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Chapter

Glycosylation on Spermatozoa, a Promise for the Journey to the Oocyte

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Abstract

Spermatozoa experience a long and tough transit in male and female genital tracts before successful fertilization. Glycosylation helps spermatogenesis, epididymal maturation, passing through cervical mucus, avoiding killing of the female immunologic system, and shaking hands between sperm and egg. Changes in glycosylations along the transit ensure that the right things happen at the right time and place on spermatozoa. Aberrant glycosylations on spermatozoa will negatively affect their fertility. Thus, we developed a lectin array method to examine the glycocalyx of spermatozoa, which will help observe glycosylations occurring on spermatozoa in a normal or abnormal conditions, such as spermatozoa with DEF126 mutation and poor freezability. Intriguingly, binding levels of ABA (*Agaricus bisporus agglutinin*), a lectin marking the inner layer of the glycocalyx, were changed in these subfertile spermatozoa, which indicates that the integrity of glycocalyx is critical for sperm fertility. In this chapter, we reviewed the impacts of glycosylations on sperm fertility, the lectin array method, and its potential application for sperm function assessment.

Keywords: spermatogenesis, sperm maturation, transit through genital tract, glycan profile, lectin microarray

1. Introduction

Before mating the oocytes, mammal spermatozoa go on a long adventure, experiencing a series of complicated events along the male and female reproductive tracts [1]. From spermatogonia, spermatozoa develop in the testis, with dramatic changes in morphology and chromatin structure. However, spermatozoa in testis could not fertilize oocytes since their motility and capabilities of recognizing and binding zona pellucida are underdeveloped.

Spermatozoa then enters into the epididymis, several meters long male reproductive tract, and undergo an additional maturation. During the epididymal maturation, also called post-testicular maturation ranges from 1 to 21 days depending on species [2], spermatozoa acquire not only capacities for progressive motility, acrosome reaction, zona pellucida binding and recognition, and spermatozoon-oocyte fusion [3–7],

but also further morphology changes [8–11], such as chromatin condensation, head size decrease, acrosome reshaping, and droplet migration.

After ejaculation, spermatozoa are transferred into the vagina. Passing through the cervix, uterus, and uterotubal junction, spermatozoa stop at a storage reservoir in the oviduct until ovulation. During ovulation, spermatozoa escape the reservoir, complete the capacitation, migrate to the site of mating, and finally achieve insemination. Despite facilitation guiding to the site of fertilization, a successful spermatozoon faces big challenges in the female genital tract, such as selection, which ensures the fittest one to fertilize, and immune defense, which fights evasion of microbial pathogens [12, 13].

2. Glycosylations on mammal spermatozoa and their physiological functions

Along the journey of spermatozoa, events, such as sperm development, maturation, transit, and fertilization, are mediated by cell–cell interaction, and interaction between spermatozoa and the microenvironment [1]. Providing promises to these events, dramatic modifications on sperm surface occur along the transit of spermatozoa [14]. For instance, high sialylation provides a negative charge surrounding the sperm surface, which results in a repulsive interaction against negatively charged mucins for bypassing cervix mucus [15]; a shell, which protects sperm antigens from immune surveillance in the female genital tract [16]; and an anchor, which helps the epithelial cells hold spermatozoa at the oviductal reservoir [17]. However, during capacitation, lipid component changes resulting in a high membrane fluidity for sperm hyperactivation [18], and desialylation occurs on the sperm surface [19, 20], both of which ensure spermatozoa reach the oocyte and fertilize successfully.

2.1 Glycoconjugates on mammal spermatozoa

A dense glycoconjugate coat with a thickness of 20–60 nm called glycocalyx on animal cells [21], is one of the major surface components on spermatozoa, which participate in such modifications and play critical roles in those interactions along the sperm's journey to the oocyte [22, 23]. The glycocalyx is synthesized during spermatogenesis in testis and is remodeled through epididymal maturation, the mixing process of sperms and seminal plasma secreted by the accessory glands during ejaculation and transit through the female genital tract [21]. Two of three types of glycoconjugates are identified in sperm glycocalyx: glycoproteins and glycolipids [24], which are classified corresponding to types of glycan-bearing carriers, proteins, or lipids. Besides those glycoconjugates inserted or anchored in the plasma membrane, others are non-covalently attached to the membrane via inter-molecule or hydrophobic interaction [21].

2.1.1 Glycoproteins

Glycosylation is a ubiquitous posttranslational modification on proteins, more than half of human proteins are deduced as glycoproteins according to their amino acid sequences [25]. By exploiting their covalently linked glycans, membrane or surface glycoproteins play roles in intercellular signaling, cell–cell interaction, and cell adhesion [26]. With nucleoside diphosphate or monophosphate glycans as precursors, glycosyltransferases create a glycosidic bond between glycan and peptide

backbone. Three types of covalent linkages exist naturally between glycans and polypeptide backbone: N-glycosylation, O-glycosylation, and GPI glycosylphosphatidylinositol [27].

As the most common posttranslational modification in the endoplasmic reticulum (ER) [28], N-linked glycosylation attaches glycans to the amide nitrogen of asparagine or arginine residues within the consensus amino acid sequences or called "sequons," Asn-X-Thr/Ser, which direct protein folding. In a manner of protein-, cell-, or species-specificity [29], remodeling in Golgi apparatus endows N-glycan structural and functional varieties, which ensure functional complexity of cell surface proteins, such as cell–cell, cell-matrix interaction, or immune responses. N-glycosylation is a prominent posttranslational modification of the protein, about 50% of the proteins have N-glycans [30, 31]. N-glycans are also major carbohydrates on the sperm glycocalyx, which are composed of three types of N-glycans, high mannose, hybrid N-glycans, and complex N-glycans [32]. High mannose N-glycans are essential for Sertoli-germ cell attachment during spermatogenesis, which is a critical cell–cell adhesion for sperm development in testis [33].

Initially in the ER then Golgi apparatus, O-glycosylation covalently links glycans to the oxygen atom at the hydroxyl group of serine, threonine, tyrosine, hydroxylysine, or hydroxyproline residue on the peptide backbone [27]. O-glycosylation has no sequon and core structure, and O-glycan moieties range from monosaccharides to sulfonated polysaccharides. β-defensin 126 (DEFB-126) is a well-known O-linked glycoprotein [34], which is acquired through epididymal maturation and is also an important component of sperm glycocalyx. On the surface of DEFB-126 knock-out sperm, the level of O-glycosylation significantly decreased, and the spermatozoa are male infertility [35].

Glycosylphosphatidylinositol (GPI)-anchor is glycolipid, containing a Mana1–6Mana1–4GlcNa1–6PI motif [36]. Performed by ER-situated transamidase in ER, the carboxyl-terminus of proteins is connected to the ethanolamine moiety of GPI-anchor via an amide bond. Located on the extracellular side of the cytoplasmic membrane, the protein is bound to the cell membrane through GPI-anchoring. Structures and functions of GPI-anchored protein are widely diverse and play important roles in many biological processes. During sperm migration in the epididymis, various GPI-anchored proteins are obtained on the surface under the mediation of epididymosomes in the epididymal fluid. A well-studied GPI-anchored protein is sperm adhesion molecule 1(SPAM1/PH-20). The SPAM1 secreted by the epididymis exists in both epididymal fluid and epididymal exosomes; SPAM1 in the latter can be attached to the epididymal sperm surface by GPI anchoring [37].

2.1.2 Glycolipids

In addition, to proteins, lipid moieties are another type of glycan carrier. Glycosphingolipids are major glycolipids in the cell membrane of animals, which are primarily synthesized in the endoplasmic reticulum, and whose carbohydrate moieties are further modified in the Golgi apparatus [38]. Glycosphingolipids contain one or more carbohydrate residues linked to a specific hydrophobic lipid moiety, sphingoids, or ceramides (N-acylated sphingoid), through a glycosidic bond [39]. According to their glycan structures, glycosphingolipids are classified into the following, namely, ganglio-, isoganglio-, lacto-, neolacto-, lactoganglio-, globo-, isoglobo-, muco-, gala-, neogala-, mollu-, arthro-, schisto- and spirometo-series.

Glycolipids participate in cellular functions in animals, and their biosynthesis and distribution change corresponding to different physiological functions and stages [40]. Glycolipids are essential for many processes of male reproduction [41]. Via manipulated deficiency of galactosyl transferase or sulfotransferase, mouse models with targeted deletion of seminolipid, a sulfoglycolipid, show suspension of spermatogenesis at the stage of meiosis [42]. And, a mouse model with complex ganglioside deficiency resulting from targeted deletion of N-acetylgalactosaminyl transferase is reported to be male infertility [43].

2.2 Physiological functions of sperm glycoconjugates

2.2.1 Spermatogenesis

Occurring in seminiferous tubules of testis, spermatogenesis is a highly regulated series of sperm development, involving in the proliferation of spermatogonia, differentiation, and maturation into spermatozoa. Sertoli-germ cell interaction is vital for spermatogenesis [44], which supports, transports, and protects germ cells. Mouse model studies targeted deletion of N-acetylglucosaminyltransferase-II (GnT-II) and α-mannosidase IIx (MX) suggested that a specific sperm N-glycan, GlcNAc-terminated triantennary, and fucosylated N-glycan, mediates the adhesion of germ cells to Sertoli cells via protein-carbohydrate interaction [45, 46].

Although N-glycans play a role in spermatogenesis has been widely admitted [32, 33], proteins containing, such as, glycans remain little known. Leukocyte differentiation antigen (basigin, BSG, also known as CD147) is an essential protein during the process of spermatogenesis, and embryo implantation [47, 48]. which is expressed in various stages of spermatogenic cells (spermatogonia, spermatocytes, and spermatids), Sertoli cells, Leydig cells, and epididymis [49]. Mature spermatozoa are absent in the testis of a mouse model with targeted deletion of BSG. Mass spectrometry and lectin studies reported that N-glycans containing fucosylated high-mannose moieties with terminated N-acetylglucosamines were identified in BSG [49–51], which is reasonably believed that BSG may participate in Sertoli-germ cell adhesion.

As sulfated glycoconjugates, seminolipids are predominant glycolipids on the plasma membrane of mammalian male germ cells [52]. With a very high cell specificity, seminolipids are synthesized in primary spermatocytes and exist in the subsequent male germ cell types [53]. Seminolipid-deficient mice illustrate male infertility and suspension of spermatogenesis before first meiotic division [42], which might result from failure of uptake of lactate for spermatocytes [54]. *In vitro* studies indicated that Sertoli membrane protein could bind seminolipids, which suggests that seminolipids may take part in Sertoli-germ cell adhesion during spermatogenesis [55].

Gangliosides are sialic acid terminated glycolipids. Complex ganglioside deficient mice showed male infertility, which might result from failure of testosterone transportation in Leydig cells [43]. Due to their reported functions on carbohydrate-dependent cell adhesion [56], gangliosides are also supposed to play roles in Sertoli-spermatocyte interaction [55].

2.2.2 Epididymal maturation

Through the maturation along the epididymal tract, spermatozoa acquire not only fertility, including progressive movement,, but further modifications of

morphology (especially, migration of the cytoplasmic droplet), molecular components and cell surface.

During the transit from caput down to cauda epididymis, a significant change in sperm surface is an increase in surface sialylation [56, 57]. Sialic acid is a strongly negatively charged acidic oligosaccharide, sialylation occurs on the terminus of N- or O-glycans. Due to the sialylation on the surface, spermatozoa are covered with a negative charge [58], which could facilitate sperm transit along the genital tracts, shield sperm antigens, and protect spermatozoa from an immune response in the female genital tract [24].

Integrating the sialylated glycoproteins or glycopeptides secreted from epididymal epithelium to the cell surface is a major means to sialylate the sperm surface. Several epididymal sialylated proteins have been reported to be associated with the spermatozoa during epididymal maturation, including proteins D and E, basigin, CD59, fertilin, HE2, HE4 [59], and HE5/CD52 [59, 60].

Secreted from the epididymal epithelium into the epididymal lumen, recent studies showed its significance in the sialylation of sperm surface [61]. CD52 is N-glycosylated mainly in a form of three-antennary oligosaccharides with a terminus of sialylation, which results in a negative charge on CD52. CD52 protects sperm from complement-dependent immune responses, facilitates the process of sperm glycocalyx remodeling, and prevent sperms from agglutination with each other [61, 62]. CD52 is a GPI-anchored protein [61], which is transported and integrated to the sperm surface via epididymosome [63].

Acquisition of membrane lipids from epididymal epithelium also exploits epididymosome [64], relative quantities of cholesterol and sphingomyelin increase during the epididymal maturation. However, other strategies are also applied for the remodeling of sperm surface components.

Originated in the epididymal epithelium and secreted into the lumen, DEFB-126 is also a highly sialylated O-linked glycoprotein, which tightly associates with the surface of human and macaque spermatozoa. It is believed that DEFB-126 is a major component of sperm glycocalyx, and a predominant contributor to the negative charge of sperm surface [34, 35, 65].

The majority of glycosyltransferase activities are found within the epididymal lumen [66]. High activities of both fucosyltransferase and sialyltransferase exist in caput epididymis and decrease gradually at cauda epididymis. It is suggested that further glycosylation may occur on the sperm surface.

2.2.3 Transit along the female genital tract

After ejaculation, spermatozoa will encounter not only selection pressure, which knocks out spermatozoa with poor motility or morphology via retrograde flow in the female genital tract [67], but immune pressures, which eliminates "foreigners," such as microbial pathogens, and even spermatozoa.

Like CD52 mentioned above, CD55, also a GPI-anchored protein, is a type of complement-regulating protein on the plasma membrane of human sperm against immune attack [68]. CD59 is an inhibitory molecule of the membrane attack complex of the complement system, which is also localized in the sperm plasma membrane [69]. These three GPI-anchored proteins with N-glycans protect spermatozoa from immunological attack in the female genital tract [70].

DEFB-126 is an O-linked glycoprotein. Like CD52, CD55, and CD59, DEFB-126 is integrated to sperm surface during epididymal maturation, but in a different

way [65]. High sialylation on DEFB-126 not only protects spermatozoa from phago-cytosis in the female genital tract, but helps penetrate through cervix mucus. It is supposed that tri- and tetra-antennary glycans with Lewis-X and Lewis-Y sequences on these glycoproteins acquired in epididymis may help escape immune detection [71], and suppress antigen-immune responses [72].

Besides pressures, the female genital tract also conducts facilitation and regulations, which help spermatozoa do the right thing at the right time, right place. Glycodelin is a secretary protein with N-glycans, which is composed of four glycoforms, namely, glycodelin-A (amniotic fluid) [73, 74], glycodelin-F (follicular fluid) [75], glycodelin-C (cumulus matrix) [76], and glycodelin-S (seminal plasma) [77], according to the tissues and fluids where the isoforms are identified. By fucosyltransferase-5 on sperm surface, glycodelin isoforms bind to spermatozoa with a spatiotemporal specificity [78], and perform diverse functions [79], with their different glycan moieties [80].

After ejaculation, spermatozoa bind glycodelin-S on the whole head [75], an abundant protein in seminal plasma secreted from the seminal vesicle, which inhibits albumin-induced cholesterol efflux and prevents sperm capacitation. After cervix penetration, glycodelin-S on spermatozoa is displaced by glycodelin-A, which is secreted from maternal endometrial epithelial cells into uterine and amniotic fluid [80], and covers the sperm acrosomal region. Glycodelin-A inhibits sperm capacitation and protects spermatozoa from immune attack. In the fallopian tube, glycodelin-F binds to spermatozoa in the acrosomal region and inhibits the progester-one-induced acrosome reaction. GdC in the cumulus matrix displaces sperm-bound glycodelin-A and -F during sperm cumulus penetration, covers spermatozoa in the equatorial region, and promotes the zona binding capacity of the spermatozoa.

Glycodelin-A carries high mannose, hybrid, and complex-type structure [73]. Whereas, glycans on glycodelin-S are much different, which are highly fucosylated, and contain a complex-type structure of bi-antennary glycans with Lewis-X and Lewis-Y antennae. Glycodelin-A, -F, -C have a similar topology of carbohydrate moi-ety, with different degrees of sialylation. Glycodelin-A and glycodelin-F are diversely sialylated, which are immunosuppressive, while non-sialylated glycodelin-S works on inhibition of capacitation, and low-sialylated glycodelin-C as successful singleton insemination [79].

In the female genital tract, spermatozoa also take part in the remodeling of the sperm surface. Ovulation triggers the release of sperm at the oviductal reservoir and sperm capacitation. During their last stage of the journey to the oocyte, spermatozoa carry out a series of desialylation on the sperm surface, including releasing high-sialylated DEFB-126 from spermatozoa to free the latter in the oviductal reservoir [17], the release of two neuraminidases (NEU1 and NEU3) to remove sialic acids on sperm surface [81], and release of sialylated GPI-anchored glycoprotein via raft redistribution due to cholesterol efflux, such as CD52 [82]. Desialylation and raft redistribution joined by glycolipids, such as ganglioside GM1and seminolipids, help present functional surface proteins for cumulus penetration, such as SPAM1 [83], for sperm-zone pullucide recognition and binding, such as dicarbonyl/L-xylose Reductase (P34H) [84], and cysteine-rich secretory protein-1 (CRISP1) [85].

3. Lectin microarray, a "sugar decipher" for sperm surface glycoconjugates

Glycosylation ubiquitously exists in cellular biology, however, it is still a poorly understood posttranslational modification, due to its complexities. One is the vast

diversity of glycan structure [86]. Due to carbohydrate structural and linkage features, it is established that all possible branched or linear oligosaccharide isomers yield 1.05×10^{12} structures.

The other is the complexity of glycan biosynthesis, due to the complicated biosynthesis machinery and no preexisting template molecule. Possibly, 1–2% of the genome produces glycan biosynthesis-related proteins, such as glycosyltransferases, glycosidases, and transporters. Carbohydrate biosynthesis does not follow "the central dogma," [87] but several factors instead [88], such as levels, activities, and trafficking of glycosyltransferases, availabilities of carriers and nucleotide sugar substrates, different physiological functions and statuses, and a variety of outer stimuli [89], such as nutrition [89], oxidative stress [90], and so on.

In other words, extreme complexity enables glycans as "the third group of bioinformative molecules," [86] which integrate panoramic information on cell biology [91, 92], including genomes encoding proteins and biosynthesis machinery of lipids or glycans, and interactions between cell and environmental factors. Glycan profiles on cell surfaces help distinguish individuals and physiological statuses. For an instance, α-linked N-acetylgalactosamine (GalNac) and β-linked GalNac on Sda/GM2 is exploited to identify spermatogonial subpopulations in cattle, pigs, and horses [93].

3.1 The lectins and other glycan-binding proteins

Lectins are a group of glycan-binding proteins that selectively bind different glycans with specific structures, which are first described back in the late of the nineteenth century [94]. As more and more plant lectin-glycan pairings have been identified [95], a sophisticated, reliable, and convenient tool set emerged (Summarized in **Table 1**). Although lectins could not acquire detailed structural information, they are capable to tell the differences between glycan profiles on different cell surfaces [96].

Animal lectins are endogenous glycan-binding proteins, which are involved in a variety of biological processes, and could provide more biological function clues for glycans (Summarized in **Table 2**). Intriguingly, summarized in **Table 3**, a group of sperm-egg receptors on spermatozoa are reported to have the capability of glycan-binding, including lectin-like proteins, glycosyltransferases, and glycosidases [132, 133]. We identified a chitobiase, lysosomal di-N-acetylchitobiase (CTBS), on mouse spermatozoa [Data unpublished]. CTBS is expressed on spermatozoa with species specificity and shows the binding ability of Lewis-X, which could inhibit mouse sperm-egg binding [134].

Microbial nontoxic B subunits and developed antibodies against specific carbohydrate structures are also glycan-binding proteins, which are exploited to probe the specific glycans [135, 136]. Cholera toxin subunit B (CTxB) is the nontoxic subunit of CTx, which specially bind to the oligosaccharide moiety of a raft-associated glycosphingolipid, ganglioside GM1, and mediates entry of microbe into the host cells [137]. In male fertility, GM1 localization on human spermatozoa detected by CTxB is used as a diagnostic tool for evaluating sperm response to stimuli for capacitation [138].

3.2 Lectin microarray methods

Originated from protein chips, the technique of lectin microarray is established in 2005 for high throughput analysis of glycans [139]. Compared with mass spectrometry-based approaches, analysis of lectin microarray is simple, convenient, and low

Lectins	Carbohydrate specificity	Sources
AAA	Fucose	*Anguilla Anguilla*
AAL	Fucα1–2,3,4	*Aurentia*
ABA	Galβ1–3GalNAc	*Agaricus bisporus*
ACA	Galβ1–3GalNAc	*Amaranthus caudatus*
AMA	Manα-	*Arum maculatum*
APA	Mannose	*Allium porum*
APP	GalNAcα-, GalNAcβ-	*Aegopodium podagraria*
ASA	Manα1–3	*Allium sativum*
BDA	GalNAcα-, GalNAcβ-	*Bryonia dioica*
BBC	GalNAcα-, GalNAcβ-	*Phaseolus vulgaris sp.*
BPL	Galβ1–3GalNAc	*Bauhinia Purpurea*
CA	Galβ1–4GlcNAc, GalNAcβ1–4GlcNAc	*Colchicum autumnale*
CAA	Complex glycans (GlcNAcβ1–2Manα1–3(GlcNAcβ1–2Manα1–6)Manβ1–4GlcNAcβ1–4GlcNAcβ-)	*Caragana arborescens*
CALSEPA	Mannose, Glucose, Glcα1–4Glc	*Calystegia sepiem*
CCA	Neu5Ac	*Cancer antennarius*
Con A	Manα-, Gluα-	*Canavalia ensiformis*
CPA	Mannose	*Cicer arietinum*
CSA	GalNAcα-	*Cytisus sessilifolius*
DBA	GalNAcα1–3GalNAc, GalNAcα1–3Gal	*Dolichos biflorus*
DSL	Neu5Ac-Gal/GalNAc	*Datura Stramonium*
ECA	Galβ1–4GlcNAc	*Erythrina cristagalli*
EEA	GalNAcβ-	*Euonymus europaeus*
GHA	Galα-, GalNAcα-	*Glechoma hederacea*
GNA	Manα-	*Galanthus nivalis*
GS-I	Galα-, GalNAcα-	*Griffonia simplicifolia*
GS-IA4	GalNAcα-	*Griffonia simplicifolia*
GS-IB4	Galα-	*Griffonia simplicifolia*
GS-II	GlcNAcα-, GlcNAcβ-	*Griffonia simplicifolia*
HAA	GlcNAcα-, GalNAcα-	*Helix aspersa*
HHA	Manα-	*Hippeastrum hybrid*
HMA	GalNAcα-, Fucα-, Neu5Ac	*Homarus americanus*
HPA	GalNAcα-	*Helix pomatia*
IAA	GalNAc	*Iberis amara*
IRA	GalNAcα-, GalNAcβ-	*Iris hybrid*
Jacalin	Galα-, Galβ-, GalNAcα-O link	*Artocarpus integrifolia*
LAA	GlcNAcβ-, GlcNAcβ1–4GlcNAc	*Laburnum alpinum*
LAL	Fucose	*Laburnum anagyroides*
LBA	GalNAcα-, complex glycans (GalNAcα1–3(Fucα1–2)Gal)	*Phaseolus lunatus*

Lectins	Carbohydrate specificity	Sources
LcA	Mannose	*Lens culinaris*
LcH	Complex glycans (Man/GlcNAc core with Fucα1–6)	*Lens culinaris*
LcH A	Manα-, Glcα-, GlcNAc	*Lens culinaris*
LcH B	Manα-, Glcα-, GlcNAc	*Lens culinaris*
LEL (TL)	GlcNAc	*Lycopersicon esculentum*
LFA	Neu5Ac	*Limax flavus*
LPA	Neu5Ac	*Limulus polyphemus*
LTL	Fucα1–2,3,4	*Lotus tetragonolobus*
MAA	Neu5Acα2–3Gal	*Maackia amurensis*
MAL-I	Galβ1–4GlcNAc	*Maackia Amurensis*
MAL-II	Neu5Acα2–3	*Maackia Amurensis*
MNA-G	Galα-, Galβ-	*Morniga G*
MNA-M	Manα-	*Morniga M*
MOA	Galα1–3	*Marasmium oreades*
MPA	Galα-, GalNAcα-	*Maclura pomifera*
NPA	Mannose	*Narcissus pseudo-narcissus*
PSA	L-Fucα1,6GlcNAc	*Pisum sativum*
PHA-E	Complex glycans (Galβ1–4GlcNAcβ1–2(Galβ1–4GlcNAcβ1–6) Man)	*Phaseolus vulgaris*
PHA-L	Complex glycans (Galβ1–4GlcNAcβ1–2Man)	*Phaseolus vulgaris*
PMA	Manα1–3	*Polygonatum mulitiflorum*
PNA	Galβ1,3GalNAc	*Arachis hypogaea*
PSL	Complex glycans (Neu5Acα2–6Galβ1–4 GlcNAc, Neu5Acα2–6Galβ1–4Glc)	*Polyporus squamosus*
PTA Gal	Galactose	*Psophocarpus tetragonolobus*
PTA GalNAc	GalNAc	*Psophocarpus tetragonolobus*
PWM (PWA)	GlcNAcβ1–4GlcNAc	*Phytolacca americana*
RCA-I	Galβ-	*Ricinus communis*
RCA-II	Galβ-, GalNAcβ-, Galβ1–4Glc (Lactose)	*Ricinus communis*
SBA	GalNAcα/β-	*Soybean*
SHA	GalNAc	*Salvia horminum*
SJA	GalNAcβ-	*Sophora japonica*
SNA	Neu5Acα2–6Gal/GalNAc	*Sambucus nigra*
SNA-I	Neu5Acα2–6Galβ1–4GlcNAc, Neu5Acα2–6 Galβ1–4Glc	*Sambucus nigra*
SNA-II	Galβ-, GalNAcβ-	*Sambucus nigra*
SSA	GalNAc-O link	*Salvia sclarea*
STL (PL)	GlcNAc, Neu5Ac	*Solanum Tuberosum*
Succinyl-Con A	Mannose	*C. ensiformis*
TKA	Galβ-, Galβ1–4Glc (Lactose)	*Trichosanthes kirilowii*

Lectins	Carbohydrate specificity	Sources
TL	α-GalNAc, β-GalNAc, GalNAc, Galatose, Fucose	*Tulipa sp.*
UDA	GalNAcβ-	*Urtica dioica*
UEA-I	Fucα-, Fucα1–2Galβ1–4GlcNAc	*Ulex europaeus*
UEA-II	GlcNAcβ-	*Ulex europaeus*
VAA	Galβ-	*Viscum album*
VFA	Manα-	*Vicia fava*
VGA	Galβ1–3GalNAc	*Vicia graminea*
VRA	Galα-,	*Vigna radiata*
VVA	GalNAcα-, GalNAcα1–3Gal	*Vicia villosa*
VVA Man	Mannose	*Vicia villosa*
WFA	GalNAcα/β-	*Wisteria floribunda*
WGA	GlcNAcβ-	*Triticum vulgaris*

The lectin carbohydrate specificities were summarized from: 1, the Consortium for Functional Glycomics (http://functionalglycomics.org); 2, Product information from EY or Vector Laboratories, Inc.

Table 1.
Incomplete list of commercial plant lectins.

time-consuming [140]. Due to its non-quantitation and incomplete determination of glycan structures, lectin microarray is more appropriate for analyzing differences between glycan profiles.

We first established the methodology of lectin microarray for the glycan profiling of sperm surface [141]. The major process is summarized in **Figure 1**. Briefly, the process includes the following steps:

1. Pre-treatment of lectin microarray and propidium iodide (PI)-labeling of fixed spermatozoa

2. Incubation of spermatozoa with the microarray

3. Rinsing the microarray to remove the excess or unbound spermatozoa

4. Air-drying the microarray, and record the signals on the chip

5. Data analysis

6. Verification of the results by using flow cytometry with fluorescence-labeled lectins.

3.3 Sugar codes on spermatozoa with lectin microarray

By using a chip with 91 plant lectins following a previous study [142], we compared surface glycan profiles of five mammalian spermatozoa, human, boar, bull, goat, and rabbit [143]. Roughly, 50 lectins were observed to bind to spermatozoa of 5

Lectin types	Sub-types	Common structures	Members	Carbohydrate specificity	Reference
R-Type	The mannose receptor family	Type I trans-membrane glycoproteins containing a single fibronectin type II domain, multiple C-type lectin domains (CTLDs), and an amino-terminal cysteine-rich domain.	The mannose receptor	C-type domain: acetylglucosamine, mannose, and fucose; C-type domain: sulfated glycans containing 3-O-sulfated galactose, 3-O-sulfated Lex, and 3-O-sulfated Lea;	[97]
			The phospholipase A2 receptor	PLA2 neurotoxins	[98]
			DEC-205	Phosphorothioated cytosine–guanosine (CpG) oligonucleotides	[99]
			Endo180	N-acetyl-glucosamine, mannose, and fucose	[100]
	UDP-GalNAc: polypeptide α-N-acetylgalactosaminyl-transferases	Type II transmembrane proteins containing an amino-terminal catalytic domain and a carboxy-terminal R-type domain	ppGalNAcT1–20	UDP-GalNAc	[101]
L-Type	Protein quality control and sorting related	Type I membrane protein with a lumenal portion containing a Ca^{++}-binding domain a proline-rich long hairpin loop called the P domain, and a L-type lectin domain.	Calnexin (CNX) and calreticulin (CRT, missing the cytoplasmic and transmembrane regions)	Misfolded or aggregated glycoproteins	[102, 103]
		Dilysine/diphenylalanine KKFF retention/retrieval motif in cytoplasmic carboxyl terminus	ERGIC-53 and related proteins ERGL, VIP36, and VIPL	Oligomannose-type glycans	[104]
	Others	Pentameric proteins containing L-type lectin folds	Pentraxins and related proteins	Phosphocholine residues on polysaccharides and on phospholipids; carbohydrate derivatives on bacterial polysaccharides	[105]
		Containing laminin G domain-like (LG) modules	Laminins	Heparin. sulfatide, and α-dystroglycan (α-DG)	[106]

P-Type	Mannose 6 -phosphate receptor	Type I trans-membrane glycoproteins containing large extracytoplasmic domains, transmembrane regions, and carboxy-terminal cytoplasmic domains.	Cation-dependent mannose 6-phosphate receptor	N-glycans containing mannose 6-phosphate	[107, 108]
			Cation-independent mannose 6-phosphate receptor		
I-Type	Sialic acid-binding immunoglobulin-like lectins (Siglecs)	Type-1 membrane proteins containing a Sia-binding domain (a V-set domain at amino terminus), and varying numbers of C2-set Ig domains that act as spacers, between Sia-binding site and plasma membrane	Subgroup1: Siglec-1(Sn), Siglec-2 (CD22), Siglec-4 (MAG), and Siglec-15; (Based on sequence similarity)	Sialic acids	[109–112]
			Subgroup2: CD33 related Siglecs. (Siglec-3, -5 to -11, -14 and -16, based on sequence similarity)		
	Others	Immunoglobulin superfamily (IgSF) members other than Siglecs containing a V-set domain.	Paired immunoglobulin-like type-2 receptors (PILRs)	Mucin-like O-glycosylated membrane proteins	[113, 114]
			Platelet endothelial cell adhesion molecule (PECAM)-1	α2–6-linked sialic acids	[115, 116]
			Neural cell adhesion molecule (NCAM) and basigin (CD147)	Oligomannose-type glycans	[117]
			Intercellular adhesion molecule (ICAM)-1	Hyaluronan	[118]

C-Type	The Ashwell-Morell receptor	Transmembrane heteroligomeric glycoprotein complex composed of ASGPR1 (HL-1) and ASGPR2 (HL-2) subunits		Exposed β-linked galactose residues on asialoglycoproteins	[119, 120]
	Collectins	Transmembrane proteins containing an N-terminal triple-helical collagenous region, an α-helical coiled-coil trimerizing neck region, and a C-terminal C-type lectin domain.	Proteins containing 185-Glu-Pro-Asn	Mannose-like sugars	[121]
			Proteins containing 185-Gln-Pro-Asp	Galactose-like sugars	
	Myeloid C-type lectins	Transmembrane proteins containing immunoreceptor tyrosine-based activation motif (ITAM) motifs in their cytoplasmic portion and extracellualr C-type lectin domains	Dectin-1 cluster, including dectin-1, CLEC-1, CLEC-2, LOX-1, CLEC12b, and CLEC9a	Variate of glycans. Dectin-1 bings β-glucans, which are polymers of a backbone of β1–3-linked glucose and side chains of β1–6-linked glucose	[122, 123]
			Dectin-2 cluster including dectin-2, blood DC antigen 2 (BDCA-2), DC immunoactivating receptor (DCAR), DC immunoreceptor (DCIR), CTL superfamily 8 (CLECSF8), and MINCLE (CLEC4E)	Variate of glycans. Dectin-2 bings α-mannans, which are polymers of α1–6-linked mannose with α1–2-linked mannose side chains	
	Selectins	Type-1 transmembrane glycoproteins, containing an N-terminal C-type lectin domain followed by a consensus epidermal growth factor (EGF)-like domain, a series of consensus repeats, and a transmembrane domain with a short intracellular tail	P-selectin on platelets	P-ctin glycoprotein ligand-1 (PSGL-1), mucins containing highly clustered O-glycans bearing SLex antigens and sulfate esters, SLex/a rich sulfated mucins	[124–128]
			E-selectin on endothelial cells	PSGL-1, other glycoproteins that express the SLex antigen on either N- or O-glycans, long-chain glycosphingolipids expressing the SLex antigen	
			L-selectin on leukocytes	PSGL-1, ligands containing sulfated glycans, such as 6-sulfo-SLex on both core-2 O-glycans and on extended core-1 O-glycans	

	Other proteins with C-type lectin domains		A number of proteins with CTLDs have been identified in the pancreas and kidney	Unknown	
Galectins	Proto-typical Galectins	Containing a carbohydrate-recognition domain (CRD)	Galectin-1, -2, -7, -10, -11, -13, -14, and -15	Glycans with terminal β-Gal residues	[129, 130]
	Chimera-type Galectins	Containing a single CRD and an amino terminus with high ratio of proline, glycine, and tyrosine residues	Galectin-3	Glycans with repeating [-3Galβ1–4GlcNAcβ-]n or poly-N-acetyllactosamine sequences	
	Tandem-repeat Galectins	Containing two CRDs connected by a peptide linker	Galectin-4, -8, -9, and -12	α2–3-sialylated glycans, blood group A determinant on a LacNAc core	
Glycosaminoglycan (GAG)-binding proteins		Do not have common folds	Various GAG-binding proteins	Glycosaminoglycan (GAG)	[131]

Classification from Varki A, Cummings RD, Esko JD, et al., editors. Essentials of Glycobiology [Internet]. 3rd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015–2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK310274/

Table 2.
Major human carbohydrate-binding proteins.

| Species	Sperm receptor	Reference
Mouse	β-1,4-galactosyltransferase (β-1,4-GalT)	[132]
	α-D-mannosidase	[132]
	sulphoglycolipid immobilizing protein (SLIP1)	[132]
	56 kDa galactose binding protein (sp56)	[132]
	α-fucosidase	[133]
	fructosyltransferase	[133]
	di-N-acetylchitobiase (CTBS)	Data unpublished
Rat	α-D-mannosidase	[132]
	galactose receptor	[132]
Human	mannose-binding protein	[132]
	α-D-mannosidase	[132]
	β-1,4-galactosyltransferase (β-1,4-GalT)	[132]
	galactose lectin	[132]
	selectin-like molecule	[132]
	PH-20	[133]
	α-fucosidase	[133]
	fucosyltransferase-5 (FUT5)	[78]
	dicarbonyl/L-xylulose reductase (DCXR)	[133]

Table 3.
Sperm surface glycan-binding receptors.

all mammalian species, which illustrates a diversity of glycan structures on spermatozoa. Although lectin binding profiles showed a similarity among the five species, a subtle difference was discovered. SBA, HPA, and VVL (binding to GalNac) and GSL I (recognizing galactose) showed strong binding to spermatozoa of boar, bull, goat, and rabbit, while MAL II, PHA-L, PHA-E + L, PHA-E and SNA bound to human spermatozoa strongly. The results suggested that complex glycan structures (recognized by MAL II, PHA-L, PHA-E + L, and PHA-E) and terminal sialic acids (recognized by SNA) are richer in human spermatozoa.

DEFB-126 is an O-linked glycoprotein, which is a major component of human sperm glycocalyx. It is postulated that DEF-126 carries terminal sialylation that is critical for sperm penetration through cervix mucus. We compared glycan profiles between human DEFB-126 mutant and wild-type spermatozoa with lectin microarray analysis [143]. Intriguingly, compared with wild-type spermatozoa, binding of ABA and Jacalin (both are O-linked glycosylation specific lectins) decreased dramatically, while binding of SNA (a lectin recognizing sialic acids deleted) did not change on DEFB-126 mutant spermatozoa. It is suggested that deficiency of DEFB-126 will cause a decrease of O-linked glycans, while the level of sialylation around spermatozoa does not change, which indicates that a more complicated mechanism may happen for subfertility resulting from DEFB-126 mutation.

We also studied the impacts of cryopreservation on sperm fertility [144]. Decreased MAA (a sialic acid-specific lectin) and increased ABA (O-linked glycosylation specific lectin, generally recognizing the inner layer of glycocalyx) were discovered. Which suggested that cryopreservation may destroy the outermost layer
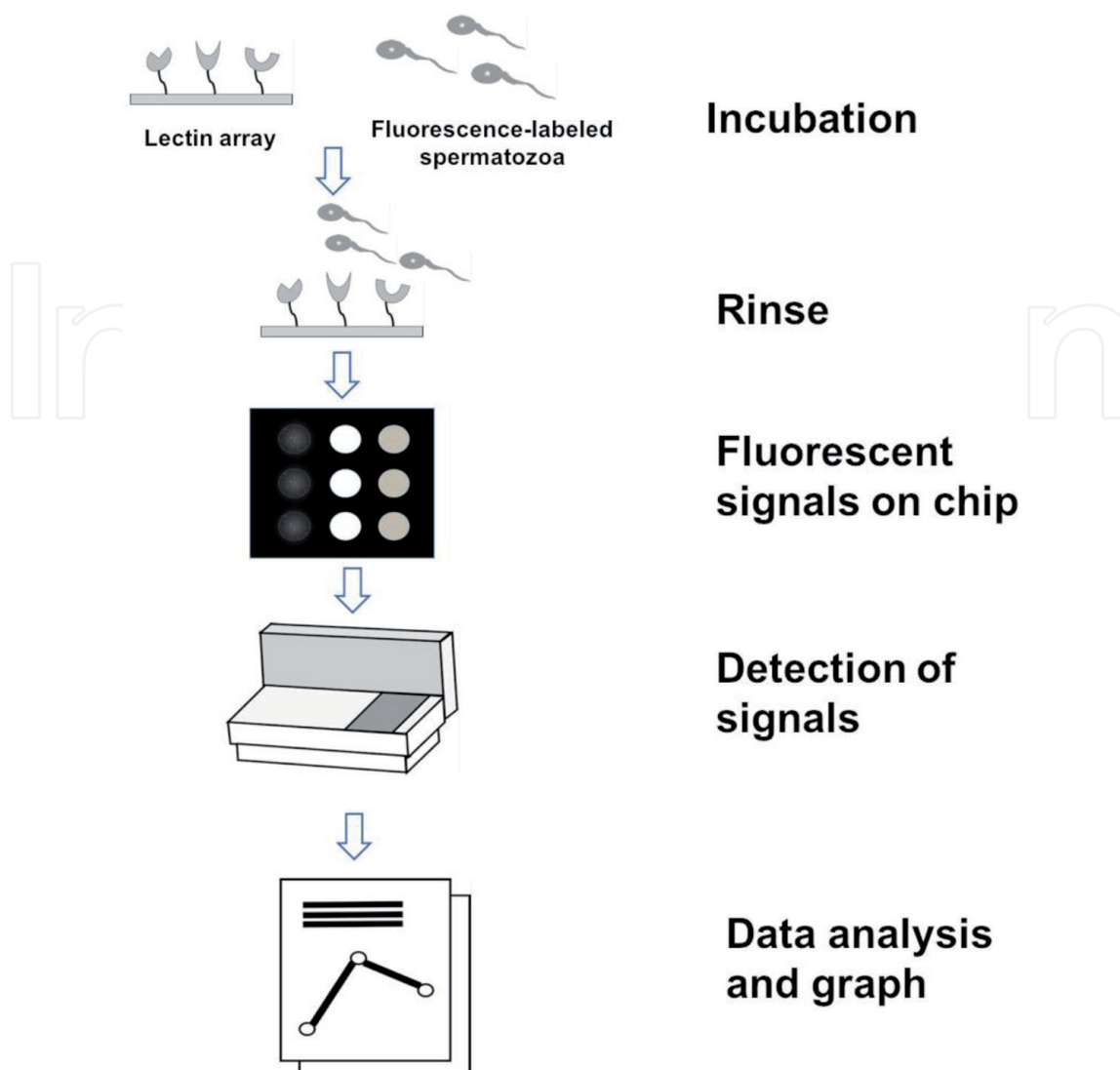
Figure 1.
Scheme of lectin microarray analysis on spermatozoa.

of sialylation on sperm glycocalyx. Furthermore, we found a weak ABA binding to cryopreservation tolerant human spermatozoa, which indicates the importance of the integrity of sperm glycocalyx on cryopreservation tolerance [145].

Due to the significance of glycan structural specificity and biological function, we utilized 60 human lectins and lectin-like proteins for microarray analysis of human spermatozoa [146]. Strong bindings were observed for 5 lectins, galectin-1, 7, 8, GalNAc-T6, and ERGIC-53 (LMAN1). Among them, galectin-8 has definite sperm physiology, which could significantly enhance the acrosome reaction.

4. Conclusion

In modern society, concerns are rising on male fertility. About 10–15% of couples worldwide suffer from infertility, of which male factors account for 50% [147]. Although spermatogenesis is a complicated process, involving in more than 2300 genes that are regulated tempo-spatially to develop spermatogonia to spermatozoa [148], only 30% of male infertility are associated with genetic abnormalities [149, 150]. Furthermore, about 50% of infertility are idiopathic [151, 152], which is a

long-term unsolved andrological question, resulting from many possible factors, such as environmental factors, lifestyle, and so on [153]. Thus, a comprehensive andrological assessment is required for a male infertility work-up, including a detailed history analysis, physical examination, well-established semen analysis, endocrine assessment, DNA and epigenetic deficiencies, and so on [154].

The specific molecular mechanism of sperm development, maturation, capacitation, and fertilization could provide clues to solve male reproductive problems. As discussed above, glycan profiling covers panoramic information about genetic background, previous and current environmental impacts, and biological functions of spermatozoa [92]. Due to the universality of glycosylation modifications along the journey of spermatozoa, it is of great significance to study the physiological function of glycosylation on spermatozoa, which could reveal the targets for diagnosis and treatment of male infertility. As a post-genome technique platform, lectin microarray is capable to decode the "sugar code," the glycoconjugate characteristics of cells [139, 155], and its research on spermatozoa can provide new ideas for diagnosis and management of male reproductive system diseases and infertility.

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Author contributions

SJW, YDL, AJX, and YY wrote the manuscript; SCT conceived the part of lectin microarray; YHG and HJS are joint corresponding authors.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

BSG	Leukocyte differentiation antigen basigin also known as CD147
CRISP1	Cysteine-rich secretory protein 1
CTBS	Lysosomal di-N-acetylchitobiase
CTx	Cholera toxin
CTxB	Cholera toxin subunit B
DEFB126	β-defensin 126
ER	Endoplasmic reticulum
GalNac	N-acetylgalactosamine
GalNAc-T6	Polypeptide N-acetylgalactosaminyltransferase 6
GM2	Ganglioside GM2
GPI	Glycosylphosphatidylinositol
GnT-II	N-acetylglucosaminyltransferase-II

LMAN1	Lectin mannose-binding 1, endoplasmic reticulum-Golgi intermediate compartment protein 53
MX	α-mannosidase IIx
NEU	Neuraminidases
P34H	Dicarbonyl/L-xylose Reductase
PI	Propidium Iodide
Sda	Sid blood group antigen
SPAM1/PH-20	Sperm adhesion molecule 1

Abbreviates of plant lectins are listed in **Table 1**.

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