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The Epic Journey of Sperm Through the Female Reproductive Tract

D. J. Miller

Department of Animal Sciences, University of Illinois at Urbana-Champaign.

Abstract

Millions or billions of sperm are deposited by artificial insemination or natural mating into the cow reproductive tract but only a few arrive at the site of fertilization and only one fertilizes an oocyte. The remarkable journey that successful sperm take to reach an oocyte is long and tortuous, and includes movement through viscous fluid, avoiding dead ends and hostile immune cells. The privileged collection of sperm that complete this journey must pass selection steps in the vagina, cervix, uterus, utero-tubal junction, and oviduct. In many locations in the female reproductive tract, sperm interact with the epithelium and the luminal fluid, which can affect sperm motility and function. Sperm must also be tolerated by the immune system of the female for an adequate time to allow fertilization to occur. This review emphasizes literature about cattle but also includes work in other species that emphasizes critical broad concepts. Although all parts of the female reproductive tract are reviewed, particular attention is given to the sperm destination, the oviduct.

Keywords

Bovine; Oviduct; Cervix; Uterus; Sperm

Introduction

Normally only one sperm fertilizes an oocyte despite that billions of sperm are deposited by natural mating into the vagina, or millions are deposited by artificial insemination into the uterus of a cow. The remarkable journey that successful sperm take to reach the oocyte is long and tortuous, filled with viscous fluid, dead ends, and potentially hostile immune cells. Rather than a simple race to get to the oocyte, there is much evidence that complex mechanisms influence sperm transport, immunological tolerance of sperm, sperm selection, sperm storage and release, all before actual fertilization. At steps along the way to the site of fertilization, sperm may interact with the fluid in which they are suspended and the epithelium lining the tract. The very dynamic process of sperm transport helps ensure that there is an appropriate number of fertile sperm at the site of fertilization so that the oocyte can be fertilized by only one sperm. This review considers sperm interaction with

Correspondence: David J. Miller, Department of Animal Sciences, University of Illinois at Urbana-Champaign, 1207 West Gregory Drive, Urbana, IL 61801, djmille@illinois.edu.

Conflict of Interest

There are no conflicts of interest.

fluid in the reproductive tract as well as sperm adhesion to the epithelium. It also reviews how sperm, foreign cells in the female reproductive tract, are tolerated by the immune system. Although it emphasizes literature about cattle, concepts developed in other species are included.

Sperm in the Vagina and Cervix

Sperm are transported through the vagina, cervix, and uterus to the oviduct where they can fertilize oocytes. In cattle and many other mammals, estrus occurs before ovulation so sperm are deposited in the female reproductive tract before ovulation. At normal copulation in cattle, semen is deposited in the cranial vagina. Vaginal fluid is the first luminal medium to which sperm are exposed after semen deposition. The acidic pH of the vagina makes it inhospitable for sperm, although buffers found in semen neutralize the local pH. The cow produces a large volume of vaginal fluid and up to 100 ml can accumulate (reviewed by Rutllant *et al.*, 2005). The rheological properties of vaginal fluid appear to influence sperm motility characteristics, although fertilizing sperm may spend only a short time in the vagina (Rutllant *et al.*, 2005).

It is likely that bovine sperm, like human sperm (Suarez and Pacey, 2006), that are candidates to fertilize oocytes enter the cervical canal quickly avoiding damage due to the low vaginal pH. The cervix contains many folds and grooves that are filled with mucus. The mucus within the canal is a major barrier to sperm, particularly those that have abnormal motility (Katz *et al.*, 1997). The composition and structure of cervical mucus changes near estrus, allowing sperm with normal motility to advance, typically through what have been called “privileged paths” that are found in the grooves produced by folds that extend through the cervical canal (Mullins and Saacke, 1989). A microfluidic model has confirmed that sperm migration through these privileged paths is controlled by microgrooves and a gentle flow of fluid (Tung *et al.*, 2015b).

Sperm are foreign cells and can induce an immune response in the cervix. In rabbits, neutrophil infiltration was observed within 30 min of mating (Tyler, 1977). Immunoglobulins IgG and IgA (Kutteh *et al.*, 1996) and complement proteins have been detected in human cervical mucus (Mathur *et al.*, 1988). Therefore, sperm retained in the cervix might be attacked by the immune system before moving into the uterus.

Sperm in the Uterus

After natural mating, sperm move from the cervical canal into the uterus. In cattle, artificial insemination (AI) is used frequently. When performing AI, the technician deposits semen directly into the uterine body, so sperm do not enter the vagina and cervix. Depositing sperm directly in the uterus reduces the number of sperm needed for routine AI to 10–20 million (Moore and Hasler, 2017). As few as 2 million sperm are often inseminated when using sperm separated based on their sex chromosome, a process used to bias the sex of offspring (DeJarnette *et al.*, 2009). Experiments in which the uterotubal junction (UTJ) in heifers was ligated at various times after mating revealed that it took 6–8 hr for sperm to move through the cervix and uterus to infiltrate the oviduct in numbers sufficient for oocyte fertilization (Wilmut and Hunter, 1984). Sperm are transported through the

uterus with the aid of uterine smooth muscle contractions in the direction of the oviduct (Hawk, 1987). To measure fluid movement and uterine contractions, technetium-labeled albumin-macrospheres were deposited in the uterus of women. These macrospheres (5–40 μm diameter) could be detected by high-resolution ultrasound. They were transported from the uterus to the oviduct more rapidly in the late follicular phase (Kunz *et al.*, 1996) which, along with other experiments, indicates that uterine contractions that transport sperm are under endocrine control. Further, this result demonstrates that materials in addition to sperm can move through the UTJ.

Sperm in the uterus of cattle and other species are retained in uterine glands in low numbers per gland (Hunter, 1995, Rijsselaere *et al.*, 2004). Retention, at least in swine, is accomplished by sperm binding to uterine epithelial cells (Rath *et al.*, 2016). Sperm attachment to uterine cells stimulates the production of both pro- and anti-inflammatory cytokines (Lovell and Getty, 1968). There is evidence that porcine sperm bind to sialic acid-containing glycans on the surface of uterine epithelial cells (Rath *et al.*, 2016). For example, a sialic acid lectin that recognizes sialic acid binds to uterine epithelial cells and blocks sperm binding, *in vitro*. Although it is not clear whether many sperm in uterine glands move into the oviduct, the fate of the majority of sperm in the uterus is elimination.

Rapid removal of sperm may help reduce the acquired immune response against sperm (Hansen, 2011). Little is known about the immune response elicited by semen deposition in cattle but it has been studied more in rodents and horses (Katila, 2012, Bromfield, 2014, Christoffersen and Troedsson, 2017). The primary function of the inflammatory response is to clear excess sperm, seminal debris and bacteria from the uterus. Following semen deposition, there is an infiltration of polymorphonuclear leukocytes. In addition to activation of innate immunity, adaptive immunity is also involved. Several classes of antibodies have been isolated from uterine fluid. In addition to cytokines released from the uterine endometrium, seminal plasma itself contains immune system modulators that affect uterine and oviduct immune cells (Robertson, 2007, Schjenken and Robertson, 2014 and 2015). There is evidence that a seminal vesicle protein may allow the uterus to tolerate sperm (Kawano *et al.*, 2014). Interestingly, the seminal fluid fraction of semen also improves preimplantation development and has interesting long-term effects on offspring (Bromfield *et al.*, 2014). This non-traditional role of seminal plasma has been studied most in rodents; the amount of seminal plasma in cattle that mate normally is low and even lower when artificial insemination is used.

Sperm Entry into the Oviduct through the Utero-tubal Junction

In the bovine UTJ, sperm move through a slit-like lumen with a mucosal pad and into the lower portion of the oviduct, the isthmus, which contains 4–8 primary grooves in tubal segment (Wrobel *et al.*, 1993). Compared to the major part of the upper oviduct, the ampulla, the isthmus has a narrower lumen with fewer folds but a thicker layer of smooth muscle. Although macrospheres seem to have the ability to pass through the UTJ (discussed above), there is evidence that sperm, at least in mice, require a specific protein to be recognized and to pass through the UTJ into the isthmus. Mouse sperm deficient in ADAM3, due to mutation of the ADAM3 gene or genes whose products affect ADAM3 are

not detected beyond the UTJ (Nakanishi *et al.*, 2004, Yamaguchi *et al.*, 2006, Yamaguchi *et al.*, 2009, Okabe, 2013). Even if sperm from a chimeric male derived from a normal and a mutant embryo were deposited, only the normal sperm moved into the oviduct (Nakanishi *et al.*, 2004). Thus, the presence of normal sperm does not aid in opening the UTJ to allow ADAM3 mutant sperm to pass into the oviduct.

In addition to ADAM3, there also appears to be a rheological barrier in the porcine UTJ, perhaps the viscous mucus present in the grooves of this structure (Hunter, 2002, Tienthai, 2015). The rabbit and mouse UTJ and oviduct fluid contain proteoglycans with sulfated glycosaminoglycan chains and hyaluronan (Jansen, 1978, Suarez *et al.*, 1997). In addition to changing the viscosity and affecting sperm motility, the abundance of hyaluronan in fluid and its receptor, CD44 on the epithelial cells of the UTJ, suggest that CD44 signal transduction might affect the function of the UTJ and lower oviduct (Bergqvist *et al.*, 2005a, Bergqvist *et al.*, 2005b).

In cattle and other species, there appears to be a valve at the UTJ that can constrict the lumen, restricting sperm entry. This valve is formed by a vascular plexus and surrounded by a thick muscle layer that, in total, can contract the lumen (Wrobel *et al.*, 1993). The physical constriction, mucus barrier and protein signature requirements emphasize how stringently entrance to the oviduct is regulated.

Sperm in the Oviduct

Once sperm enter the lower oviduct, the isthmus, they can bind to the epithelial cell surface or remain in oviduct fluid. Many studies of the intact oviduct have been performed in mice because the uterus and oviduct can be transilluminated so that sperm can be observed (Demott and Suarez, 1992). Sperm from transgenic mice that have enhanced green fluorescent protein in their acrosomes and red fluorescent protein in their midpiece mitochondria have been followed in the female tract after natural mating (La Spina *et al.*, 2016). The location of live sperm and their acrosomal status can be followed using fluorescence microscopy.

When sperm in the lumen of the isthmus were observed, groups of sperm were carried by fluid that was moved alternately toward the uterus and then toward the ampulla (back and forth) by contractions of oviduct smooth muscle (Ishikawa *et al.*, 2016). These contractions were not observed in the ampulla. Most of the sperm in the isthmus were acrosome-intact (La Spina *et al.*, 2016). Relatively few sperm were found in the ampulla and most were acrosome-reacted (La Spina *et al.*, 2016, Muro *et al.*, 2016), consistent with the recent evidence that the acrosome reaction of fertilizing mouse sperm occurs prior to contact with the cumulus-oocyte complex (Jin *et al.*, 2011, La Spina *et al.*, 2016).

Oviduct Fluid Affects Sperm Function—The fluid in the oviduct is highly viscous, unlike the culture medium in which studies of mammalian fertilization are usually performed. Fluid viscosity is often overlooked in studies of sperm function within the oviduct. More viscous fluid has more internal friction so the wake from a sperm swimming in viscous medium is relatively small compared to less viscous medium (Kirkman-Brown and Smith, 2011). Studies of human sperm demonstrate that resistance of the fluid to be

in sperm bound to the oviduct epithelium, it might contribute to their release prior to fertilization (Topfer-Petersen *et al.*, 2008, Hung and Suarez, 2010).

In addition to losing proteins, sperm also gain proteins while they reside in the oviduct. The first of two examples is Oviduct Specific Glycoprotein (OGP) or oviductin, also known as OVGPI, found in oviducts of many mammals. Although it has homology to the chitinase family of proteins, OGP does not have enzymatic activity (Jaffe *et al.*, 1996, Araki *et al.*, 2003). Bovine sperm incubated in OGP have improved motility and viability (Abe *et al.*, 1995). Hamster sperm treated with recombinant OGP have increased phosphorylation of tyrosine residues on proteins, an indication that capacitation was enhanced (Yang *et al.*, 2015). There is also evidence in mice and swine that OGP binds to the zona pellucida to increase fertilization success by rendering the zona matrix more permissive to penetration by sperm (Lyng and Shur, 2009, Algarra *et al.*, 2016).

A second example of an oviduct protein that affects sperm is osteopontin. Although it is already bound to bovine sperm before semen is deposited in females (Erikson *et al.*, 2007), addition of osteopontin during *in vitro* fertilization reduces polyspermy (Goncalves *et al.*, 2008). Neither osteopontin nor OGP is necessary for fertility in mice because animals deficient in each are fertile (Rittling *et al.*, 1998, Araki *et al.*, 2003).

In addition to oviduct fluid proteins being added as peripheral membrane proteins, integral membrane proteins could be added by fusion with sperm of oviductosomes secreted by the oviduct. For example, a portion of the major Ca^{2+} efflux pump is added to mouse sperm by oviduct exosomes (Al-Dossary *et al.*, 2015). The proteins secreted by bovine oviduct cells and found in oviduct fluid have recently been profiled and include growth factors, metabolic regulators, immune modulators, enzymes and extracellular matrix components (Lamy *et al.*, 2016, Pillai *et al.*, 2017). They function in immune homeostasis, gamete maturation, fertilization and early development (Pillai *et al.*, 2017). The abundance of some depend on the stage of the estrous cycle and whether they were found in oviducts ipsilateral or contralateral to the ovary that ovulated (Lamy *et al.*, 2016).

The Oviduct as a Functional Sperm Reservoir—The oviduct, along with the UTJ in some species, appears to be the major location in which sperm are stored before fertilization. In contrast, although sperm are retained in the cervix or uterus, it is not clear that they are eventually released to move to the oviduct. So the UTJ and oviduct appear to be the major sperm storage sites in many mammals. To be a true “functional sperm reservoir”, as coined by Hunter, (Hunter *et al.*, 1980), in addition to retaining sperm, the oviduct must affect sperm function and lengthen sperm lifespan beyond the inherent longevity of sperm (Orr and Zuk, 2014). More than simple adhesion occurs because binding to the oviduct epithelium prolongs the lifespan of sperm and suppresses capacitation and motility (Pollard *et al.*, 1991, Rodriguez-Martinez, 2007, Hung and Suarez, 2010)(Rodriguez-Martinez *et al.*, 2005). Thus, the oviduct isthmus meets these requirements. But the ability of sperm reservoirs described in a variety of species to prolong the lifespan of a highly differentiated and transcriptionally inactive cell is enigmatic.

The reservoir also releases a finite number of stored sperm, acting as a buffer for sperm number to prevent polyspermy but still provide an appropriate number of fertile sperm to the upper oviduct (Hunter and Leglise, 1971b). The isthmic epithelium binds and retains preferentially sperm that have intact acrosomes and normal morphology (Teijeiro and Marini, 2012)(Teijeiro *et al.*, 2011). All together, the isthmus functions to increase the probability that a suitable number of fertile sperm are present at the site of fertilization.

The Oviduct Epithelium Retains Sperm and Modulates Sperm Function—

In mammals, the oviduct epithelium binds and retains sperm so they accumulate to form the reservoir. Adhesion is very specific. The sperm head binds to oviduct epithelial cells but not all cells (Pacey *et al.*, 1995, Kervancioglu *et al.*, 2000). And the ability of sperm binding to maintain viability is not a common property of all cells (Boilard *et al.*, 2002). The ability to maintain viability requires direct contact between sperm and oviduct epithelial cells (Dobrinski *et al.*, 1997, Murray and Smith, 1997, Smith and Nothnick, 1997). Adhesion to the oviduct regulates sperm capacitation (Dobrinski *et al.*, 1997, Boilard *et al.*, 2002, Fazeli *et al.*, 2003) and suppresses the normal increase in sperm intracellular free calcium that occurs during capacitation (Dobrinski *et al.*, 1996, Dobrinski *et al.*, 1997).

Studies performed in several mammals have concluded that glycans are the components in oviduct epithelial cells that bind sperm (Lefebvre *et al.*, 1997, Green *et al.*, 2001, Suarez, 2001, Cortes *et al.*, 2004, Topfer-Petersen *et al.*, 2008). The evidence in most studies underpinning a role for oviduct glycans is a competition assay in which different glycans are added to sperm before challenging these sperm by allowing them to bind oviduct epithelial cells *in vitro*. If few sperm bind to oviduct cells, this result is interpreted as an indication that the specific glycan is related to the authentic oviduct glycan that binds sperm. A frequent problem with these studies is that most test high concentrations of a small number of monosaccharides or small oligosaccharides.

Identification of Glycans that Bind Porcine Sperm Using a Glycan Array—

The development of glycans immobilized on an array provided an opportunity to test hundreds of glycans for their ability to bind sperm. Using such an array, nearly 400 glycans were tested for their ability to bind porcine sperm (Kadirvel *et al.*, 2012). All the glycans that bound sperm contained one of two glycan motifs, either a Lewis X trisaccharide (Le^X) or a structure with with core mannose and two antennae terminating in the sialylated lactosamine trisaccharide bi-SiaLN or in simply lactosamine (Figure 1). There were several examples demonstrating that sperm bound these two motifs with high specificity. In all sialic acid-containing structures that bound sperm, sialic acid was linked to the 6 position of galactose; structures that were identical except that sialic acid was attached to galactose at the 3 position did not bind sperm. Furthermore, the branched structure on a mannose core was required because single sialylated lactosamine trisaccharides (Neu5Ac α 2–6Gal β 1–4GlcNAc) did not bind sperm (Kadirvel *et al.*, 2012).

The Le^X trisaccharide was found as a monomer, dimer or trimer in the remaining glycans that bound sperm (Kadirvel *et al.*, 2012). This trisaccharide is composed of Gal and Fuc linked to GlcNAc (Figure 1). The Le^X trisaccharide also bound sperm with high specificity; the closely related Lewis A trisaccharide (Le^A, a positional isomer; the carbons in GlcNAc

to which Gal and Fuc are linked are exchanged) did not bind porcine sperm. Contrarily, bovine sperm bind Le^A but not Le^X (Suarez *et al.*, 1998). Binding specificity was further supported because porcine sperm did not bind to Galβ1–4GlcNAc; fucose substitution on Le^X was necessary (Kadirvel *et al.*, 2012).

To confirm that the glycans on the array that bound sperm were present in the oviduct isthmus and to determine the complete structures of the oviduct glycans that bound sperm, oviduct glycans and glycolipid structures were identified by tandem mass spectrometry (Kadirvel *et al.*, 2012). The Le^X and branched sialylated motifs (bi-SiaLN) that bound sperm were found on larger structures that were the most abundant of the complex-type glycans on epithelial cells (Kadirvel *et al.*, 2012). Most of the complex-type oligosaccharides linked to proteins through asparagine residues were branched with two antennae and several had a sialyl residue on at least one terminus. Some biantennary glycans had both motifs, a sialyl residue on one terminus and a Lewis structure on the second. This kind of hybrid glycan was not present on the array but it is possible that, because it includes both motifs, it might bind sperm with highest affinity.

Because tandem mass spectrometry did not distinguish between Le^A and Le^X and between glycans with sialyl residues attached to the 6-carbon and the 3-carbon of Gal, an additional strategy was used. An antibody and specific lectin, *Sambucus nigra* agglutinin (SNA) were used that recognize sialic acid attached to galactose in an α-2,6 linkage preferentially and not sialic acid attached to galactose in an α-2,3 linkage (Naito *et al.*, 2007, Song *et al.*, 2011). Both reagents detected 6-sialylated structures that were abundant on the epithelium throughout the oviduct including on ciliated and non-ciliated cells (Kadirvel *et al.*, 2012).

Similarly, an antibody to Le^X was also used to confirm the identity of the oviduct Lewis trisaccharide structures identified by MS (Kadirvel *et al.*, 2012). Interestingly, Le^X was found in a punctate pattern at the luminal surface of porcine isthmus epithelial cells (Machado *et al.*, 2014) but was not found in the ampulla.

bi-SiaLN and Le^X Glycan Motifs Bind to the Porcine Sperm Head—The head is the portion of sperm that binds to the oviduct epithelium and is where (Suarez *et al.*, 1991) authentic receptors for glycans with bi-SiaLN and/or Le^X motifs should be localized. Fluorescein-labeled Le^X and bi-SiaLN bound preferentially to the apical edge of the head in 60–70% sperm prior to capacitation (Kadirvel *et al.*, 2012, Machado *et al.*, 2014). Binding of fluoresceinated glycans could be displaced by an excess of the same glycan that did not have a fluorescent tag. The binding specificity was confirmed by testing sperm binding to oviduct glycans attached to Sepharose beads (Figure 2). Tethering a motile cell to a solid phase glycan rather than a soluble glycan more closely mimics sperm binding to the oviduct and requires a higher affinity.

Porcine Sperm Binding to Oviduct Cells Requires Glycans with bi-SiaLN and Le^X—Experiments using immobilized glycans (i.e. the glycan array and glycans linked to Sepharose) showed that bi-SiaLN and Le^X were each sufficient to tether a motile sperm. Necessity experiments were performed in which either the glycans or putative receptors were blocked. The result of blocking was assessed by sperm binding to aggregates of

epithelial cells stripped from the isthmus (Figure 2). Results of these experiments indicated that each glycan or glycan receptor was necessary for sperm to bind oviduct cells.

Receptors on Sperm for Oviduct Glycans—The identity of the sperm molecules that mediate binding to the oviduct is controversial. It appears that different species may use different adhesion molecules. Using bovine tissues, one group found that two oviduct proteins, the chaperones GRP78 and HSP60, bound to sperm (Boilard *et al.*, 2004). In contrast, a second group, also using bovine sperm, proposed that oviduct plasma membrane annexins containing fucose bind to accessory gland proteins deposited on sperm at ejaculation (Ignatz *et al.*, 2007). This result was a bit surprising because annexins are usually considered as cytosolic proteins and they lack signal peptides that would direct them through the secretory pathway to become fucosylated. A proteomic study found that annexin A1 is the most abundant protein in oviduct fluid (Lamy *et al.*, 2016). Perhaps it is released into fluid without passing through the secretory pathway. But in the fluid, it would be expected to compete with annexin A1 located on oviduct epithelial cells and decrease sperm binding to the oviduct.

Studies of porcine sperm also implicated accessory gland secretions added to sperm (Ekhlesi-Hundrieser *et al.*, 2005, Topfer-Petersen *et al.*, 2008). The spermadhesin AQN1 originating from accessory gland secretions is a glycan-binding protein (Ekhlesi-Hundrieser *et al.*, 2005, Topfer-Petersen *et al.*, 2008). Spermadhesins represent 90% of the total boar seminal plasma protein and they become peripherally associated with the sperm plasma membrane after ejaculation (Sanz *et al.*, 1993). Sperm AQN1 is reported to bind mannose and galactose residues on oviduct cells, but not Le^X or bi-SiaLN structures (Ekhlesi-Hundrieser *et al.*, 2005).

The observation that the accessory gland proteins do not bind Le^X and bi-SiaLN motifs (Ekhlesi-Hundrieser *et al.*, 2005) and sperm obtained from the cauda epididymis are still able to bind oviduct cells, although in reduced number (Petrunkina *et al.*, 2001), suggested that other glycan receptors were important. Indeed, in cattle there is no evidence that the fertility of epididymal sperm, not exposed to accessory gland proteins, is lower than normal ejaculated semen that includes accessory gland secretions (Amann and Griel, 1974). The fertility of cauda epididymal sperm motivated the investigation of glycan receptors on porcine sperm from the epididymis, which also avoided interference from the very abundant accessory gland proteins (Silva *et al.*, 2014).

Membrane lysates from porcine cauda epididymal sperm were separated chromatographically and each fraction was subjected to SDS-PAGE, transferred to nitrocellulose and incubated with biotinylated Le^X and bi-SiaLN. This “glycan blot” was used to identify proteins with appropriate glycan affinity. Several proteins were identified including the peripheral membrane protein MFG-E8, also known as lactadherin, P47 or SED1 (Silva *et al.*, 2017). Competition experiments showed that lactadherin bound to oviduct cells and that inhibition reduced sperm binding (Silva *et al.*, 2017).

Although there is compelling evidence that oviduct glycans are at least partially responsible for sperm binding, there is also evidence that sperm binding to oviduct epithelial cells is

mediated to some degree by other interactions. Perturbation of glycans or their candidate receptors decreases sperm binding to oviduct cell aggregates by a maximum of 60% (Kadirvel *et al.*, 2012, Machado *et al.*, 2014). Protein-based interactions may account for the residual binding. For example, fibronectin from oviduct cells can bind $\alpha 5\beta 1$ integrin on bovine sperm (Oszycka-Salut *et al.*, 2017) and the adhesion protein E-cadherin is found in both sperm and oviduct epithelial cells (Caballero *et al.*, 2014). (Pollard *et al.*, 1991, Lefebvre *et al.*, 1995)

Oviduct Epithelial Cells Respond to Sperm Binding—In addition to the effect of adhesion on sperm, sperm adhesion to the oviduct modifies the transcriptional profile of oviduct epithelial cells (Fazeli *et al.*, 2004, Georgiou *et al.*, 2005, Georgiou *et al.*, 2007, Lopez-Ubeda *et al.*, 2015). Genes related to the inflammatory response, molecular transport, protein trafficking, and cell-to-cell signaling are among those most affected by sperm (Lopez-Ubeda *et al.*, 2015). In the sow, there is evidence that the ovary has a local effect on the transcriptome of the oviduct. Unilateral ovariectomy reduces expression of genes believed to be involved in sperm survival and early embryonic development (Lopez-Ubeda *et al.*, 2016). The effect of sperm on the sperm reservoir appears conserved between birds and mammals. Infiltration of porcine sperm into the UTJ and rooster sperm into the chicken utero-vaginal junction alters the expression of genes involved in pH regulation and immune-modulation (Atikuzzaman *et al.*, 2017). Even more surprisingly, the transcriptional response of oviduct cells is different in response to insemination of either X chromosome- or Y chromosome-bearing sperm (Alminana *et al.*, 2014). Thus, the presence of sperm changes the behavior of oviduct cells in addition to its consequences for sperm. The result of altered production of specific proteins by oviduct cells is not clear.

Sperm Release from Oviduct Epithelial Cells—The fertilizing sperm must be released from the oviduct reservoir to move to the ampulla and meet the oocyte. There are several models to explain sperm release. One paradigm is that there is a controlled release of stored sperm near ovulation in response to a signal, perhaps from the ovulated oocyte or the released follicular fluid. There is an alternative hypothesis that a subset of sperm are released more stochastically at all times so that there is always a small number of sperm in the ampulla prepared to fertilize an oocyte. But even in this paradigm, it seems likely that there is some control over release of the heterogeneous population of sperm. Sperm release may be due to a change in the oviduct epithelial cells, in the sperm or in the fluid surrounding the cells.

The observation that capacitated sperm have reduced ability to bind oviduct glycans (Kadirvel *et al.*, 2012, Machado *et al.*, 2014) supports a model in which capacitation, the programmed maturation that sperm undergo, causes a change in oviduct glycan receptors so that sperm are released from the oviduct epithelium. Because capacitation does not occur synchronously, in this model sperm release would be expected to be stochastic. The mechanism behind this reduced binding during capacitation is not clear but it may be related to modification in the function of oviduct glycan receptors on sperm, perhaps by proteolysis. There is some preliminary evidence for proteolysis because one candidate glycan receptor, MFG-E8, co-precipitates in sperm lysates with a proteasomal subunit (Miles *et al.*, 2013).

Another alternative is that the development of hyperactivated motility may be sufficient to detach a sperm from the oviduct epithelium (Curtis *et al.*, 2012). In support of this, mouse sperm deficient in CatSper calcium channels that cannot hyperactivate do not detach from the oviduct (Ho *et al.*, 2009).

There is evidence that the cumulus cells of the ovulated cumulus-oocyte complex can release chemical signals, such as progesterone (Schoenfelder *et al.*, 2003, Tosca *et al.*, 2007), which might activate localized sperm release by promoting Ca^{2+} influx through CatSper channels (Lishko *et al.*, 2012). Release may also be controlled by components from the oviduct itself, such as disulfide reductants (Talevi *et al.*, 2007, Brussow *et al.*, 2008), glycosidases that cleave oviduct glycans from the epithelium (Carrasco *et al.*, 2008a, Carrasco *et al.*, 2008b), and oviduct smooth muscle contractions (Chang and Suarez, 2012). There is evidence that locally produced anandamide activates cannabinoid receptors and TRPV1 to induce a Ca^{2+} influx and sperm release (Gervasi *et al.*, 2016). Anandamide may also activate nitric oxide production by sperm to promote their release (Osycka-Salut *et al.*, 2012). Finally, the production of unknown sulfated glycoconjugates may release sperm by competing for binding sites on the oviduct epithelium (Talevi and Gualtieri, 2010). The dynamic nature of sperm interaction with the oviduct suggests that a variety of factors may regulate sperm release that may aid in providing a constant supply of competent fertilizing sperm.

Immunological Tolerance of Sperm in the Oviduct—The oviduct lumen must maintain an aseptic state for successful fertilization and early embryonic development while regulating maternal responses to allogenic sperm and semi-allogenic embryos (Marey *et al.*, 2016). Under pathologic conditions, the mucosal immune system produces a proinflammatory response. But sperm binding to oviduct epithelial cells induces an upregulation of *IL-10*, *TGF β* and increased production of prostaglandin E_2 , inducing an anti-inflammatory response (Marey *et al.*, 2016, Yousef *et al.*, 2016). This produces an environment that suppresses sperm phagocytosis by PMNs and allows sperm greater opportunity to survive in the oviduct and fertilize oocytes. In essence, sperm induce their own protection from an immune response in the oviduct.

Practical Applications of Unraveling the Complexities of Sperm-Female Tract Interaction

Understanding how the oviduct stores sperm should provide insight into how we could improve semen diluents to store sperm for longer periods of time without cryopreservation (McGetrick *et al.*, 2014). This would be of great benefit to species whose sperm are stored as liquid semen for a few days because they do not survive cryopreservation. It would also benefit regions of the world that have poor infrastructure for storing cryopreserved semen (i.e. irregular delivery of liquid nitrogen) or use fresh semen routinely due to easy transportation and rapid use of the semen (Vishwanath and Shannon, 2000). As proof of principle, addition of a specific soluble heat shock protein (HSPA8) to bull, boar and ram sperm can improve viability after a 24–48 hr incubation (Elliott *et al.*, 2009, Lloyd *et al.*, 2009, Lloyd *et al.*, 2012, Holt *et al.*, 2015).

A second application of this line of research is that it may lead to methods to lengthen sperm lifespan in the oviduct. This ability would improve fertility of females if semen deposition

was not properly timed with ovulation, a significant problem with AI in cattle and many other species. It may be possible to reduce the need for estrus detection in females because an accurate estimate of ovulation time might be less critical. Fertility despite the uncoupling of mating with ovulation has been accomplished by some mammals, notably some species of bats that store sperm for months, as well as snakes, reptiles and insects (Holt and Fazeli, 2016). Although the opportunity to reduce estrus detection by lengthening sperm lifespan may be overly optimistic, the examples in nature of species that store sperm for a long duration suggest that it may be possible.

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Implications

Sperm interaction with the cow reproductive tract after semen deposition has a profound influence on pregnancy rates and provides perplexing fundamental questions that are unresolved despite considerable study. The fertilizing sperm are selected by the tract from the millions or billions of sperm deposited at mating or artificial insemination. Successful sperm interact with luminal fluid and epithelia, while evading destruction by the immune system. They respond to rheotactic, chemical and adhesive stimuli to undergo functional changes and arrive at the site of fertilization. An understanding of how these processes are coordinated can improve *in vitro* fertilization success, contraception effectiveness, sperm lifespan in the oviduct, improved semen storage, and fertility.

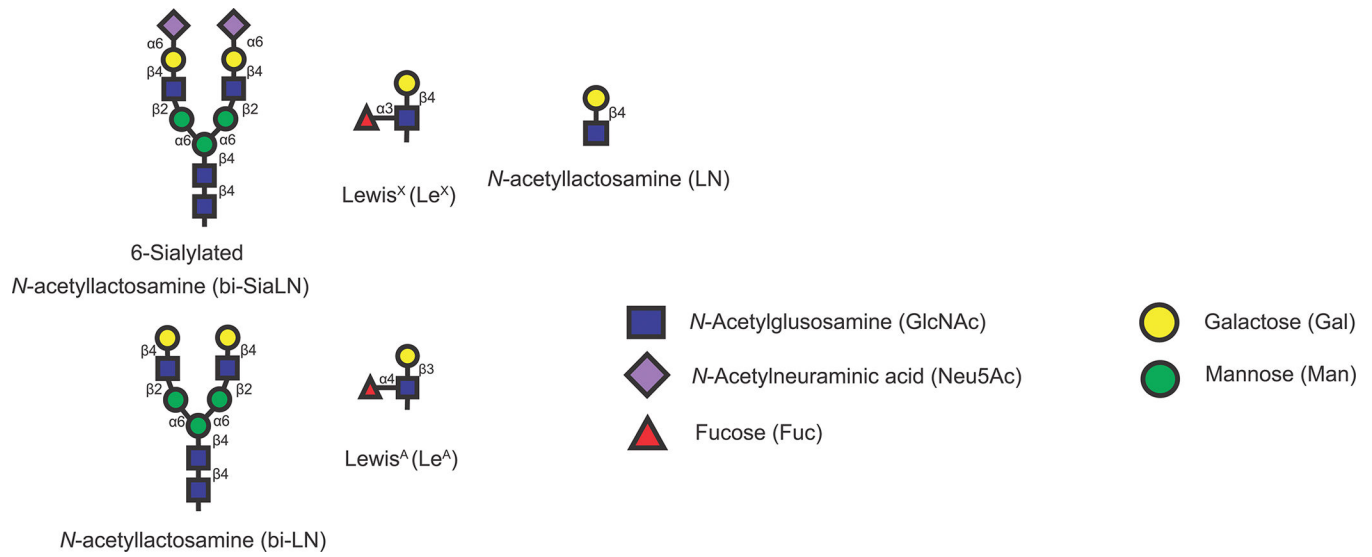


Figure 1.

Structures of glycans that bind bovine (Le^A) and porcine sperm (bi-SiaLN, bi-LN, and Le^X), and the related glycan that does not (LN). Le^A is found on the bovine oviduct epithelium. bi-SiaLN is abundant on the epithelium of the porcine ampulla and isthmus including ciliated and non-ciliated cells. Le^X is found in the porcine isthmus but not the ampulla.

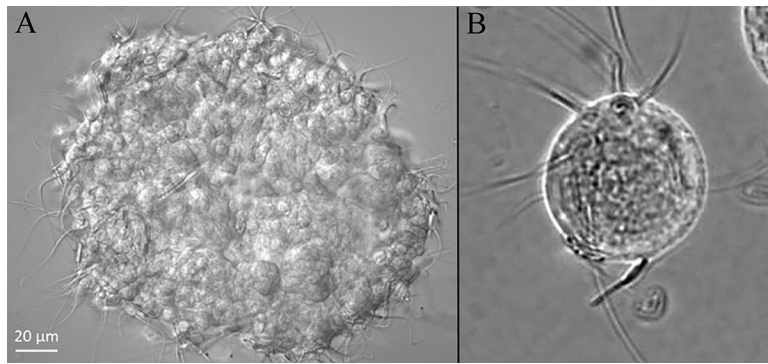


Figure 2. Sperm bind to oviduct cell aggregates isolated from the isthmus (A, porcine sperm) and beads to which Le^A trisaccharide has been attached (B, bovine sperm).