

Review Article/Artículo de Revisión

# INVOLVEMENT OF SPECIFIC OVIDUCT CELL GLYCANS IN SPERM BINDING TO FORM A SPERM RESERVOIR

## PARTICIPACION DE LOS GLICANOS ESPECIFICOS DE LAS CELULAS DEL OVIDUCTO EN LA UNION DEL ESPERMATOZOIDE PARA FORMAR EL RESERVORIO ESPERMATICO

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**ABSTRACT**

After semen deposition, sperm are retained in the isthmus region of the oviduct forming a reservoir. The formation of the oviduct sperm reservoir is mediated, at least partially, by sperm recognition of glycan structures present on the oviduct epithelium. The aim of this review is to discuss sperm molecules involved in sperm-oviduct interaction.

**Keywords:** glycans, sperm, oviduct, reservoir  
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**RESUMEN**

Luego de la deposición del semen, los espermatozoides son retenidos en la región del istmo del oviducto formando un reservorio. La formación de reservorio oviductal de espermatozoides es mediado, al menos parcialmente, por el reconocimiento espermático de estructuras de glicanos presentes en el epitelio del oviducto. El propósito de esta revisión es discutir acerca de las moléculas involucradas en la interacción espermatozoide-oviducto.

**Palabras clave:** glicanos, espermatozoide, oviducto, reservorio  
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**INTRODUCCIÓN**

The apical portion of epithelial cells have an elaborate glycocalyx that includes many types of protein and lipid-based structures to which a variety of complex carbohydrates are covalently attached. Sometimes referred to as glycans, they can be polysaccharides (long repeating chain of carbohydrates) or oligosaccharides (smaller carbohydrate containing 2-20 monosaccharides). There is increasing interest in studies of saccharide chains and their biological activities. Recognition of glycan structures by membrane proteins mediates cell-cell adhesion and plays an important role in reproductive processes (Miller, 2015). The best-known examples of sperm interaction with glycans occurs at two points prior to the fertilization process: (1) sperm-oviduct cell binding and (2) sperm-zona pellucida binding. The aim of this review is to discuss recent findings that support a remarkable and specific mechanism by which mammalian sperm bind to oviduct epithelial cells, focusing on porcine sperm. Another review has discussed the sperm recognition of zona pellucida glycans (Shur, 2008).

## THE OVIDUCT RESERVOIR

After semen deposition, sperm pass through the uterine tubal junction within minutes (Baker and Degen, 1972) reaching the oviduct before the uterine immune response occurs (Hunter, 1981). The oviduct is divided into the caudal portion known as isthmus and the cranial portion known as ampulla. During sperm movement in the oviduct, uncapacitated sperm can attach to the isthmus region establishing a reservoir (Hunter 1981). Sperm-oviduct interaction is dynamic; close to ovulation time sperm are released from the reservoir moving to the ampullary segment where fertilization occurs (Miller, 2015).

There are three main functions commonly attributed to the oviduct reservoir. First, the reservoir prolongs sperm lifespan. While bound to the oviduct, sperm are resistant to capacitating stimulatory components such as bicarbonate (Satake et al. 2006) and are maintained in a state of inactivity characterized by inhibition of capacitation, motility, calcium influx and hyperactivation (Rodriguez-Martinez et al. 2005). Because capacitation destabilizes the plasma membrane and consequently reduces sperm life span (Rodriguez-Martinez, 2007), attachment to the oviduct epithelium ensures sperm viability until close to ovulation time. This period may also be considered as a “pre-ovulatory arrest phase” (Hunter 1984).

Second, the reservoir reduces polyspermy. Close to ovulation time, sperm are gradually released from the epithelium controlling the number of sperm reaching the oocyte for fertilization (Mburu et al. 1996). Insemination after ovulation, when oviduct binding is greatly reduced, increases number of accessory sperm bound to oocyte indicating higher number of sperm reaching the oocyte for fertilization (Soede et al. 1995).

Third, the reservoir selects a superior sperm subpopulation. The oviduct acts as an additional checkpoint for the selection of higher quality sperm. Oviduct cells preferentially bind non-capacitated sperm with an intact acrosome, stable chromatin and reduced protein phosphorylation (Ardon et al. 2008; Fazeli et al. 1999; Luno et al. 2013). This sperm population is protected in the oviduct crypts whereas damaged/capacitated sperm are maintained on folds near the central part of the lumen (Mburu et al. 1997). In this region, interaction of capacitated sperm with the oviductal protein deleted in malignant brain tumor 1 triggers acrosome alterations in a sub population of prematurely capacitated/damaged sperm reducing the chances of a suboptimal sperm to fertilize an oocyte (Teijeiro and Marini, 2012; Teijeiro et al. 2012).

## GLYCANS: OVIDUCT COMPONENT OF RESERVOIR FORMATION

In several mammalian species, there is evidence suggesting that the oviduct reservoir is mediated by sperm recognition of glycan structures present on the oviduct epithelium (Suarez, 2001). The type of oviduct carbohydrate motif recognized by the sperm may be species-specific as sialyl, galactosyl and fucosyl residues competitively inhibit binding of hamster, equine and bull sperm to oviduct epithelium (DeMott, et al. 1995; Lefebvre et al. 1997; Sabeur and Ball 2007). Early studies in pigs concluded that boar sperm bind oligomannose with high affinity and galactose with low affinity (Wagner et al. 2002). This study was based on a competitive assay using glycans and glycoproteins containing galactose and mannose residues (asialofetuin and ovalbumin, respectively). Other glycans were not examined.

Competitive assays that test inhibition of sperm binding to oviduct cells after sperm pre incubation with specific glycan candidates are limited; only a small group of selected candidates can be tested. Because of the great diversity of oviduct glycans (Walter and Bavdek 1997), a methodology that tests a broader number of candidates is necessary to identify the authentic glycan recognized by the sperm. Recently, a groundbreaking approach using an array with 377 glycans identified with great accuracy the glycans uncapacitated porcine sperm bind (Kadirvel et al. 2012). Noncapacitated sperm bind with great affinity glycans with either one of two motifs: 1) 6-sialylated N-acetyllactosamine or 2) Lewis X trisaccharide. Binding was highly specific, since isomers of each motif did not bind sperm.

Both Lewis X and sialylated N-acetyllactosamine (SiLN) motifs have been shown to be involved in other reproductive events. In humans, the sialylated version of Lewis X is present at the terminus of zona pellucida glycans and is implicated in sperm attachment to the zona pellucida during fertilization (Pang, et al. 2011). Lewis X trisaccharide has been detected attached to glycoproteins in mouse uterine fluid that may influence sperm function (Kuo, et al. 2009). In pigs, branched sialylated N-acetyllactosamine and Lewis X structures are present on the isthmus epithelial cells and it has been suggested that these motifs are part of the sperm recognition mechanism of oviduct cells (Kadirvel, et al. 2012, Machado, et al. 2014). Inhibition of oviduct SiLN and LeX significantly reduces sperm binding (Kadirvel et al. 2012) supporting the involvement of these carbohydrates as ligands for sperm surface proteins.

In summary, there is evidence that formation of the sperm reservoir involves recognition of a variety of glycans. In addition to mannose, oviduct Lewis X and multi-antennary sialylated N-acetylglucosamine also play an important role in sperm reservoir formation (Kadirvel et al. 2012; Machado et al. 2014; Miller 2015; Silva et al. 2014).

## SPERM COMPONENTS OF RESERVOIR FORMATION

Glycans are recognized by lectin-like proteins that have a defined carbohydrate-recognition domain and tend to recognize specific monosaccharide residues by fitting them to a binding pocket (Varki et al. 2009). Even if part of the sperm binding mechanism is present before ejaculation, maximal binding capability to oviduct epithelium is achieved after sperm contact with seminal fluid proteins (Petrunkina et al. 2001). At ejaculation in bull sperm, bovine seminal plasma (BSP) proteins originating from accessory gland fluids interact with choline phospholipids in the plasma membrane to mediate binding to the oviduct epithelium (Gwathmey et al. 2003, Manjunath and Therien 2002). In the pig, the search for mannose-binding proteins present on the sperm plasma membrane identified members of the spermadhesin family as the proteins mediating sperm adhesion to oviduct epithelium. Spermadhesins are the major proteins present in boar seminal plasma; members of this family includes Awn, Aqn1, Aqn3, PSP-I and PSP-II and their glycosylated forms (Topfer-Petersen, et al. 1998). Spermadhesins bind to the sperm surface during passage through the epididymis (Awn) or after exposure to accessory gland fluids (Aqn1, PSPI and II) coating the membrane covering the acrosomal cap (Dostalova et al. 1994). In pigs, some spermadhesin genes are expressed in the caput and cauda epididymis, but most are highly expressed in seminal vesicles accounting for the great amount of these proteins in ejaculated fluid (Song et al. 2010). Spermadhesin proteins also bind to phospholipids present on the sperm surface (Dostalova et al. 1995). Spermadhesin Aqn1 recognizes glycoproteins with either galactose or oligomannose termini and has been proposed to be the main sperm molecule responsible for oviduct reservoir formation (Ekhlas-Hundrieser et al. 2005). After capacitation, when sperm adhesion to oviduct cells is greatly reduced, Aqn1 is lost from the plasma membrane. This may contribute to sperm release from the isthmus. Antibodies against Aqn1 inhibit sperm-oviduct binding, supporting the role of this protein as a glycan-receptor.

Even though early studies have proposed that molecules mediating reservoir formation include carbohydrates with mannose termini and spermadhesin proteins, it is still unclear if other factors are perhaps involved. As previously discussed, branched glycans with SiLN termini and Lewis X play a role in sperm adhesion to oviduct epithelium but the protein/s recognizing these glycan motifs have not been identified. It is uncertain if current known sperm glycan-binding proteins (Awn, Aqn1, Aqn3) bind Lewis X or SiLN motifs. Recently, a complex containing disintegrin and metalloprotease (ADAM) proteins was proposed as a sperm receptor for zona pellucida N-acetylglucosamine disaccharide (not branched) but not Lewis X (Mori et al. 2012). It is not known if ADAM proteins also recognize the sialylated form of N-acetylglucosamine and furthermore if these proteins have a role in oviduct attachment.

Some Lewis X-binding proteins have been identified in other systems. Selectin proteins recognize a diverse number of Lewis X structures and play a critical role in cancer metastasis through promotion of cell-cell adhesion (Taylor and Drickamer, 2007). An unknown sperm selectin-like protein is proposed to recognize fucose residues in sialylated Lewis X motifs present in human zona pellucida, but the identity of this protein is only speculated (Pang et al. 2011). It is necessary to verify the presence and characteristics of glycan binding proteins specific to SiLN and Lewis X on both epididymal and ejaculated sperm plasma membranes to potentially elucidate different mechanism present in each sperm maturation stage.

## CONCLUSIONS

After insemination, sperm can be held in the isthmus region of the oviduct forming a reservoir. The formation of the oviduct sperm reservoir is mediated, at least partially, by sperm recognition of glycan structures present on the oviduct epithelium. In the pig, a current proposed model for oviduct reservoir formation includes carbohydrates with terminal mannose and spermadhesin proteins (Ekhlas-Hundrieser et al. 2005; Wagner et al. 2002). A large scale screen using a glycan array elucidated that noncapacitated boar sperm bind carbohydrate structures containing Lewis X or SiLN motifs (Kadirvel et al. 2012). Based on these results, it is proposed that a complex adhesive mechanism coordinates sperm-oviduct binding which potentially includes multiple oviduct carbohydrate structures and sperm glycan-binding proteins (Silva et al. 2014).

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