Regulation of sperm storage and movement in the ruminant oviduct

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Three regions of the ruminant oviduct play different roles in the progress of sperm: the uterotubal junction, isthmus, and ampulla. The uterotubal junction acts as a point of selection of sperm, requiring that sperm are progressively motile and express specific proteins in order to enter the oviduct. The isthmus stores sperm, preserving motility and viability until ovulation. Sperm are stored in the isthmus by binding to its mucosal epithelium. In bovine sperm, binding to the oviductal epithelium is promoted by proteins that are secreted by the seminal vesicles and coat the heads of sperm by associating with plasma membrane phospholipids. Putative oviductal receptors for the seminal vesicle proteins are members of the annexin protein family. Release of sperm from the storage site in the isthmus is gradual, which serves to ensure that sperm in the proper physiological state reach the oocytes at the appropriate moment and also to reduce incidence of polyspermic fertilization. The ampulla supports fertilization and may participate in guiding sperm toward the eggs. Further studies are needed to improve our understanding of the interactions between sperm and the female reproductive tract, in order to develop means to improve fertility in ruminants.

Introduction

Mammalian sperm travel a long distance relative to their size in order to reach the site of fertilization in the oviduct. When sperm attempt to enter and pass through the oviduct, they are subjected to selective processes to eliminate those of poor quality. Selective processes also regulate the numbers of sperm that reach the fertilization site, thereby reducing the incidence of polyspermic fertilization (Hunter et al. 1982, Hunter & Wilmut 1984). The oviduct acts as well to support fertilization, for instance, by storing sperm and maintaining their viability during storage. Furthermore, the timing of capacitation of sperm may be influenced by the oviduct in order to ensure that capacitated sperm are available at ovulation.

Three regions of the oviduct, uterotubal junction, isthmus, and ampulla (Figure 1) play different roles in selecting and supporting sperm. In this review, the current understanding of the roles of each region will be discussed, particularly with regard to ruminant reproduction.

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The uterotubal junction regulates sperm passage into the oviduct

Regardless of the billions of sperm in the ejaculates of most ruminants, only a minority of several thousand enters the uterotubal junction and passes through it. There may be only a small window of opportunity for sperm to pass through the junction, due to the apparent ability of the junction to close down. In the mouse, patency of the junction can be seen using transillumination of the oviduct and the junction has been observed to close about an hour after mating (Suarez 1987). Three morphological characteristics of the ruminant oviduct indicate that it may do likewise. First, the connective tissue in the bovine junction wall is heavily vascularized and engorgement of the vascular beds may act to compress the lumen (Wrobel et al. 1993). Second,
the thick smooth muscle layer of the junction could contract to compress the lumen. Third, the tube of the junction is bent into a sigmoidal shape and the muscular ligaments attached to the junction could increase the flexure of the curve to further impede passage of sperm (Hafez & Black 1969, Hook & Hafez 1968).

In the cow, the uterotubal junction is lined with mucosal folds that form channels ending in cul-de-sacs rather than continuing into the isthmus (Figure 2) (Yaniz et al. 2000). When muscular and/or vascular action compresses the lumen, these dead ends might form a plug. On the other hand, when the uterotubal junction is not being compressed, the dead ends of the channels could act more like funnels to direct sperm into the isthmus.

![Fig. 2. Scanning electron micrographs of the bovine uterotubal junction and isthmus. A. The uterotubal junction is lined with mucosal folds that form channels ending in cul-de-sacs. The bottom of the figure represents the prouterine end of the uterotubal junction. B. Mucosal folds in the lower isthmus form pockets in the wall. C. Bull sperm binding primarily to cilia on the oviductal epithelium in the lower isthmus to form a reservoir. Figure 2A and 2B are courtesy of Dr. JL Yaniz (Yaniz et al. 2000).](image)

When the uterotubal junction is not being closed down by vascular and/or muscular action, the junction is equipped with mechanisms to cause it to act as a point of sperm selection. There are mucous secretions in the lumen of bovine uterotubal junction (Suarez et al. 1997) that might filter out sperm with weak motility, as does mucus in the cervical canal (Katz et al. 1990). Furthermore, based on null mutant mouse models, there is evidence that mouse sperm must possess certain proteins in order to swim into the uterotubal junction (Nakanishi et al. 2004, Yamaguchi et al. 2009). For example, calmegin is a spermatogenesis-specific chaperone protein, and the sperm produced by calmegin knockout mice are unable to pass through the uterotubal junction, despite having normal morphology and motility (Ikawa et al. 2001). A subsequent study confirmed that each sperm must possess the proteins chaperoned by calmegin, because when females were mated with chimeric males that produced a mixture of developing germ cells in the testis that contained or were missing calmegin, the only sperm that entered into the uterotubal junctions where those in which calmegin had been expressed during development (Nakanishi et al. 2004). Calmegin is known to be required for the expression of ADAM3 (a disintegrin and metallopeptidase domain 3) on mature sperm, and sperm from male mice deficient in ADAM3 are also unable to pass through the uterotubal junction (Yamaguchi et al. 2009). It is not yet known whether similar proteins are required for ruminant sperm to pass through the uterotubal junction.
In cattle (VanDemark & Moeller 1951), sheep (Mattner & Braden 1963), humans (Kunz et al. 1996), and rabbits (Overstreet & Cooper 1978), a small fraction of sperm are transported through the uterus and oviduct to the site of fertilization within a few minutes of mating. This phenomenon, which has been called “rapid sperm transport”, may be a consequence of muscular contractions in the female tract that occur during mating, because it occurs too rapidly for it to be the result of sperm swimming and it may not serve to bring competent sperm to the site of fertilization (Hays & VanDemark 1953). For instance, rabbit sperm that underwent rapid transport were found to have sustained damage and thus would be unlikely to fertilize (Overstreet & Cooper 1978, Overstreet & Tom 1982). It is not known whether rapid transport serves any purpose.

Sperm are stored in the isthmus

After sperm pass through the intramural portion of the uterotubal junction, most bind to the epithelium in the extramural portion and/or the lower part of the isthmus of the oviduct to form a storage reservoir. Oviducal reservoirs of sperm have been identified in a broad array of mammals, including sheep (Hunter et al. 1982), cattle (Hunter & Wilmut 1984), mice (Suarez 1987), and hamsters (Smith & Yanagimachi 1991). There is evidence that binding to the oviducal epithelium prolongs the motile life span of ruminant sperm. When sperm of cattle (Chian et al. 1995, Pollard et al. 1991), sheep (Lloyd et al. 2008, Lloyd et al. 2009), or red deer (Berg et al. 2002) were incubated with oviducal epithelium in vitro, higher percentages of the sperm remained motile over time. The mechanism for sustaining motility has been proposed to involve suppression of capacitation, because equine sperm showed delayed capacitation when incubated with oviducal epithelium (Dobrinski et al. 1996). Elucidating the mechanism of preservation of motility during storage might suggest new methods for storing sperm in vitro.

There is evidence that sperm at least begin to capacitate while in the oviducal isthmus (Smith & Yanagimachi 1991). It is not fully understood how sperm become capacitated in the oviduct. In vitro, it has been shown that capacitation involves loss of decapacitating factors including cholesterol, a rise in intracellular Ca^{2+}, and an increase in activity of a sperm-specific adenyl cyclase known as SACY (Sinclair et al. 2000). SACY activation results in increased cAMP, which, in turn, activates protein kinase A (PKA) (reviewed by Visconti et al. 2002). Through a poorly understood process, activation of PKA, a serine and threonine kinase, leads to increased tyrosine phosphorylation of several sperm proteins (reviewed by Visconti et al. 2002), such as testis-specific serine/proline-rich protein, calcium binding protein, and others (Platt et al. 2009). Studies in mice (Visconti et al. 1995) and cattle (Galantino-Homer et al. 1997) have shown that protein tyrosine phosphorylation is correlated with capacitation, confirmed by the ability of sperm to undergo induced acrosome reactions and to fertilize eggs in vitro. Most of the roles of tyrosine phosphorylated proteins in sperm capacitation remain to be determined.

Bull sperm capacitation is facilitated in vitro when heparin is present (Parrish et al. 1988). There is evidence that the effect of heparin on sperm is mediated by bovine seminal plasma proteins (binder of sperm, BSP) (Manjunath et al. 2009) on the sperm surface, particularly PDC109 (also known as BSPA1/A2) (Manjunath & Therien 2002, Therien et al. 1997). BSP proteins are produced by the bovine seminal vesicles and adhere to the sperm surface by associating with phospholipids in the sperm plasma membrane (Desnoyers & Manjunath 1992). BSP proteins are small in mass (14-30 kDa) and each contains a unique N-terminal domain followed by two fibronectin type II domains (Calvete et al. 1999). The type II domains contain heparin binding sites (Chandonnet et al. 1990) that could be responsible for the stimulation of capacitation by heparin, and phospholipid binding sites that are responsible for binding the BSPs to the
sperm surface (Wah et al. 2002). BSP homologs have been reported in other ruminant species, including goats (Villemure et al. 2003), sheep (Bergeron et al. 2005), and bison (Boisvert et al. 2004), and some of the homologs have been demonstrated to bind heparin (Bergeron et al. 2005, Boisvert et al. 2004, Villemure et al. 2003).

The process of capacitation is considered to include hyperactivation of sperm motility. Hyperactivated sperm display asymmetrical, high-amplitude flagellar beating patterns, causing vigorous, and sometimes circular, movement of free-swimming sperm (Suarez et al. 1983, Yanagimachi 1970). Hyperactivated motility is required for the penetration of viscoelastic substances in the oviduct (Suarez et al. 1991), such as mucus and the matrix of the cumulus oophorus, and for sperm penetration of the zona pellucida (Stauss et al. 1995). Hyperactivation is triggered by a rise of intracellular Ca\(^{2+}\), primarily from extracellular sources (reviewed by Suarez 2008). In cattle, sperm can be induced to hyperactivate in vitro by procaine in the presence of extracellular Ca\(^{2+}\) and therefore is presumed to operate by stimulating Ca\(^{2+}\) influx (Ho & Suarez 2001, Ho & Suarez 2003). In mouse sperm, sperm-specific CatSper proteins are known to form Ca\(^{2+}\) channels in the plasma membrane of the principal piece of the flagellum (Carlson et al. 2005, Jin et al. 2007). Sperm from CatSper-null mice do not hyperactivate (Carlson et al. 2005, Jin et al. 2007, Qi et al. 2007) and cannot fertilize zona pellucida-intact eggs (Quill et al. 2003, Ren et al. 2001).

**BSP proteins play a role in sperm storage**

In hamsters (DeMott et al. 1995), cattle (Lefebvre et al. 1997), and pigs (Wagner et al. 2002), studies have shown that sperm-oviductal epithelium interaction is mediated by carbohydrate-recognition mechanisms. Fucose, specifically in the Lewis-A trisaccharide, was identified to be a key component of the receptor for bull sperm binding in the oviduct (Suarez et al. 1998). Lewis-A was used in an affinity column to pull proteins from sperm extracts that would play a role in binding sperm to the oviductal epithelium. The main protein identified by this method was PDC109 (Gwathmey et al. 2003, Ignotz et al. 2001), which is one of the BSP proteins. Gwathmey et al. (2006) later reported that two other BSP proteins (BSPA3 and BSP30K), each acting alone, can also induce sperm binding to oviductal epithelium in vitro. This leads to the question: Why do bull sperm need three BSP proteins when they can bind to the oviductal epithelium with any one of the BSP proteins alone? Key functions are often protected by redundancy; however, this apparent redundancy may also provide more intricate control over sperm movement out of the reservoir. Differences in the amino acid sequences of the three BSP proteins result in different patterns of surface charges on the BSP molecules (Gwathmey et al. 2006); therefore, it is proposed that each BSP molecule adheres to the surface of sperm and binds sperm to the oviductal epithelium with different affinities and kinetics. Altogether, the BSP proteins may act to provide a gradual release of sperm from the reservoir, in order to ensure that sperm capacitate and reach eggs at the ideal time for fertilization and yet not too many reach the egg at once, which might result in polyspermic fertilization. It is possible that BSP homologs in other ruminant species are also involved in the formation of sperm reservoir; however, this remains to be determined.

**Oviductal annexins are possible binding partners of BSP proteins**

When BSP proteins were used as bait to pull down oviductal ligands from a mixture of proteins extracted from apical plasma membranes of bovine isthmic epithelium, four annexin proteins (ANXA1, 2, 4, and 5) were captured (Ignotz et al. 2007). ANXAs comprise a large, diverse
family of Ca\(^{2+}\)- and lipid-binding proteins. In other cell types, there is evidence that ANXAs serve as membrane scaffold proteins and are involved in vesicle transport and protein secretion, but much of their functions and secretory pathways are still poorly understood (reviewed by Rescher & Gerke 2004).

In support of the proposal that ANXAs are the oviductal receptors for sperm, all four ANXAs were immunolocalized on the apical surfaces of oviductal epithelium (Ignotz et al. 2007). Western blot analysis confirmed that oviductal ANXAs contain fucose, and sperm binding to oviductal epithelium can be inhibited in vitro in the presence of ANXA antibodies (Ignotz et al. 2007). There is also evidence that one of the ANXAs (ANXA2) is a sperm binding protein on the porcine oviductal epithelium (Teijeiro et al. 2009). Like BSP proteins, ANXAs were also found to bind to heparin (Ishitsuka et al. 1998, Shao et al. 2006). This interesting finding implicates heparin, or a similar glycosaminoglycan, in the process of binding or release of sperm, particularly since heparin-like molecules have been identified in oviductal fluid (Parrish et al. 1989) and have been shown to trigger release of bovine sperm from monolayers of oviductal epithelium in vitro (Gualtieri & Talevi 2000).

**Sperm movement beyond the reservoir to the ampulla**

In cattle, sperm are stored in the isthmic region of the oviduct for 18-20 hours or more before being released to ascend to the ampulla (Hunter & Wilmut 1984). The release of sperm from the reservoir begins prior to ovulation, which occurs 28-31 hours after the onset of estrus in cattle (Hunter & Wilmut 1984). It is unlikely that the release of sperm is due to the loss of binding sites on the oviductal epithelium, as sperm can bind to oviducts from different stages of estrus cycle (Lefebvre et al. 1995). Rather, it is likely that hormonal changes that trigger ovulation also stimulate the release of factors in the oviduct that cause changes in sperm which enable them to release themselves from the oviductal epithelium.

There is evidence that sperm release themselves from the reservoir by two mechanisms: hyperactivation and shedding of sperm surface proteins during capacitation. First, hyperactivation was proposed to play a role in release of mouse and human sperm, (DeMott & Suarez 1992, Pacey et al. 1995), because sperm were seen to hyperactivate before pulling away from the epithelial surface. In addition, sperm from CatSper null mice, which are unable to hyperactivate, fail to move beyond the sperm storage reservoir (Ho et al. 2009). It is not yet known whether hyperactivation plays such a role in ruminants. Second, sperm may lose binding affinity for the oviductal epithelium by shedding BSP proteins during capacitation. It has been reported that bull sperm shed the BSP protein PDC109 during capacitation in vitro and capacitated sperm are less able to bind to epithelium unless they are treated with purified PDC109 (Gwathmey et al. 2003). Less is known of the roles of BSPA3 and BSP3OK; however, because capacitated sperm lose binding affinity for the epithelium, one would predict that these two proteins are also shed during capacitation. Because BSPA3 and BSP3OK differ from PDC109 in molecular surface charges (Gwathmey et al. 2006), we predict that the kinetics of loss during capacitation differs from that of PDC109. Differential loss of BSP proteins could serve to spread out the release of sperm from the reservoir and thus assure that sperm reach eggs shortly after they enter the oviduct, but that not so many reach eggs that polyspermy occurs.

After sperm are released from the reservoir, they are still required to travel a long distance before they reach the fertilization site. Furthermore, as sperm move up the isthmus into the ampulla, the diameter of the tube increases and the shape of the lumen becomes even more complicated by elaboration of the mucosal folds that create narrow, labyrinthine passages (Figure 1). How sperm find their way to the egg is still largely unknown. It has been proposed that
chimotaxis serves to guide sperm toward eggs (reviewed by Kaupp et al. 2008). The existence of chemotaxis has been well documented in several species of marine invertebrates (reviewed by Hildebrand & Kaupp 2005); therefore, it has been hypothesized that chemotactic factors direct mammalian sperm to eggs. In humans (Cohen-Dayag et al. 1995, Spehr et al. 2003, Villanueva-Diaz et al. 1990) and rabbits (Fabro et al. 2002), sperm reportedly turn to swim up a gradient of follicular fluid or putative chemotactic agents, indicating that chemotaxis plays a role in mammalian fertilization; however, unlike the massive response shown by various species of marine invertebrate sperm (Cook et al. 1994, Yoshida et al. 2003), only small percentages of mammalian sperm (2-12% in humans) have shown this response in vitro (Gakamsky et al. 2008). In ruminant species, 8-10% of frozen-thawed bull sperm were reported to orient into a gradient of follicular fluid (Gil et al. 2008). Some follicular fluid escapes from the oviduct with the egg mass during fertilization and thus could be present in the ampulla to attract sperm toward the site of fertilization.

Conclusions

Studies from different animal models have helped researchers to better understand the regulation of sperm storage and movement in the oviduct; however, not much research has been done in ruminant species other than Bos taurus. There is still much to be learned about how sperm entry through the uterotubal junction is regulated, how sperm fertility is maintained during storage in the oviductal reservoir, how sperm are released from the reservoir, and whether sperm are guided toward eggs in the ampulla by chemotaxis. Such information could prove valuable for developing new methods to improve sperm storage and the success rate of artificial insemination of domestic ruminants and endangered wildlife species.

References


Dobrinski I, Suarez SS & Ball BA 1996 Intracellular


**Gwathmey TM, Ignotz GG & Suarez SS** 2003 PDC-109 (BSP-A1/A2) promotes bull sperm binding to oviductal epithelium in vitro and may be involved in forming the oviductal sperm reservoir. *Biology of Reproduction* 69 809-815.


**Ho HC & Suarez SS** 2001 An inositol 1,4,5-trisphosphate receptor-gated intracellular Ca2+ store is involved in regulating sperm hyperactivated motility. *Biology of Reproduction* 65 1606-1615.

**Ho HC & Suarez SS** 2003 Characterization of the intracellular calcium store at the base of the sperm flagellum that regulates hyperactivated motility. *Biology of Reproduction* 68 1590-1596.

**Ho K, Wolff CA & Suarez SS** 2009 CatSper-null mutant spermatozoa are unable to ascend beyond the oviductal reservoir. *Reproduction, Fertility, and Development* 21 345-350.


**Ignotz GG, Cho MY & Suarez SS** 2007 Annexins are candidate oviductal receptors for bovine sperm surface proteins and thus may serve to hold bovine sperm in the oviductal reservoir. *Biology of Reproduction* 77 906-913.

**Ignotz GG, Lo MC, Perez CL, Gwathmey TM & Suarez SS** 2001 Characterization of a fucose-binding protein from bull sperm and seminal plasma that may be responsible for formation of the oviductal sperm reservoir. *Biology of Reproduction* 64 1806-1811.


Teijeiro JM, Ignotz GG & Marini PE 2009 Annexin A2 is involved in pig (Sus scrofa) sperm-oviduct interaction. Molecular Reproduction and Development 76 334-341.


