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Glycoconjugates in sperm function and gamete interactions: how much sugar does it take to sweet-talk the egg?

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Abstract. Glycoconjugates in the mammalian reproductive tract are critical components of the molecular mechanisms that control sperm maturation, sperm transport and gamete interactions. In the oviduct of many species, sperm transport and maturation are regulated by protein-carbohydrate interactions that form a sperm reservoir. Subsequently, gamete interactions are mediated by the binding of lectin-like sperm proteins with carbohydrate moieties on the zona pellucida. The sperm glycocalyx is extensively modified during sperm transport and maturation. Multiple functions have been proposed for this dense carbohydrate layer overlying the sperm plasmalemma, and sperm-surface carbohydrates have been implicated in immune-mediated human infertility. The structure and function of glycoconjugates in the oviductal sperm reservoir, the zona pellucida, and on the sperm surface are reviewed.

Key words. Spermatozoa; oocyte; fertilization; glycosylation; lectin.

Introduction

Mammalian spermatozoa undergo a series of maturation events as they journey through the male and female reproductive tracts with the ultimate goal of fertilizing the egg [1]. Sperm maturation, sperm transport and fertilization are mediated by a series of complex sperm-surface modifications, cell-cell interactions and soluble factors in the reproductive tract lumen. Glycoconjugates from both the male and female reproductive tracts are critical components of the molecular mechanisms underlying the control of these reproductive processes.

Spermatozoa develop in the testis and proceed into the epididymis where further maturation involves modification of the sperm plasma membrane, including alteration of the dense carbohydrate coat on the cell surface, the glycocalyx [2]. Upon ejaculation, spermatozoa are deposited in the vagina and travel through the cervix and uterus (fig. 1). In the oviduct of many mammalian species, a sperm reservoir is formed by spermatozoa binding to the oviductal epithelium via protein-carbohydrate interactions [3], thus regulating sperm transport in this region of the female reproductive tract. Spermatozoa proceed further into the oviduct following capacitation, a cascade of sperm maturation events that is required for successful fertilization. Upon encountering the oocyte, proteins on the spermatozoon bind to carbohydrate moieties on the extracellular glycoprotein matrix, known as the zona pellucida, that encapsulates the egg [1]. Binding to the zona pellucida induces the acrosome reaction, an exocytotic event involving the release of hydrolytic enzymes from a vesicle (the acrosome) in the anterior region of the sperm head, and thus enables the spermatozoon to penetrate the zona pellucida and complete fertilization.

The protein-carbohydrate interactions mediating sperm transport in the female reproductive tract and gamete binding are described in this review with specific regard to the investigation of the glycoconjugates involved in these binding interactions. The sperm glycocalyx is discussed with consideration given to its modification during sperm maturation, its function and its contribution to immune-mediated infertility. Furthermore, a discussion of CD52 is included to review the characterization and potential function(s) of this major component of the sperm glycocalyx.

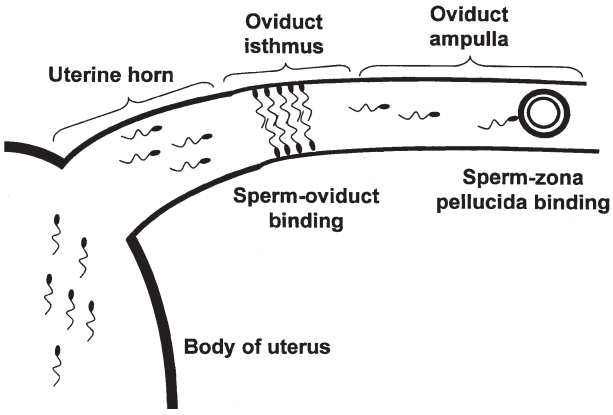

Figure 1. Sites of protein-carbohydrate interactions that regulate sperm function in the female reproductive tract. Upon ejaculation, spermatozoa are deposited in the vagina and travel through the cervix and uterus and into the uterine horns. In the oviduct of many mammalian species, but not in humans, spermatozoa bind to the oviductal epithelium via protein-carbohydrate interactions, forming a sperm reservoir. Following capacitation, a cascade of maturation events that are required for successful fertilization, spermatozoa are released from the reservoir and proceed further into the oviduct. Sequestration in the sperm reservoir mediates sperm transport into the oviduct and insures that spermatozoa are present at the right time to fertilize the egg. Upon encountering the oocyte, proteins on the spermatozoon bind to carbohydrate moieties of the extracellular glycoprotein matrix, known as the zona pellucida, that encapsulates the egg.

Role of carbohydrates in maintenance of the oviductal sperm reservoir

Upon reaching the isthmus of the oviduct, spermatozoa of many mammalian species, but apparently not the human, bind to ciliated epithelial cells lining this region of the oviduct via carbohydrate-protein interactions [4, 5]. These tight interactions form a sperm reservoir that prevents further progression into the oviductal ampulla until a combination of sperm maturational changes and signals from the female reproductive tract permit sperm release. The storage of spermatozoa in the oviductal isthmus has been proposed to have multiple functions including (i) to serve as a site for sperm capacitation; (ii) to maintain sperm viability; (iii) to synchronize the timing of sperm maturation with ovulation and (iv) to select for the most viable spermatozoa with the best morphology.

Binding to and release from the oviductal sperm reservoir appear to be involved in the regulation of capacitation, the series of maturational changes that occur to the spermatozoon in the female reproductive tract [6]. These molecular, biochemical and physiological changes are prerequisite to successful fertilization of the oocyte [7]. Noncapacitated spermatozoa bind to the oviductal epithelial lining with greater frequency than do capacitated spermatozoa, while release from the sperm reservoir is apparently coincident with the acquisition of capacitated status [6, 8]. The oviductal epithelium also appears to select for spermatozoa with superior morphology [9], intact acrosomes [10] and normal chromatin [11]. Furthermore, the binding of porcine spermatozoa to oviductal epithelial cells in vitro was shown to maintain sperm viability [12]. Significantly, sperm release from the oviductal epithelium also appears to be affected by the estrus cycle such that the arrival of spermatozoa and the oocyte in the oviductal ampulla is synchronized [13]. Thus, interaction of spermatozoa with the oviductal epithelium regulates sperm maturation and sperm progress into the oviduct in order to place high-quality spermatozoa in the oviductal ampulla at the right time for fertilization to occur.

The first evidence that carbohydrates were involved in sperm-oviduct binding came from inhibition studies with glycoconjugates. In the hamster, fetuin, an N- and O-glycosylated protein, was shown to inhibit sperm-oviduct binding [14], and other glycoconjugates or free sugars inhibited binding in other species [4]. Although both spermatozoa and oviductal epithelial cells exhibit surface-associated glycoconjugates and lectin-like molecules, sperm-oviduct binding appears to be mediated by the interaction of sperm-surface lectins with defined oviductal glycans [12]. During capacitation, these sperm-surface lectins are shed, effectively undocking the spermatozoa and permitting their ascension further into the oviduct.

The oviductal carbohydrates involved in maintaining the sperm reservoir and their complementary sperm-surface lectins have been most extensively characterized in the porcine and bovine models [15, 16]. Inhibition studies with oviductal explants implicated oligomannose as the as the high-affinity binding site for sperm binding in the porcine oviduct with low-affinity binding to galactose [16]. Suarez et al. [15] demonstrated sperm lectin binding to a Lewis-a trisaccharide in cattle. In the hamster, sialic acid was shown to play a pivotal role [14], while galactose was a significant recognition signal in the horse [8]. Thus, the glycans that serve as sperm-oviduct recognition signals vary among species.

Candidate sperm-surface lectins also vary among species. In the pig, proteins of the spermadhesin family are candidates for sperm-surface lectins that mediate sperm-oviduct binding as well as zona pellucida binding. The spermadhesions are low molecular weight (12–14 kDa) proteins that coat the sperm surface from the seminal plasma and have a wide range of carbohydrate specificities [4, 17]. The spermadhesin AQN-1 has an affinity for mannose, the key recognition signal in the porcine sperm reservoir, and was shown to inhibit porcine sperm-oviduct binding [12]. In cattle, a protein homologous to the porcine spermadhesins, acidic seminal fluid protein (aSFP), has been identified [18]; however, this bovine spermadhesin does not exhibit carbohydrate bind-

ing affinity. In this species, the Lewis-a trisaccharide recognition sequence was shown to bind a Ca^{2+}-dependent lectin on the bovine sperm-surface [15]. Putative sperm lectins have also been identified in the hamster and horse based on their binding affinities with sialic acid-containing glycoconjugates and galactose, respectively [14, 19]. Furthermore, loss of the identified hamster lectins from the sperm surface coincided with the inability to bind to the oviduct.

Glycoconjugates in oviductal fluid may also be involved in regulating the sperm reservoir. Talevi and Gualtieri [13] demonstrated that sulfated glycoconjugates inhibited bovine sperm binding to oviductal monolayers in vitro as well as promoted sperm release from the monolayers. These results led to the hypothesis that alterations in the normal sulfated glycoconjugate concentration of oviductal fluid during the estrus cycle modulate sperm-oviduct binding. Thus, sperm release from the oviductal epithelium is putatively mediated by a combination of free glycoconjugates in the oviductal lumen and alteration of carbohydrate binding activities on the sperm surface.

In the human, sequestration of uncapacitated spermatozoa in an oviductal reservoir apparently does not occur [4]. However, co-culture of spermatozoa with oviductal epithelial cells maintains the viability of human spermatozoa [20]. Furthermore, human homologues of the spermadhesins have been identified [21]. A model describing the role of carbohydrates in the events mediating sperm transport through the female reproductive tract has not been developed.

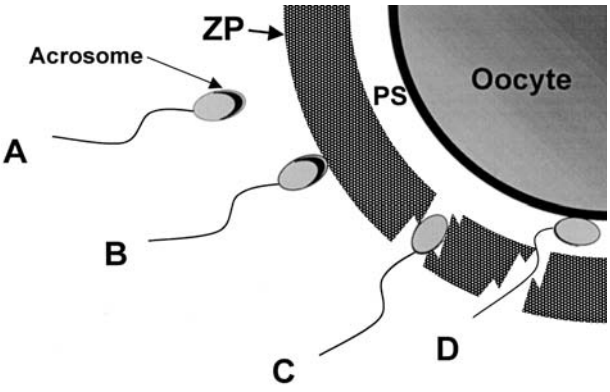

Figure 2. Protein-carbohydrate interactions in sperm-zona pellucida binding. (*A*) Sperm are transported into the oviduct and encounter the oocyte encapsulated by the zona pellucida, a polysulfated glycoprotein matrix that mediates initial gamete interactions. (*B*) Spermatozoa bind to the zona pellucida via the interactions of lectin-like molecules on the sperm surface and carbohydrate moieties on the zona pellucida. (*C*) The binding of sperm receptors on the zona pellucida to sperm-surface moieties aggregates these factors and induces the sperm acrosome reaction. The acrosome is a vesicle overlying the nucleus and underlying the plasma membrane in the anterior region of the sperm head. During the acrosome reaction, the outer acrosomal membrane and the plasma membrane fuse in an exocytotic process that releases hydrolytic enzymes required for penetration of the zona pellucida. This alteration in cellular architecture results in the exposure of the inner acrosomal membrane to the extracellular environment, thus redefining the sperm surface, including the glycocalyx, over the anterior sperm head. Sperm penetration of the zona pellucida is mediated in part by secondary binding events involving lectin-like molecules on the sperm head and carbohydrate moieties on the zona pellucida. (*D*) Following successful penetration of the zona pellucida, spermatozoa enter the perivitelline space and fuse with the oocyte plasma membrane for fertilization to occur. ZP, zona pellucida; PS, perivitelline space.

Role of carbohydrates on the zona pellucida in sperm-egg binding

The zona pellucida is a highly organized, three-dimensional glycoprotein matrix that encapsulates the mammalian egg [22, 23]. This sulfated glycoprotein coat serves to protect the unfertilized oocyte and preimplantation embryo. Spermatozoa must bind to and then penetrate this outer egg investment for fertilization to occur. Upon initial binding to the zona pellucida, spermatozoa are induced to undergo the acrosome reaction, the exocytotic event involving release of hydrolytic enzymes from the sperm head and exposure of additional sperm-surface moieties required for penetration of the zona pellucida and secondary sperm-zona interactions. In many species these recognition, initial binding and secondary binding events are modulated by carbohydrate moieties on the zona pellucida and complementary lectins on the sperm surface [24]. However, a limited number of studies indicate that carbohydrate moieties on the sperm surface may also be involved in sperm-zona pellucida interactions (see below).

The mammalian zona pellucida glycoproteins are encoded by three conserved genes: ZPA, ZPB and ZPC [25]. In the mouse and human, their gene products are designated ZP2, ZP1 and ZP3, respectively [26]. For other species, the nomenclature of the encoded zona pellucida proteins has been revised. To give one example, the gene product of ZPB in the pig, previously designated ZP3α, is now referred to as ZP1 by many investigators [26].

In mice, zona pellucida proteins were thought to be expressed exclusively by the oocyte, while granulosa cells contribute to the expression and construction of the zona pellucida in other species [27, 28]. Recent evidence reported by Dunbar et al. indicates that in the mouse zona pellucida proteins are also expressed in the granulosa cells of the developing follicle [26]. The extracellular zona pellucida matrix in the mouse is formed by filaments of ZP2-ZP3 heterodimers cross-linked by ZP1 [29].

Characterization of zona pellucida glycans has been hampered by the enormous complexity of zona pellucida glycosylation that is due to the inter- and intraspecies heterogeneity in glycan structures [4]. This complexity is

further compounded by the multiple potential glycosylation sites present in each zona pellucida protein. For example, the acidic *N*-glycan of porcine and bovine zona pellucida are similar in that they contain fucosylated complex-type core structures with sialylated and sulfated poly-*N*-acetyllactosamine chains [30, 31]. Furthermore, the *O*-glycans of porcine zona pellucida have the same sulfated polylactosamine structures as on the *N*-glycans attached to the Galβ1-3GalNAc *O*-glycan core [32]. However, these structures differ in the extent of branching, extent of lactosamine repeats, sialylation and sulfation between the two species as well as within each species. The bovine zona pellucida also contains a neutral high mannose *N*-glycan not present in the porcine zona pellucida.

Conflicting data have been reported for the glycan structure of the mouse zona pellucida. Nagdas et al. [33] described the characterization of N-linked polylactosamine chains on mZP2 and mZP3 and an O-linked trisaccharide terminated by *N*-acetyl glucosamine (GlcNAc). Noguchi and Nakano [34] reported that the *N*-glycans of mZP2 and mZP3 were similar with tri and tetraantennary complex chains containing polylactosamine. In an extensive study of total mouse zona pellucida glycans, Easton et al. [35] identified over 25 possible N-linked glycans and more than 12 O-linked glycans. Easton et al. found that the zona pellucida *N*-glycans were composed of a range of structures of both the high-mannose and complex types with the predominant structure being the high-mannose structure $Hex_5HexNAc_2$. The identified bi-, tri-, and tetraantennary *N*-glycan structures were terminated with multiple antennae, including the Sd^a antigen and terminal GlcNAc. The *O*-glycans were composed of the core 2 type; however, terminal GlcNAc was not identified on the *O*-glycans, in contrast to the results of Nagdas et al.

Identification of carbohydrate moieties involved in sperm binding and their complementary receptors on the sperm surface

The nature of the zona pellucida carbohydrate moieties that mediate sperm binding and their receptors on the sperm surface remain a matter of considerable debate. Investigation of the carbohydrate-protein interactions mediating sperm-zona binding has been complicated by the complexity of zona pellucida glycan structures as well as the apparent species specificity of these interactions [24]. Thus, among different species, various glycan structures on different zona pellucida proteins appear to bind to nonhomologous sperm proteins. Discussion of the multiple sperm proteins proposed to be involved in zona binding is beyond the scope of this review. Examples of such sperm surface lectins will be discussed in context with the identification and characterization of specific zona pellucida carbohydrate moieties implicated in sperm-zona binding.

The most extensive investigation of sperm-binding carbohydrates has been performed in the mouse model. Multiple lines of evidence indicate that the receptor for initial sperm binding in this species resides on the *O*-glycans of ZP3 (reviewed by Benoff [24]). Multiple studies have shown that chemical and enzymatic treatment to destroy protein and N-linked structures does not affect sperm-binding activity. Removal of O-linked oligosaccharides diminishes the ability to inhibit sperm-zona binding, and released *O*-glycans bind to spermatozoa and block sperm-zona interactions. Furthermore, mutation of serine residues in mZP3 to prevent O-linked glycosylation was shown to abrogate sperm-zona binding [36]. Removal of polylactosamine chains did not interfere with sperm-zona binding, indicating that the sperm receptor activity does not lie within these extensions [37]. However, the specific sperm-binding carbohydrate sequence(s) within the mZP3 *O*-glycan has not been clearly elucidated.

The involvement of mZP3 *O*-glycans terminated with GlcNAc in initial sperm binding has been proposed. Shur et al. have described β-1,4-galactosyl transferase (GalTase), an enzyme that transfers galactose to GlcNAc and that has been identified on spermatozoa from multiple species, including mouse and human [38–40]. GalTase has been shown to transfer galactose to mZP3, but not to mZP1 or ZP2, indicating ZP3 binding specificity. It is thought that sperm-surface GalTase functions as a lectin by binding to terminal GlcNAc or GalNAc residues on ZP3. Aggregation of GalTase on the sperm surface induces the acrosome reaction, an event mediated by ZP3 binding in vivo [41]. Significantly, sperm from GalTase knockout mice demonstrated a diminished ability to bind ZP3 and undergo the ZP3-induced acrosome reaction [42]. A wealth of data support the involvement of GalTase in ZP3 binding; however, the presence of the proposed GalTase ligand, O-linked terminal GlcNAc or *N*-acetyl galactosamine (GalNAc), on mZP3 remains a matter of debate. While Nagdas et al. [33] reported terminal O-linked GlcNAc on mZP3, Easton et al. [35] detected terminal GlcNAc only on N-linked zona pellucida glycans, and sperm-binding activity has been clearly localized to mZP3 O-glycans [24]. Furthermore, lectin-binding studies suggest that terminal GalNAc is only present on the inner surface of the mouse zona pellucida [35]. Nonetheless, neoglycoproteins with terminal GlcNAc and GalNAc residues were shown to induce the acrosome reaction in mouse spermatozoa [43].

Tulsiani et al. have suggested that the binding of ZP3 mannose residues by an α-D-mannosidase activity identified on mouse, rat, hamster and human spermatozoa mediates initial sperm-zona interactions [44–46]. Mannose and mannose-containing ligands have been shown to in-

hibit mouse sperm-zona pellucida binding [46], and neoglycoproteins exhibiting terminal mannose residues induced the mouse acrosome reaction [43, 47]. However, mannose-containing oligosaccharides are present on N-linked, not O-linked, glycans in the mouse [35]. Thus, the role of mannose and mannosidase activity in sperm-zona adhesion requires further investigation.

Significantly, mouse spermatozoa have been shown to bind tightly and specifically to a carbohydrate moiety on the surface of rabbit erythrocytes [48]. This binding requires acrosome intact spermatozoa, suggesting that it mimics initial sperm-zona interactions. The oligosaccharide sequence common to the rabbit erythrocyte and O-linked glycans of the mouse zona pellucida is Galβ1-4GlcNAcβ1-6 [35]. The sperm receptor activity of this carbohydrate sequence needs to be tested directly, and the complementary sperm-surface lectin must be identified.

In the porcine model, conflicting reports provided evidence that the sperm receptor activity resides in the *N*-glycans of the ZPB gene product [49] and in the *O*-glycans of the ZPB/ZPC complex [50]. As in the mouse, the sperm-binding activity does not reside in the polylactosamine extension of these N- or O-linked glycans [30, 51]. Although the specific carbohydrate ligands have not been elucidated, sperm-surface lectins, the spermadhesins, have been identified that appear to mediate initial sperm-zona interactions [4]. The spermadhesins are a family of multifunctional proteins with wide range of carbohydrate-binding activities and are acquired by pig spermatozoa during epididymal maturation and from the seminal plasma [52]. These molecules have been shown to bind to porcine zona pellucida proteins, and characterization of their carbohydrate binding affinities has provided valuable, though indirect, insights into potential sperm-binding ligands. For example, the spermadhesins AQN-3 and AWN exhibit affinities for Galβ1-3GalNAc- and Galβ1-4GalNAc-containing glycoproteins [52], and PSPII displays affinity for mannose-6-phosphate [53]. Whether the spermadhesins are directly involved in pig sperm-zona binding requires additional investigation.

In humans, the carbohydrate structures of zona pellucida have not been directly characterized, most likely due to the limited quantity of human zona pellucida available for research purposes. However, mannose, GalNAc, fucose and complex carbohydrates containing fucose have been demonstrated to inhibit sperm-zona binding in vitro (reviewed by Benoff [24]), thus identifying these carbohydrate moieties as potential zona pellucida sperm receptors. The Benoff and Tulsiani research groups have proposed a role for a mannose lectin [24] and an α-mannosidase [45], respectively, in binding to mannose-containing glycans of the human zona pellucida. Furthermore, mannose-containing neoglycoproteins have been shown to induce the acrosome reaction of human spermatozoa [43, 47]. Inhibition of sperm-zona binding by fucose-bearing ligands such as fucoidan [54] and glycodelin-A [48] implicate the involvement of a selectin-like activity in the binding of spermatozoa to fucose moieties on the zona pellucida. Significantly, monoclonal antibodies (mAbs) against L-selectin, a selectin expressed on lymphocytes, block sperm-zona binding in vitro and were used to identify a 90-kDa protein on the human sperm head [55]. These data have led some investigators to hypothesize a convergence in immune and gamete carbohydrate and protein recognition mechanisms in the human [56].

Following initial sperm-zona binding and the acrosome reaction, spermatozoa continue to bind with zona pellucida glycoproteins as they penetrate this extracellular matrix [57]. These secondary binding events are mediated, at least in part, by carbohydrate and protein interactions. The acrosomal enzyme acrosin has been proposed to function as a lectin in binding sulfated polysaccharides of the porcine, mouse and human zona pellucida [57–59]. Also in the mouse, a mAb that recognizes terminal GalNAcβ1-4Gal inhibits secondary, but not initial, sperm-zona binding [60]. Significantly, Easton et al. identified this sequence in mouse zona pellucida as a component of the Sd^a antigen [35]. Further investigation will be required to identify the specific carbohydrate moieties involved in secondary sperm-zona binding.

The sperm glycocalyx

As described above, the composition and function of zona pellucida carbohydrate structures have received considerable attention. In contrast, little is known about the structure and function of the sperm glycocalyx, in part due to the extreme complexity of this carbohydrate layer [61]. While the zona pellucida is composed of three glycoproteins, literally hundreds of glycoconjugates contribute to the carbohydrate coat overlying the sperm. Component moieties of the sperm glycocalyx reside on integral and peripheral membrane glycoproteins as well as on glycolipids [62]. At least 300 proteins are present on the human sperm surface, and it is anticipated that the vast majority of these are glycosylated [63]. Furthermore, the sperm glycocalyx is two to six times as thick (20–60 nm) as that of the typical somatic cell [62]. Thus, the presence of this dense carbohydrate coat over the sperm surface suggests that interactions between spermatozoa and other cells or the extracellular environment must, at least initially, involve the sperm glycocalyx.

Compositional analyses for the general structure of the sperm glycocalyx have mainly involved lectin-binding studies (reviewed in Schröter et al. [62]) to identify component carbohydrate moieties and to follow their fate during sperm maturation. A limited number of studies have attempted to directly characterize the composition of the

sperm glycocalyx. Calzada et al. [64] reported that glycans on the surface of ejaculated human sperm are composed of 40% sialic acid, 27% hexosamines and 30% fucose. This composition is notably high in sialic acid as compared with somatic cells, and this high level of sialic acid contributes to the net negative charge observed for spermatozoa. Furthermore, this glycoproteic layer was shown to be composed of protein and carbohydrate at an equal mass-to-mass ratio.

The sperm glycocalyx is incredibly dynamic in structure. Although mammalian spermatozoa derive from the seminiferous epithelium of the testis, the composition of the sperm surface undergoes considerable posttesticular modification. During transport through the epididymis, additional glycoconjugates associate with the sperm surface integrally and/or peripherally, and existing sperm-surface glycans are altered by glycosidases and glycosyltransferases in the epididymal lumen [2, 65]. Subsequently, other glycoconjugates, sometimes referred to as 'coating antigens', are acquired from the seminal plasma [66]. In the female reproductive tract, the sperm glycocalyx is further modified during membrane modification events of capacitation [7]. Glycoconjugates, such as sialoglycoconjugates [67], are shed during capacitation, and glycans that remain bound on the sperm surface are modified to prepare the spermatozoon for the acrosome reaction and fertilization. Furthermore, loss of the plasmalemma during the acrosome reaction results in removal of the anterior head glycocalyx and exposes the glycans on the inner acrosomal membrane to the extracellular environment [1], thus forming a new glycocalyx in that region of the sperm head. In addition, a given sperm glycan may be presented in multiple conformations over time due to the high flexibility of large glycans. These observations led Schröter et al. [62] to suggest that as a general structure the sperm glycocalyx is four-dimensional, rather than three-dimensional in nature.

The function(s) of the sperm glycocalyx has remained speculative. Immunosuppresive properties have been attributed to glycoconjugates in the reproductive tract. Thus, certain components of the sperm glycocalyx may protect non-self epitopes on spermatozoa from both the male and female immune systems [48, 62]; however, such properties have not been demonstrated directly. Conversely, sperm-surface carbohydrates are immunogenic and implicated in immune infertility (see below), although it is doubtful that this immunogenicity is required for normal sperm function. Another potential function involves a stabilizing/protective effect or other influence on the components and biophysical properties of the sperm membrane [61, 62].

Modification of sperm-surface carbohydrate moieties during sperm maturation indicates a functional, though to date undetermined, significance. For example, extensive modification occurs to the glycocalyx as spermatozoa pass through the epididymis, and spermatozoa also acquire fertilizing ability at this time, suggesting a correlation between these maturational events [2]. Spermatozoa also attain an increased negative charge during maturation [68] that may serve to prevent nonspecific interactions with soluble proteins and genital tract epithelia. Significantly, Banerjee and Chowdhury [69] have described a uterine sialic acid-binding protein (SABP) that induces human sperm capacitation in vitro. Furthermore, carbohydrate modifications during capacitation would seem to suggest these alterations are prerequisite to the acrosome reaction and fertilization.

The model accepted by most investigators for sperm-zona pellucida binding involves the recognition and binding of carbohydrate moieties of the zona pellucida by lectin-like proteins on the sperm surface [24]. However, a limited number of studies indicate a role for carbohydrate moieties of the sperm glycocalyx in early gamete interactions. Tanphaichitr et al. reported that Fab antibody fragments against sulfogalactosylglycerolipid (SGG), the major sulfoglycolipid in mammalian spermatozoa, inhibited human and mouse sperm binding and that SGG-bearing liposomes bound to the human and mouse zona pellucida [70, 71]. Mahony et al. [72] demonstrated that mAbs against carbohydrate epitopes of CD52, a glycosylphosphatidyl inositol (GPI)-anchored sperm-surface glycoconjugate, inhibit human sperm-zona binding. Furthermore, D'Cruz et al. [73] reported that incubation of sperm with mAbs against CD15, a carbohydrate epitope implicated in cell adhesion, partially inhibited human sperm-zona binding. Further investigation is required to determine whether sperm-zona binding is mediated by a combination of protein and carbohydrate moieties on the sperm surface and to identify potential receptors for these sperm carbohydrate moieties in the zona pellucida.

CD52: The major maturation-associated sperm membrane antigen

Although the mammalian sperm glycocalyx is composed of numerous glycoconjugates, multiple groups have identified the CD52 glycoprotein as a dominant component, both quantitatively and immunologically, of this complex structure. Human CD52 is a 12-amino acid, GPI-anchored glycoprotein the expression of which is restricted to the male reproductive tract and lymphocytes [61]. Originally identified in lymphocytes with the mAb CAMPATH-1, CD52 was subsequently identified in the epididymal epithelium, in male reproductive tract fluids and on the sperm surface [74, 75]. CD52 is secreted with its GPI anchor intact by the epididymal epithelium into the epididymal lumen, where it is recruited to the surface of spermatozoa [75, 76]. CD52 has been termed the 'major maturation-associated sperm membrane antigen'

because the appearance of sperm-surface CD52 in the epididymis coincides with the appearance of fertilization-related abilities such as forward motility and zona pellucida-binding ability [76].

Herr et al. produced the anti-CD52 mAb S19 via the immunization of mice with human sperm homogenates, and this mAb exhibited multiple sperm-inhibitory effects [72, 75, 77]. The cognate S19 antigen [designated sperm agglutination antigen-1 (SAGA-1)] was immunoaffinity purified, microsequenced and shown to have the same core peptide sequence as CD52 [75]. In ejaculated spermatozoa, CD52 is present over the entire sperm surface [77]. High-resolution, two-dimensional electrophoresis identified sperm CD52 as a series of discrete molecular mass and isoelectric variants ranging from 15 to 25 kDa and pI 2.5–3.5 [77]. Thus, sperm CD52 represents one of the most acidic, microheterogeneous glycoproteins identified to date. Schröter et al. [78] reported the structural characterization of N-linked glycan moieties on seminal plasma CD52 and identified a heterogeneous series of di-, tri- and tetraantennary structures that explain the identification of multiple molecular mass and isoelectric variants of CD52 in spermatozoa. Furthermore, the high sialic acid content of these structures potentially explain the high negative charge of sperm CD52.

The S19 mAb recognizes an N-linked carbohydrate epitope present on sperm CD52 that is absent on lymphocyte CD52, indicating that the N-linked glycans on sperm CD52 and on lymphocyte CD52 are immunologically distinct [75]. Mass spectrometry analyses of the carbohydrate moieties on CD52 revealed significant structural differences between the N-linked oligosaccharides on CD52 isolated from human seminal plasma and those on CD52 isolated from human spleen [78, 79]. Treumann et al. [79] determined that the single N-linked glycosylation site at Asn-3 of human splenic CD52 is occupied by large core-fucosylated, tetraantennary oligosaccharides of the complex type. These structures are sialylated, contain variable numbers of polylactosamine repeats and in some cases have an additionally branched outer antenna creating a five-branched glycan. In contrast to splenic CD52 *N*-glycans, seminal plasma CD52 exhibits a heterogeneous series of complex type structures at Asn-3 composed of di-, tri- and tetraantennary oligosaccharides with the triantennary forms predominating [78]. The larger tetraantennary forms have additionally branched outer antennae resulting in five- and six-branched structures. As for splenic CD52, these seminal plasma CD52 glycans contain polylactosamine chains of variable length and are core fucosylated. However, these *N*-glycans are almost fully sialylated, resulting in a high negative charge for the molecule, and the polylactosamine branches of a subset of structures contain peripheral fucose. Thus, these data indicate that male reproductive tract and lymphocyte CD52 represent glycoforms, glycoproteins with the same core protein but with different carbohydrate structures.

The generation of numerous anti-CD52 carbohydrate mAbs in multiple laboratories by immunization with total sperm or seminal plasma preparations points to the immunodominance of the CD52 N-linked carbohydrate structure [66, 75, 77]. The S19 mAb and other anti-CD52 mAbs were shown to strongly agglutinate human spermatozoa, immobilize spermatozoa in the presence of complement, impede sperm penetration of cervical mucus and inhibit human sperm-zona pellucida binding [66, 72, 74, 75, 77]. Furthermore, the S19 mAb induced the shaking phenomenon when the mAb was incubated with human spermatozoa in bovine cervical mucus [A. B. Diekman and J. C. Herr, unpublished observations].

Role of CD52 in sperm function

Although CD52 is a major component on the sperm surface and multiple functions have been proposed, the functional role of sperm CD52 remains a subject of conjecture. The small size of the CD52 peptide core and its divergent nature among mammalian species led Kirchhoff and Schröter [61] to suggest that the peptide core acts as a scaffold for the *N*-glycan, while the chief functional and physiochemical properties of the molecule reside in the tissue-specific carbohydrate and lipid moieties. The high acidity of sperm CD52, conferred by the high degree of sialylation on its *N*-glycans, coupled with its surface localization suggests that CD52 contributes to the overall net negative charge observed for the sperm surface [61, 77]. Yeung et al. [80] demonstrated that a change occurs in the structure of CD52 during capacitation, resulting in increased exposure of sialic acid residues; whether this modification is required for the acrosome reaction and fertilization has not been determined. It has also been suggested that the CD52 carbohydrate structure provides a stabilizing and protecting effect for other sperm-surface moieties or may otherwise impact the biophysical properties of the sperm plasmalemma [61]. Furthermore, CD52 may represent one of the so-called 'decapacitation factors' [1]. Such molecules are acquired by spermatozoa from the epididymis and seminal plasma and are shed prior to capacitation. Interestingly, Della Giovampaola et al. [81] reported that a non-GPI-anchored subpopulation of CD52 was lost from the sperm surface during capacitation in vitro.

Perhaps the most significant insight into sperm CD52 function was obtained through the inhibition of sperm-zona pellucida binding by the anti-CD52 S19 mAb. Mahony et al. [72] used the hemizona assay to demonstrate that S19 Fab fragments inhibited tight binding of human spermatozoa to the human zona pellucida by 70%. Fab fragments are monovalent; therefore, sperm agglutina-

tion was not a likely factor in the inhibition of sperm-zona adhesion. These results suggested that the S19 mAb blocked a portion of the zona-binding receptor on the sperm surface and implicated CD52 itself or a CD52-containing complex in sperm-zona interactions. The role of sperm-surface CD52 carbohydrates in zona-binding requires further investigation.

Immune infertility

The correlation of antisperm antibodies with some cases of unexplained infertility indicates a role for these antibodies in blocking fertilization, and numerous investigators have sought to identify the sperm-surface antigens involved [82]. A significant number of these sperm-inhibitory activities from infertile patient sera have been shown to involve sperm-surface carbohydrates [83]. Antibodies against the sperm glycocalyx may arise following the breakdown of immunosuppressive mechanisms in the reproductive tract that serve to protect non-self epitopes from the immune system of either sex [82]. An immune response against sperm carbohydrates may also occur following genital tract infection as a result of cross-reactivity of bacterial and sperm oligosaccharide epitopes [84, 85].

CD52 has been identified as a potential target for antisperm carbohydrate antibodies involved in immune infertility [75]. In a benchmark study, Isojima et al. [86] established a human-mouse heterohybridoma using peripheral blood lymphocytes from an infertile woman who exhibited high sperm-inhibitory titers. The resulting immunoglobulin (Ig)M mAb, H6-3C4, localized to the entire sperm surface and demonstrated sperm-agglutinating and -immobilizing activities, thus implicating the H6-3C4 epitope as a target for antibodies involved in the etiology of clinical immune-mediated infertility. The H6-3C4 epitope was identified as an N-linked oligosaccharide on a 15–25 kDa cognate sperm glycoprotein. Furthermore, Tsuji et al. [87] demonstrated H6-3C4 reactivity with poly-*N*-acetyllactosamine carbohydrate structures and detected a cross-reactivity with a related, but not identical, carbohydrate epitope of the i blood group glycolipid antigen. Immunohistochemical analysis suggested that the cognate H6-3C4 antigen was expressed in the epididymis and transported to the sperm surface [88]. Diekman et al. [75] demonstrated identity of the cognate H6-3C4 antigen with CD52 via H6-3C4 immunoreactivity with S19 immunoaffinity-purified CD52. Significantly, Koyama et al. [89] subsequently demonstrated that sperm-immobilizing antibodies in some infertile patient sera reacted with CD52. Therefore, the CD52 glycoprotein and its carbohydrate side chains are implicated in the etiology of immunologic infertility.

Opportunities for contraceptive development

In an effort to identify new contraceptive alternatives, sperm-specific antigens have been investigated as the basis for immunological regulation of fertility through immunocontraception [90]. Most studies have focused on sperm protein epitopes due to the relative ease of characterization and recombinant production of proteins as compared with the study and synthesis of carbohydrate epitopes. However, new technologies facilitating carbohydrate characterization and synthesis and the implication of anti-carbohydrates in the etiology of immune infertility have advanced sperm-surface oligosaccharides as potential immunocontraceptive targets.

The unique glycan structure of sperm CD52 is an attractive candidate for immunocontraceptive development. CD52 is localized on the entire sperm surface and is accessible to antibody binding that inhibits sperm transport and sperm-zona binding. The N-linked glycan on CD52 is recognized by a sperm-agglutinating mAb that was immortalized from an infertile woman. Furthermore, the S19 carbohydrate epitope is specific to sperm CD52. Opportunities for CD52 immunocontraceptive development include targeting a contraceptive vaccine against the unique epitopes of the sperm CD52 glycoform and using the S19 mAb as an intravaginal agent. Towards production of an intravaginal contraceptive, the S19 mAb has been expressed as a recombinant miniantibody that was shown to exhibit anti-CD52 immunoreactivity and sperm-inhibitory activity [91].

In order to establish a CD52 animal model for immunocontraceptive development, McCauley et al. [92] investigated expression of the S19 carbohydrate epitope in non-human primates. The S19 carbohydrate epitope was identified in the chimpanzee epididymis and spermatozoa, but not in the baboon, marmoset, bonnet, cynomolgus macaque or pigtailed macaque. CD52 was detected in the chimpanzee spleen, but the S19 carbohydrate moiety was not identified, paralleling tissue-specific glycosylation of human sperm CD52. The presence of this distinctive carbohydrate epitope in the human and chimpanzee and its absence in other primate species suggests that specific patterns of glycosylation at the sperm surface may be conserved evolutionarily among closely related species.

Conclusions

The oviductal sperm reservoir and sperm-zona binding is mediated predominately by protein-carbohydrate interactions involving carbohydrate moieties on the oviductal epithelium and on the zona pellucida, and lectin-like proteins on the sperm surface. Further investigation will be required to identify the specific glycoconjugates and lectins involved. Due to the apparent evolutionary divergence in these binding events, the specific moieties must

be identified independently in each species of interest. The identification of multiple candidate molecules suggests that multiple carbohydrate ligands and sperm lectins are involved in each species. Further study is necessary to investigate the indications that molecules present in the oviductal fluid modulate binding in the sperm reservoir and that carbohydrate moieties on the sperm surface are involved in zona pellucida.

The sperm glycocalyx is composed of multiple glycoconjugates, and this complexity has somewhat hindered the characterization of its structure and function. The carbohydrate coat on the sperm surface differs in size and composition compared with the glycocalyx of somatic cells. Although multiple functions have been proposed for this structure, additional investigation will be required to ascertain its roles in sperm function. Furthermore, sperm-specific carbohydrate moieties are implicated in the etiology of antibody-mediated infertility and are being investigated for immunocontraceptive development. In general, the further study of glycoconjugates on the sperm surface, as well as in other cell types of the male and female genital tracts, will provide a greater understanding of the molecular mechanisms that control the process of mammalian reproduction.

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