation in the hamster was demonstrated (Tulsiani et al., 1996). The aforementioned changes, concerning modifications in surface properties of mammalian sperm, could reflect the variability in the identity of egg receptors observed in different species.

Currently, several candidates have been suggested for the sperm component that binds to ZP3 and serves as the ZP receptor on the sperm plasma membrane. The nature of this component is still controversial, and a few of the more prominent candidates proposed, are described hereby (Fig. 3).

a. Sp56

Sp56 is a 56 kDa MW, ZP3 binding protein. It was identified in mouse sperm and was shown to be localized to heads of acrosome-intact, but not acrosome-reacted, sperm (Bleil and Wassarman, 1990). The protein sp56 binds to mZP3 but not mZP2 affinity columns. It binds galactose but not GlcNAc affinity columns, and it binds specifically to those OS that inhibit mouse sperm-egg interaction in vitro (Cheng et al., 1994). Recently the complementary DNA encoding sp56 was isolated and its primary sequence indicates that sp56 is a member of a super-family of protein receptors. Sp56 expression is restricted to mouse spermatids and spermatozoa, implying that transcription activation of the sp56 gene occurs in haploid cells. The presence or absence of sp56 on sperm from different species accounts for the species specificity of sperm binding to the ZP (Bookbinder et al., 1995). Therefore sp56 has been suggested as the specific receptor that binds to Gal residues on the mouse ZP.

b. ß-1,4-Galactosyltransferase

The enzyme ß-1,4-Galactosyltransferase (GalTase) is traditionally found in the endoplasmic reticulum and Golgi apparatus, where it participates in biosynthesis of glycoconjugates by catalyzing the transfer of Gal from the sugar nucleotide (UDPGal) to terminal N-acetyl-glucoseamine (GlcNAc) residues of glycoproteins and glycolipids. It was reported that GalTase is also located on the plasma membrane of mouse sperm, directly overlying the acrosome (Lopez and Shur, 1987; Shur and Neely, 1988). It was suggested that the enzyme GalTase serves as the sperm receptor by binding to terminal GlcNAc residues of mZP3 OS. The sugar donor is absent from the extracellular environment, thus the enzyme functions as a lectin (Shur and Hall, 1982). It

was demonstrated that sperm GalTase interacts specifically with ZP3 but not ZP1 or ZP2. In contrast, non-sperm GalTase (bovine-milk GalTase) interacts with all ZP glycoproteins, thus demonstrating a more strict substrate specificity for the sperm enzyme (Miller et al., 1992). ZP-sperm binding was shown to induce aggregation of GalTase in the plasma membrane, which is responsible for induction of the acrosome reaction (Macek et al., 1991). After the AR, the enzyme is redistributed to the lateral surface of the sperm, where it can no longer interact with ZP3 OS (Lopez and Shur 1987; Miller et al., 1992). Competitive GalTase substrates, as well as anti-GalTase antibodies, affinity-purified soluble GalTase or UDP-Gal, all inhibit sperm-egg binding (Lopez et al., 1985; Miller et al., 1992). Furthermore, removal of ZP3 terminal GlcNAc residues by N-acetylglucosaminidase digestion, caused a loss of ZP3 sperm receptor activity (Miller et al., 1992). Manipulation of GalTase levels in transgenic mice indicated the importance of optimal, but not maximal, levels of surface GalTase expression. Sperm from transgenic mice over-expressing significantly high levels of GalTase bound more ZP3 than the wild-type sperm, but were relatively unable to bind eggs. This phenomena resulted from binding of epididymal glycoside substrates, thus masking GalTase residues, or by hypersensitivity of transgenic sperm to ZP3, resulting in precocious acrosome reaction (Youakim et al., 1994). On the other hand, overexpression of GalTase caused higher rates of G protein activation than in that of the wild-type sperm, implying that the cytoplasmic domain of the cell surface GalTase enables it to function as a signal-transducing receptor during the process of sperm-egg interaction (Gong et al., 1995).

c. P95

P95 is a 95 kDa MW sperm protein localized on mouse sperm heads. Iodinated ZP3 of mouse binds specifically to p95 of sperm proteins on Western blots (Leyton and Saling, 1989a). It is localized at the acrosomal region of the mouse sperm head, and its aggregation was demonstrated as the initiating signal leading to acrosomal exocytosis (Leyton and Saling, 1989b). P95 is a phosphotyrosin-containing protein that possesses intrinsic tyrosine kinase activity, stimulated by solubilized ZP proteins (Leyton et al., 1992). In view of all this data, it was proposed that p95 in mouse sperm is a protein tyrosine kinase (PTK) receptor that is a member of the family of PTK receptors involved in signal transduction in somatic cells. Recently, a human 95 kDa homologue protein was demonstrated, and its structure and the primary sequence was determined, as well as its biological activity (Burks et al., 1995). In a recently published paper it was argued that the amino acid sequence of p95 corresponds to mouse hepatoma hexokinase, and that p95 is actually a unique phosphorylated form of this glycolytic enzyme present in the mouse sperm (Kalab et al., 1994). Generation of mouse monoclonal antibodies raised against p95 (LL95) demonstrated a unique binding of the antibodies to the PTK, but not to hexokinase. By using LL95 and anti-hexokinase antibodies it was also shown that hexokinase and PTK activity can be distinguishable on the basis of cellular localization and biological affects (Leyton et al., 1995).

d. Other candidates for the ZP receptor on sperm

Another sperm surface molecule that has been suggested as the potential sperm receptor is α-D-mannosidase. This enzyme activity was recognized in the plasma membrane of rat, mouse, hamster and human sperm (Tulsiani et al., 1989; Chen et al., 1995). In the rat it was shown to be localized on the periacrosomal region of the sperm head, synthesized in the testis and converted to its mature form during the sperm epididiymal maturation (Tulsiani et al., 1995). This mannosidase differs from other mannosidases identified in other tissues, including the mannosidase present in the acrosome (Tulsiani et al., 1989). Incubation of mouse spermatozoa in the presence of inhibitory sugars such as α-methyl Man, α-methyl Glc or D-Man resulted in a dose-dependent decrease in sperm-egg binding (Cornwall et al., 1991). This theory is in agreement with another study (Tulsiani et al., 1992) where both mouse ZP2 and ZP3 were shown to possess N-linked high mannose/hybrid OS chains.

Another potential ZP-binding molecule in the sperm is the galactosyl receptor. The rat and rabbit testis galactosyl receptor (54kDa) was identified in the plasma membrane overlying the dorsal portion of the sperm head (Abdullah and Kierszenbaum, 1989; Abdullah et al., 1991). Recently a similar molecule was characterized in human testis and sperm (Goluboff et al., 1995). This protein functions as a lectin to bind Gal residues, and thus could have a role in sperm-ZP binding during fertilization.

A different candidate to serve as the sperm receptor to the ZP is the sperm adhesin AWN-1 that was recognized on the external surface of the acrosomal cap of boar sperm (Sanz et al., 1992; Dostalova et al., 1994; Topfer-Petersen and Calvete 1995). AWN-1 was also shown to bind preferentially to [±NeuAcα(2-3/6)]-Galß(1-3)-GalNAc O-linked OS structures (Dostalova et al., 1995), which are similar to OS residues found in minor O-linked carbohydrate chains of the porcine ZP glycoproteins (Hokke et al., 1994).

Various studies suggest the existence of more than one candidate for the ZP and sperm receptors, even in the same species. This variability can indicate the existence of more than one specific gamete interaction molecule for each species, and may represent redundant functions to ensure that the important event of gamete interaction occurs without fail. Unique binding molecules found in the ZP or sperm of different mammalian species may represent the existence of a species-specificity barrier at the level of gamete

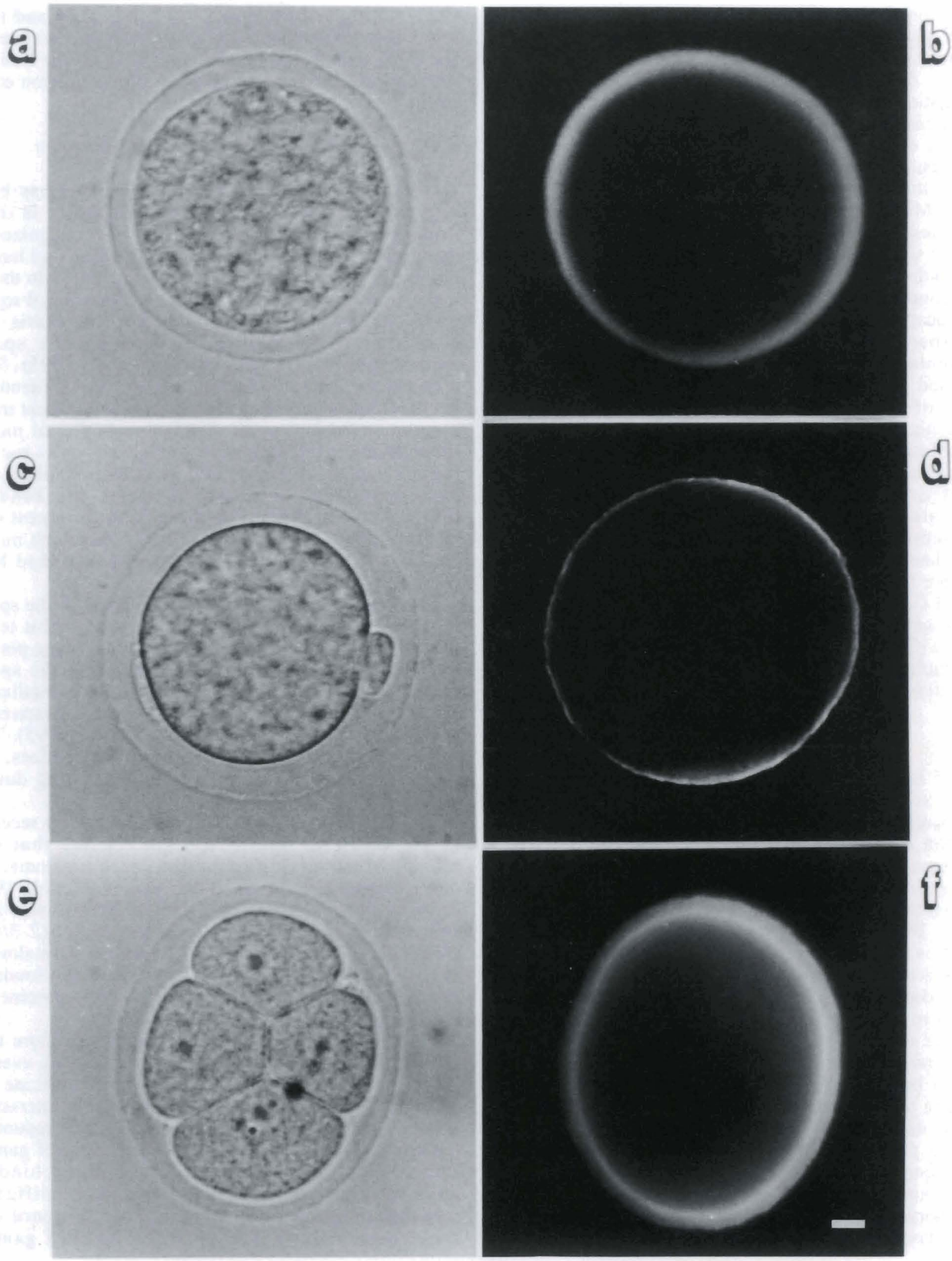
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Fig. 4. Changes in the binding pattern of the lectin RCA-I to ZP of rat eggs after fertilization. Paired light (left) and epifluorescent (right) photomicrographs of fixed eggs incubated with the lectin *Ricinus communis* (RCA-I). **b.** Homogeneous binding (ovulated egg) **d.** Uneven binding to the outer zone of the ZP (parthenogeneticaly activated egg). **f.** Uneven binding to the inner and outer zones of the ZP (fertilized, 4-cell embryo). Bar: 10 μm. (Raz et al., 1996).

interaction.

Post-fertilization changes in the carbohydrate residues of the ZP

Fusion of the spermatozoon with the egg plasma membrane induces exocytosis of cortical granules (CG reaction) (Szollosi 1967). The content of the CG alters the ZP glycoproteins, thus establishing a block to polyspermy (Wolf and Hamada, 1977; Ducibella et al., 1990; Ducibella, 1991). The nature of the alterations which the ZP undergoes during fertilization is only partially understood. ZP2 undergoes limited proteolysis and is converted to ZP2f (Bleil et al., 1981; Moller et al., 1990), while ZP3, which acts as the primary sperm receptor, losses its ability to bind acrosome intact sperm (Hedrick et al., 1987; Moller et al., 1990; Kalab et al., 1991). The exact nature of the CG content and the mechanism by which they modify the ZP is yet not fully understood. Several studies have suggested the involvement of different enzymes in this process: trypsin like proteinase (Gwatkin et al., 1973, Wolf and Hamada 1977; Lindsay and Hedrick 1989), serine-like proteinase (Tawia and Lopata 1992), chymotrypsin-like proteinase (Lindsay et al., 1992) or cystein-like proteinase (Kalab et al., 1991). Some such proteinases may be involved in ZP2 conversion to ZP2f, causing ZP hardening (Moller and Wassarman, 1989). In contrast to ZP2, ZP3 modification is indistinguishable by SDS-PAGE. The loss of ZP3 sperm-binding activity must, therefore, result from subtle, but crucial, modifications in its structure and/or biological activity.

A few studies suggest that post-fertilization modifications of ZP3 include biochemical alterations in carbohydrate residues. These modifications can alter the sperm receptor in a way that will prevent additional sperm binding, thus assisting to the establishment of the block to polyspermy (Florman et al., 1984). It has been shown that ZP3 from fertilized mouse eggs loses its GalTase binding sites, as a result of N-acetyl-glucosaminidase activity released from the CG. The enzyme removes terminal N-acetylglucosamine residues from ZP3, thereby inactivating the ability of ZP3 to serve as a sperm GalTase receptor, and leading to a loss of the sperm binding activity of ZP3 (Miller et al., 1992, 1993).

A modification in the murine ZP3 reactivity to various lectins was demonstrated, implying to a possible decrease in GalNAc (Shimizu and Ito 1986). Immuno-histochemical studies in the light and electron microscopic levels revealed post-fertilization modifications in the distribution of ß-Gal residues on the rat ZP (Fig. 4) (Raz et al., 1996), as well as a shift in the binding density of various lectins (Aviles et al., 1996). These post-fertilization changes in ZP carbohydrate residues may represent different aspects of the general concept of carbohydrate residues being part of the sperm receptor.

Conclusions

The field of mammalian fertilization, and in particular the identity of the molecules participating in gamete interaction has progressed in the recent years to the molecular level. Carbohydrate-protein interactions appear to be part of the species-specific binding site of the free-swimming sperm and the egg ZP. The enormous heterogeneity of carbohydrate structures and their potential for structural diversity, permits the existence of variable and specific recognition determinants. This feature of carbohydrate-mediated cellular adhesion is believed to play an important role in cellular adhesion in general, and is extremely appealing in regard to the species-specific gamete recognition and interaction. Multiple gamete recognition receptors may exist in each species in order to assure successful binding and fertilization.

Future studies may shed more light on the identity of ZP and sperm receptors in the different mammalian species, leading to better understanding of the nature of these interactions. Further knowledge may be important in clarifying some unexplained infertility etiologies, and in developing new contraceptive methods.

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