

Impact of glycosylation on the unimpaired functions of the sperm

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One of the key factors of early development is the specification of competence between the oocyte and the sperm, which occurs during gametogenesis. However, the starting point, growth, and maturation for acquiring competence during spermatogenesis and oogenesis in mammals are very different. Spermatogenesis includes spermiogenesis, but such a metamorphosis is not observed during oogenesis. Glycosylation, a ubiquitous modification, is a preliminary requisite for distribution of the structural and functional components of spermatids for metamorphosis. In addition, glycosylation using epididymal or female genital secretory glycans is an important process for the sperm maturation, the acquisition of the potential for fertilization, and the acceleration of early embryo development. However, nonenzymatic unexpected covalent bonding of a carbohydrate and malglycosylation can result in falling fertility rates as shown in the diabetic male. So far, glycosylation during spermatogenesis and the dynamics of the plasma membrane in the process of capacitation and fertilization have been evaluated, and a powerful role of glycosylation in spermatogenesis and early development is also suggested by structural bioinformatics, functional genomics, and functional proteomics. Further understanding of glycosylation is needed to provide a better understanding of fertilization and embryo development and for the development of new diagnostic and therapeutic tools for infertility.

Keywords: Competence; Glycosylation; Infertility; Maturation; Sperm

Introduction

Research into reproduction has advanced from the identification of specific compounds to detailed structural and functional analysis of proteins, lipids, and carbohydrates. Glycosylation, which is the enzy-

matic process attaching glycans or carbohydrate to proteins, lipids, or other organic molecules, has become recently a popular field in basic life science as well as in medicine. Because it is a key step in structural and functional regulation of biological components. In addition, abnormal glycosylation is associated with various diseases and malfunctions [1-3].

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Glycosylation is essential in the process for spermatogenesis, extracellular quality control of sperm, and early embryo development through the building and remodeling of glycosylated cytosolic factors such as PLC ζ [4,5] and of glyocalyx. It is estimated that > 50% of all human proteins are glycoproteins [1]. Using analytic tools developed in functional proteomics, many glycoprotein candidates have been investigated including 1,196 proteins [6] and 26,000 transcripts [7] in mouse testis, 415 transcripts in bovine sperm [8], and 19,229 transcripts in human [9]. In addition, chemical analyses indicate that a typical mammalian cell contains as many as 10,000 glycolipids [10,11]. Advances in analytical techniques have enabled differences in proteins and lipids between fertile and infertile groups to be de-

terminated [12,13].

Although the distribution of proteins in a sperm membrane or matrix is directed by the given zip code at Golgi complexes during spermatogenesis, such a directed distribution of proteins on the plasma membrane or acrosomal membrane is insufficient for travel to the female reproductive tract and recognition and penetration of the oocyte. These abilities are acquired during epididymal maturation and further biochemical maturation in the female genital tract, and the specific biochemical reactions are observed in the plasma membrane and acrosomal membrane. The polarized localization of membrane proteins and lipids in a specific area of sperm during spermiogenesis is not permanent; and, to maintain sperm fertility, it is essential for their relocation and redistribution in sperm to progress correctly [2,14]. This includes migration, removal from the anterior head and major part of the flagellum, or addition to the special parts of spermatozoa [15-17].

It remains difficult to apply sperm glycosylation in the diagnosis of infertility because of high economic, labor, and time cost even though there have been major developments in carbohydrate analysis methods and machines [18-21]; however, evaluation of sperm glycosylation is becoming an important field in the diagnosis and treatment for infertile couples. The purpose of this review is to provide the basic information of glycosylation during spermatogenesis and fertilization as a useful indicator of competent sperm and of sperm function.

Glycomics and sperm

Glycosylation of proteins can be species specific, tissue specific, cell specific, or a combination of these [22]. The structural and functional diversity of glycoproteins depends on the combination of monosaccharides because this determines the chain length, branching points, linkages, type of anomery (α , β), and/or covalent attachment of modifying groups such as sulphate, phosphate, acetyl, and methyl. However, the terminal sequences are under the control of their specific biological roles (Table 1) [23-25].

Carbohydrate-protein links can be divided into four main categories: links with proteins through a nitrogen of asparagine or arginine residues (N-linked glycosylation; endoplasmic reticulum [ER] or Golgi apparatus); through β -hydroxyl group of serine, threonine, tyrosine, hydroxylysine, or hydroxyproline residues (O-linked glycosylation; various from the subregional ER to beyond an intermediate ER-Golgi compartment of the Golgi apparatus); C-glycosylation (C-mannosylation); or through glycosyl-phosphatidylinositol anchor attachment. In C-glycosylation, a glycan binds to the first tryptophan in Trp-X-X-Trp, Trp-X-X-Cys, and Trp-X-X-Phe [25-27]. In addition, carbohydrates can form links with protein through indirect glycosidic link-

Table 1. Glycosylation linkages between amino acids and carbohydrates

Type of linkage	Linkage amino acid	Carbohydrate (Monosaccharide code)	
N	Arginine	Glucose (Glc)	
	Asparagine	Glucose (Glc) N-Acetylgalactosamine (GalNAc) N-Acetylglucosamine (GlcNAc) Mannose (Man) L-Rhamnose (Rha)	
O	5-Hydroxylysine 4-Hydroxyproline	Galactose (Gal) N-Acetylglucosamine (GlcNAc) L-Arabinose (Ara) Galactose (Gal)	
		Serine	N-Acetyl-fucosamine (FucNAc) Galactose (Gal) Glucose (Glc) Xylose (Xyl)
	Threonine	N-Acetylglucosamine (GlcNAc) L-Fucose (Fuc) Galactose (Gal) Glucose (Glc) Mannose (Man)	
		Serine/Threonine	N-Acetylgalactosamine (GalNAc) N-Acetylglucosamine (GlcNAc) DiActrideoxyhexose Fucose (Fuc) Galactose (Gal) Mannose (Man) Pseudaminic acid (Pse)
			Tyrosine
	C-mannosylation		
	Alkali labile ester	Glutamic acid	Glucose (Glc)
	Ethanolamine	Carboxy-terminus	Glycosyl-Phosphatidylinositol (GPI)
	Glypiation	Pr-C-(O)-EthN-6-P-Man	Mannose (Man)
	Phosphate covalent (Phosphoglycosyl)	Amino acid (Xaa)	ADP-ribose (monomeric or polymeric)
Arginine			ADP-ribose (monomeric or polymeric) Fucose (Fuc)
Aspartic acid		ADP-ribose (monomeric or polymeric) Mannose (Man)	
		Cysteine	ADP-ribose (monomeric or polymeric) Xylose (Xyl)
Glutamic acid		ADP-ribose (monomeric or polymeric)	
		Serine	N-Acetylglucosamine (GlcNAc) ADP-ribose (monomeric or polymeric) Fucose (Fuc) Mannose (Man) Xylose (Xyl)

age (e.g., glycation); linkage of 3-hydroxyl of ADP-ribose to a variety of amino acids (ADP ribosylation) or ethanolamine phosphate to tyrosine, 5-hydroxylsine, and 4-hydroxyproline. Carbohydrates also form links non-enzymatically in vivo to the epsilon amino group of lysine [23-27].

The expansion of knowledge on glycosylation in sperm is the result of the development of a variety of methodologies such as glyco-filtered aided sample preparation (glyco-FASP) coupled with the tandem mass spectrometry (MS/MS) method. Glycoproteins are synthesized during spermatogenesis and involved in various sperm functions [28]. Glycosylation of sperm occurs during spermatogenesis, maturation process during epididymal transit, and capacitation. Recently it has been revealed that there are 554 N-glycosylation sites and 297 N-glycosylated proteins in human sperm with about 91% of them localized in plasma membrane, extracellular region, and lysosomes [29]. Some of the membrane N-linked glycosylated proteins are recognized as important factors for gamete binding [29].

Glycosylation for the approach and recognition of the gamete and fertilization

Sperm can pass the cervical mucus and migrate to the oocytes, and the importance for this function of glycosylation of some proteins has been revealed recently. Sperm defensin (DEFB126) derives from the epididymis and its adsorption onto the sperm is important for cervical mucus penetration by sperm [30-32].

The navigation of sperm to the oocyte depends on guidance by thermotactic attraction, chemotactic attraction, and anatomical characters and physiological control of the female genital tract. Although the thermoreceptor is not clear, phospholipase C (PLC) and inositol 1,4,5-trisphosphate receptor (InsP3R) mediate thermal guidance. PLC and InsP3R are glycoproteins involved in Ca²⁺ regulation [33]. The odorant protein receptor GRK3 (alternative name: beta-adrenergic receptor kinase 3) that is localized in the midpiece of elongating spermatids plays a chemosensory role for sperm chemotaxis during sperm migration to oocytes [34,35]. Rheotaxis requires rotation and CatSper channels. CatSper glycoproteins form the sperm-specific voltage-gated Ca²⁺ channels localized along the membrane of the sperm flagella. CatSper channels are a member of glycoprotein that are involved in positioning regulation [36]. Targeted disruption of CATSPER 1, CATSPER2, CATSPER3 or CATSPER4 affects hyperactivated motility [37].

Getting and maintaining the motility occurs during epididymal maturation as well as during travel the female genital tract. The nicotinic acetylcholine receptor CHRNA7 is localized mainly on the mid-piece and faintly on the principal piece of sperm. Chrna7-null sperm have impairment in maintenance of normal motility [38]. The angio-

tensin-converting enzyme (ACE), a zinc-containing dipeptidyl carboxypeptidase, is present in many tissues including spermatids and sperm surface. ACE-deficient mice show greatly reduced fertility because of poor sperm motility for migration into the oviduct [39]. Calreticulin (CALR) is expressed in germ cells and directly required for alpha disintegrin-associated metalloprotease (ADAM3) maturation. Calr3^{-/-} males are infertile because, although males produce apparently normal sperm, there is a defect in sperm migration from the uterus into the oviduct [40]. Adam1a null results in infertility as sperm cannot migrate through the uterotubal junction [41]. Tyrosyl-protein sulfotransferase 2 (TPST-2) is localized in the acrosomal cap and equatorial segment, and Tpst2-null sperm show a defect in motility at genital tract [42]. Pmis-2 and Press 37 are localized in the sperm surface, and Pmis2-knockout male is sterile because of defects in sperm motility with the failure of sperm transport into the oviducts [43,44]. Lymphocyte antigen 6 complex, locus K (LY6K)-deficient sperm show impaired sperm migration into the oviduct [45] (Table 2) [40-61].

A few glycoproteins have been evaluated as mediators for capacitation and cumulus penetration. Cystein-related epididymal spermato-

Table 2. Glycoprotein of sperm which are involved in zona pellucida (ZP) binding or sperm-egg membrane fusion

Protein	Defects or antibody		References
	ZP binding ability	Sperm-egg fusion	
ADAM1a	Impaired	-	[41]
ADAM2	Impaired	-	[41]
ADAM3	Impaired	-	[40]
Angiotensin-converting enzyme	Impaired	-	[46]
Amyloid-precursor-like protein 2	-	Impaired	[47]
β1,4-galactosyltransferase I	Impaired		[48]
CD9	-	Impaired	[49]
CD52	-	Impaired	[50]
CD81	-	Impaired	[49]
Fertilin	Impaired	Impaired	[41,51,52]
GLIPR1L1	Impaired	-	[53]
HAP2	-	Impaired	[54]
HSPD1	Impaired	-	[55,56]
Izumo1	-	Impaired	[52]
M29 and M37	-	Impaired	[52]
M5 and M42	Impaired	Impaired	[52,57]
PH20	Impaired	-	[58,59]
PMIS-2	Impaired	-	[44]
PRSS37	Impaired	-	[43]
SED1	Impaired	-	[52,60]
SP17	Impaired	-	[59]
TPST2	Impaired	Impaired	[42,44]
Tumor rejection antigen gp96	Impaired	-	[55]
ZPBP1 (C38)	Impaired	-	[52,61]

genic protein (CRES) is involved in capacitation. CRES is a serine protease inhibitor for prohormone convertase 2, and its KO mice show reduced fertility owing to a defect in capacitation [62]. SPAM1, glycosylphosphatidylinositol (GPI)-anchored membrane sperm hyaluronidase, functions by enabling the acrosome-intact sperm to pass through the layer of cumulus cells to reach the zona pellucida [63]. HYALP1 localizes on the plasma membrane of the anterior head and has neutral hyalase activity. It is involved in cumulus penetration [64]. PH-20 is a glycosylphosphatidylinositol-anchored protein localized on both the plasma membrane and the inner acrosomal membrane. It is involved in penetration of the cumulus oophorus [58].

Recognition between sperm and oocyte is highly specific and some glycoproteins are essential in this process. Chaperone glycoproteins, heat shock protein 1 (HSPD1) and tumor rejection antigen 1 (TRA1, endoplasmic), are expressed in most germ cells and involved in recognition of the zona pellucida. They are localized on the surface of the sperm head and following capacitation [56,58]. Sperm surface protein 17 (SP17, cancer testis antigen 22) is a mammalian testis- and sperm-specific glycoprotein. It localizes along the length of the principal piece of the fibrous sheath and over the acrosomal head region and is a sperm autoantigen [65] that is involved in binding between the zona pellucida and sperm with high affinity. Sperm β 1,4-galactosyltransferase I (B4GALT1) is a receptor for the residue of the zona pellucida. Sperm of B4galt null mice bind less to the zona pellucida and penetrate it poorly [48]. Fertilin is an alpha:beta dimer encoded by two genes disintegrin and metalloproteinase domain-containing protein 1 (ADAM1) and ADAM2. Adam1a null results in infertility as sperm cannot migrate through the uterotubal junction and there is impairment in binding to the zona pellucida. Also Adam2 KO mice show strong inhibition in binding of sperm to the zona pellucida [41]. ADAM3, which requires CALR3 for maturation, is also involved in sperm-zona pellucida binding. Calr3^{-/-} sperm have a defect in zona pellucida binding [40]. Pmis-2 is involved in zona pellucida binding. Pmis2-deficient sperm fail to bind to the zona pellucida [44]. Prss37-deficient sperm fail to recognize and bind to a zona-intact egg [43] (Table 2).

Glycine receptor (GlyR) and nicotinic acetylcholine receptor containing an alpha 7 subunit (alpha7nAChR) are important for acrosome reaction. GlyR mutant sperm or alpha7nAChR mutant sperm do not undergo the acrosomal reaction on zona pellucida intact mouse eggs, although they exhibit normal capacitation [66]. TESP5 is a candidate enzyme involved in sperm penetration through the zona pellucida. Tesp5-deficient mice are fertile, but sperm penetration of the zona pellucida is at a very low rate because of a reduced ability to bind to the zona pellucida [67]. ACs is a membrane-associated AC isoforms (mAC), and it is regulated by activated cell surface receptors and associated G proteins leading to modulation of cAMP generation. It is

detected in round and elongating spermatids in a region corresponding to the developing acrosome. AC2, AC3, and AC8 are present in the head and flagellum of sperm [34]. Sperms of AC3 null mice have decreased motility and show increased spontaneous acrosome reactions [68]. MC31 present in the midpiece of cauda epididymal sperm moves to the sperm head after the acrosome reaction and facilitates sperm-egg interactions [69]. Also, sp56 is a part of the acrosomal matrix and is involved in penetration of the zona pellucida [70].

Amyloid-like protein 2 (alternative name: sperm membrane protein YWK-II) is a component of the sperm membrane. In mature sperm, it localizes in the plasma membrane overlying the acrosome. It participates in the binding and fusion of sperm with the egg plasma membrane [47]. Izumo (Izumo 1–4), a molecule with a single Ig domain, is essential in sperm-egg membrane fusion. Sperm lacking Izumo protein are unable to fuse. Izumo1 is also involved in the prevention of polyspermy [71]. Tetraspanins CD9 and CD81 are also essential in sperm-egg membrane fusion [49]. TPST2-null sperm are unable to fuse with the egg membrane [42]. HAP2 is essential for gamete fusion. It has 9 putative N-glycosylation sites and the cytoplasmic region is specialized for fusion [54]. Equatorin (MN9) translocates to the equatorial region during the acrosome reaction, and it is involved in fusion between sperm-oolemma [72]. Fertilin (PH-30) is also involved in sperm-oolemma fusion [51] (Table 2).

One pathological phenomenon is immune infertility. It is present during the fertilization process and affects both men and women. One of the causes of immune infertility is the presence of antisperm antibody, which disturbs fertilization by reducing sperm motility, and making it more difficult for sperm to pass through the cervical mucus [73]. Although it is believed that a mutation of some gene and abnormal glycosylation is the cause of infertility, so far there has been a failure to unmask any related modulation or patterns of glycoproteins or glycolipids. There is a need for additional studies in this area to address these issues [28,74].

Modification of glycoproteins in the epididymis and female reproductive tract

Molecules and mechanisms that are modified during sperm maturation have been studied recently with the aid of the development of high-throughput methodologies, functional genomics, and proteomics. Although the importance of glycosylation for sperm fertility is recognized, specific structural details are lacking. Modification of the plasma membrane includes changes in lipids, protein composition, modifications of surface proteins, and increased total negative charge of the extracellular surface [75]. It has been revealed that the epididymal fluid causes modifications of the glycosylation status of spermatozoa through interaction with various glycotopes [76,77].

Cellular processes associated with glycosylation include host defense and modifications of the cell surface. Posttranslational modification of glycoprotein in the epididymal lumen is mediated by two sets of glycan-modifying enzymes: glycosyltransferases that add sugars to proteins and glycosidases that cleave sugar residues from glycoproteins in the epididymal fluid [78]. There are differences between the caput, corpus, and cauda of the epididymis in the amount and kinds of glycan in the epididymal luminal fluid. Glycosylation shows a maturation-dependent manner and is associated with spermatozoa. Glycoproteins formed by glycosylation of proteins present in the epididymal fluid include 150, 116, 68, 64, 58 (N- and O-linked) and 170 kDa (O-linked) [79].

A comparative glycomics analysis of the male genital tract fluids reveals that there is a gradient of glycomic complexity from the cauda to caput regions of the epididymis, varying from high mannose to sialylated complex type N-glycans and mostly devoid of fucosylation. Meanwhile, the seminal vesicle fluid glycome carries equally abundant multimeric Lewis X structures, but there is a distinctive lack of additional fucosylation of the terminal galactose to give the Lewis Y epitope that typifies the glycome of female uterine luminal fluid (ULF). Some of these changes are correlated with the acquisition of the recognition affinity of the zona pellucida ligands [80]. Monkey epididymal fluid protein 3 (MEF3) is important in sperm fertility as the cause of posttranslational modifications in glycosylation status during epididymal passage. It exhibits strong affinity for N-linked α -D-mannose groups and O-linked N-Ac-galactosamine linkages in epididymal fluids and exhibits moderate affinity for N-Ac-glucosaminylated, fucosylated, and N-Ac-galactosamine residues on more mature corpus and caudal sperm in a maturation dependent manner [81].

PH-20, which is synthesized in the spermatocytes and spermatids, is a glycosyl phosphatidyl inositol-anchored glycoprotein located on the surface of the sperm plasma membrane and on the outer acrosome membrane [82]. A well-known function of PH-20 is its hyaluronidase activity, which is required for sperm penetration of the cumulus oophorus and zona pellucida. Mannose is a major sugar within or at the terminal end of the linked glycan of sperm surface PH20, while fucose and sialic acid are not found as terminal sugars of sperm surface PH-20 [83]. Deglycosylation of sperm surface PH-20 or reduction of its disulfide bonds by beta-mercaptoethanol or dithiothreitol results in a complete loss of its hyaluronidase activity [83]. Glioma pathogenesis-related 1-like protein 1 (GliPr1L1) localized in the sperm equatorial segment and neck is produced by the N-glycosylation process during transition in the caput. It is involved in sperm-zona pellucida interaction [53].

Uterine fluid is also a resource for glycotopes [84]. Lopocalin 2 (Lcn2), an abundant mouse ULF glycoprotein, is highly fucosylated in the context of carrying multiple Lewis X and Y epitopes on complex

type N-glycans at its single glycosylation site. The Lewis X/Y epitopes contribute to the sperm motility-enhancing activity of 24p3, whereas lactotransferrin is largely inactive in this context despite being similarly glycosylated [84].

Possible roles of glycosylation in embryo development

Sperm is no longer thought of as being merely a truck for paternal genome transporter to the egg. Several studies suggest that a specific set of functional RNAs and glycoproteins may be delivered into oocytes and support early embryonic development [85,86]. NL1, a neprilysin-like peptidase, is a member of the M13 family of zinc metalloendopeptidases. NL1 null mice display normal spermatogenesis and sperm parameters, but they produce smaller litters because of decreased oocyte fertilization and perturbed early development of fertilized eggs [87].

Protein convertase subtilisin/kexin-like 4 (PC4) is a testis-specific serine protease and a glycoprotein. It is localized in the acrosome and on the sperm plasma membrane overlying the acrosome [88]. PC4 null mice do not show any altered spermatogenesis, but their sperm exhibit accelerated capacitation, precocious acrosome reaction, and reduced sperm-egg binding ability. Eggs fertilized by these sperm fail to grow to the blastocyst stage [89].

Phospholipase C zeta (PLC ζ) is a member of the phosphoinositide-specific phosphatase C family. It is a soluble sperm factor that is transferred from the sperm into the egg cytoplasm following sperm-egg membrane fusion [90]. It triggers Ca^{2+} waves at egg activation and supports early development. PLC ζ transgenic mice exhibit autonomous intracellular free calcium oscillations and parthenogenetic development [91].

Also, glycation of sperm by some female reproductive glycoprotein is a candidate factor for embryo development. Oviductins in oviduct-specific glycoproteins suspected as a factor for fertilization and/or early development. Oviductin binds to the sperm membrane and has a positive effect on cleavage and blastocyst formation [92,93].

Nonezymatical linking of glucose to protein and abnormality

Unexpected glycosylation can result in functional changes. It was revealed recently that various diseases are derived from abnormal glycosylation. Sustained hyperglycemia leads to non-enzymatic glycation of free amino groups of proteins [94] and increased production of advanced glycosylation end products [14,95]. Glucose reacts nonenzymatically with the NH_2 -terminal amino acid and forms a stable covalent linkage [96,97]. This leads to structural and functional

changes and adversely affects their function. Such a glycation is an irreversible process. In streptozotocin-induced diabetes Wistar rat, the ratio of spontaneous acrosomal reaction is duration dependently higher than the control and fertility is decreased dramatically [98]. Although it has not been shown yet that hyperglycemia is a direct cause of spontaneous acrosomal reaction in diabetes rat, it is suggested that controlled glycosylation is important in the normal physiology of sperm and fertilization.

Sperm fertility decreased dramatically in the diabetes rats [99]. Diabetes is thought to give rise an increase of the spontaneous acrosome reaction and immune infertility [99,100]. Alteration of sperm glycosylation may result in the spontaneous acrosome reaction, but it is not clear yet why diabetes causes this phenomenon. Immune infertility caused by diabetes may also result from the abnormal glycosylation of immune cells [98].

Conclusion

The sperm has been simply considered as paternal genome transporter, but it is no longer restricted to transporter. It is revealed that sperm is a functional mediator of fertilization and early development. Sperm gains full competence during spermatogenesis and epididymal and female-genital-tract transit. During these periods, biochemical modifications including glycosylation are tightly controlled. As discussed above, glycosylation is main reaction and indispensable for competence of sperm. Developed analysis tools and their application to the sperm analysis showed that the useful information is very limited, and suggested that the evaluation of huge candidate glycoproteins and glycolipids is needed to fully understand the getting-competence of sperm. Besides, unspecific adding of carbohydrate also should be solved for clinical application.

There has been little consideration of glycosylation in the approach and treatment for infertile couples. Recently new diagnostic approaches are being prepared using developed methodologies based on molecular technologies. They will be helpful to evaluate the glycosylation during the processes for getting-competence. More accurate and specific information about glycosylation in the diagnosis of male infertility is essential in control or keeping for fertility.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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