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Sperm Movement, Storage, and Release from the Oviduct – an epic journey

Movimento espermático, estocagem e liberação do oviduto – uma jornada épica]

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Abstract

At mating or artificial insemination, sperm begin their movement towards the site of fertilization. However, most sperm are lost due to retrograde flow and very few ultimately reach the upper oviduct where fertilization occurs. The complex structure of the oviduct functions to store sperm, to increase their lifespan, and to release and move sperm towards the oocytes in the ampulla. To accomplish this feat, the oviduct has complex folds that provide direction to sperm, waves of fluid that carry sperm and also provide sperm direction, adhesive glycans that retain sperm in the isthmus, and a signaling sperm to promote sperm release from the isthmus for fertilization. A better understanding of how the oviduct accomplishes these goals will likely lead to improved sperm storage and release and enhanced fertility.

Introduction

The oviduct is a complex tube that captures oocytes from the ovary at one end and connects at the other end to the uterine horn. It can be separated into the infundibulum, ampulla, isthmus, and the utero-tubal junction, although the major portions are the ampulla and isthmus. It is coiled and has a complex structure lined with ciliated and non-ciliated cells. The isthmus has a thick layer of smooth muscle and folded epithelium while the ampulla has even more folds and more ciliated cells but a much thinner smooth muscle layer. The oviduct contains fluid that sloshes like waves but also has microcurrents within the waves. The volume, protein, and hormone content of oviduct fluid varies considerably during the estrous cycle.

For sperm to reach the oviduct at fertilization, they have a long journey through the female tract. The site of semen deposition varies depending on whether artificial insemination or normal mating is performed because semen is usually deposited in the uterus during artificial insemination. And natural mating, the site of semen deposition varies between species. So, sperm may need to pass through the vagina and cervix before entering the uterus. Those that enter the uterus and avoid phagocytosis then enter the utero-tubal junction (UTJ) and finally the oviduct. Consequently, most sperm do not reach the site of fertilization in the oviduct. This was demonstrated well in a classic experiment in which sperm were inseminated into the uterine body of cows. Twelve hrs later, sperm were flushed and scraped sperm from different parts of the tract and counted. Only 6.4% of sperm inseminated were recovered in the tract; most sperm were in the mucus discharged by retrograde flow. The authors counted sperm in the other parts of the tract and found only 5.7% of the total inseminated in the vagina, 0.3% in the cervix, 0.1-0.2% in the uterus, and 0.002-3% in the oviduct. Interestingly, although very few sperm were in the oviduct, the sperm that were collected from the oviduct all had normal morphology and motility.

A variety of experiments using mouse sperm demonstrated that sperm require specific proteins to pass from the uterus through the UTJ and enter the isthmus. For example, mouse sperm deficient in ADAM3 are not found beyond the UTJ. Although the UTJ, at least in the mouse, appears to serve as a selection gate, it is not clear how gating is accomplished.

The location of sperm storage, fertilization, and preimplantation development in mammals, the oviduct is an essential reproductive organ. Despite that, due to difficulties in observing gametes within the oviduct, many details of its function are still unclear. There has been some progress in studying sperm interaction with an intact oviduct in the mouse oviduct, due to its thin walls and the ability to use genetics to add fluorescent tags to sperm proteins. But studies of the oviduct have are problematic in other mammalian species. We and others have endeavored to provide some understanding of oviduct function and its interaction with gametes so that ultimately, we can use this information to improve fertility or and to develop novel contraceptives.

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Sperm Storage

The isthmus and in some species, the UTJ, are the major sites of sperm storage in female mammals. In addition to mammals, females from a wide variety of phylogenetically distinct animal species store sperm as a method of reducing the need for synchrony between ovulation and mating/semen deposition. Chicken and turkey hens store sperm for several weeks in specialized sperm storage tubules found in the uterovaginal junction. Females of some insects copulate only early in their life and store sperm in specialized organs called spermathecae for their lifetimes, which may be several years or a decade in some ant species. The ability to store cells that are not active in mitosis, transcription, or translation for a decade is fascinating.

Some female mammals also demonstrate a remarkable ability to store sperm. For example, some species of bats, such as the big brown bat (Eptesicus fuscus), mate in the autumn and store sperm during hibernation until they ovulate in the spring in North America. Most mammals store sperm for much shorter periods than bats; cattle and pigs store sperm for a couple of days. Sperm storage is particularly consequential in domestic animals in which artificial insemination is practiced, such as swine and dairy cattle. For success using artificial insemination in cattle and swine, semen deposition needs to be a few hours before ovulation. The time of ovulation is often predicted by observing estrus because ovulation usually occurs in the last half of or just after estrus, depending on the species. But the length of the estrous cycle is variable, making predicting ovulation challenging. In fact, in swine, a common practice is to inseminate every 24 hrs until the female is no longer in estrus, emphasizing the deficiency in sperm lifespan in the oviduct. If sperm lived longer in the oviduct, fewer inseminations and less semen could be used while maintaining fertility.

The ability of bats, insects, and other animals to store sperm for months or years inspired us to consider if we could somehow lengthen sperm storage time in cattle and swine. By understanding better how sperm are stored in a variety of animals, could we use what we learn to improve sperm storage in farm animals? By learning the characteristics of effective sperm storage, could we apply them more broadly to more animals?

Free Sperm in the Oviduct

In the isthmus, some sperm remain free in oviduct fluid and others are retained in the isthmus by attaching to the epithelium. The free sperm are moved by fluid that, in turn, is moved in waves by peristaltic contractions of smooth muscle surrounding the oviduct. These peristaltic contractions are greatest in the isthmus. Fluid movement can be observed by the use of fluorescent microspheres in the mouse oviduct. The observation that these microspheres sloshed back and forth in the oviduct indicated the effect of peristaltic contractions on fluid movement. There was greater movement of the microspheres in the region of the oviduct closest to the uterus and movement progressively decreased in segments of the oviduct closer to the ovary. The fluid flow rates in the mouse oviduct ranged from 10-60 μm/sec.

In addition to being carried by oviduct fluid, free sperm are motile themselves, providing movement that is superimposed on the movement of oviduct fluid. This has been likened to a surfer paddling on a wave. The direction of sperm swimming is influenced by the shape of the walls that confine sperm, the oviduct folds. In experiments using microchannels, sperm hugged the walls as they swam, due to the tail beat having greater amplitude further from the head, which provides directionality to sperm movement. It was also observed that sperm could hug the walls on gradual turns of the channel but when the channel turned sharply, sperm crossed the channel and then swam along the wall on the opposite side. Thus, sperm could hug the surface of a gradual oviduct fold but would be released by a very sharp fold.

Another observation made using channels is that sperm tended to swim longitudinally along the 90° angle corners of a rectangular channel rather than only along one wall. These experiments demonstrate that the oviduct walls can influence the direction of sperm movement; the grooves and folds in the oviduct wall could influence sperm to swim along the deep folds of the oviduct.

The fluid in which sperm are suspended influences sperm swimming behavior. Sperm orient their swimming direction upstream in a flow of fluid at fluid flow rates of 10-25 μm/sec, a phenomenon known as rheotaxis. The temperature of the fluid in the oviduct also provides direction to sperm. It is believed that the ampulla is slightly warmer than the isthmus, which will draw sperm to the site of fertilization. Finally, fluid viscosity can affect the beat pattern of the sperm tail. In aqueous medium, the tail has fewer bends and the head moves more from side to side during each tail beat stroke. The majority of the force that propels sperm tends to be localized to the tail bend point. But in higher viscosity medium

like oviduct fluid, the tail has more bends during its beat stroke and there is a more uniform force generated by the tail beat pattern. The tail beat pattern is less extreme and forms a more consistent pattern. This results in less turbulence and less wake during sperm swimming.

Interestingly, the progressive velocity of sperm is similar in viscous and aqueous medium despite the greatly increased resistance to movement of viscous medium. Unfortunately, many studies of sperm motility ignore the observation that oviduct fluid is more viscous than normal aqueous culture medium.

Sperm Adhesion to the Oviduct Epithelium

Some sperm in the oviduct are retained in the same location by binding to the oviduct epithelium. There was evidence that sperm from many species bind to glycans but the specific glycans that bound sperm had not been identified. For example, bovine sperm bind to glycans containing the Lewis A trisaccharide and, notably, have a higher affinity for a sulfated version of Lewis A but only a few glycans had been tested. So, we developed a process to screen many more glycans for their ability to bind porcine sperm using an array of about 400 different glycans immobilized onto microscope slides. These glycans were immobilized mimicking the glycans on the surface of oviduct epithelial cells. All the glycan spots that bound porcine sperm had one of two glycan motifs, either a Lewis X trisaccharide (Le^X)or a 6-sialylated biantennary glycan (Figure 1). 6-Sulfated Lewis X structures ($suLe^X$) had a higher affinity for sperm than non-sulfated Lewis X structures. Lewis X trisaccharide and 6-sialylated biantennary glycans (bi-SiaLN) were both very abundant among the oviduct asparagine-linked glycans from the isthmic epithelial cells. We used fluoresceinated lectins and specific glycan antibodies to localize the glycan motifs and discovered that they were found on the epithelial cell surface of the isthmus. Their abundance was not affected by the stage of the estrous cycle, and they were present before puberty. Sperm binding was very specific because Le^A, an isomer of Le^X, did not bind sperm, nor did a 3-sialylated biantennary glycan. Additional experiments confirmed binding affinity. Fluoresceinated versions of $suLe^X$ and bi-SiaLN in solution bound to the sperm head, the region of sperm that binds oviduct cells. And if $suLe^X$ and bi-SiaLN were immobilized by attaching them to beads, they also bound sperm. Because tethering a motile sperm requires a high affinity, we concluded that these oviduct glycans had a high affinity for sperm.

The glycan arrays results showed that Le^X and bi-SiaLN motifs in glycans are sufficient by themselves to bind sperm. We also designed experiments to determine if these glycans were necessary for sperm to bind oviduct cells. We collected epithelial cells from the isthmus and allowed them to form aggregates. During these experiments, we observed ciliary activity on the outside of these aggregates, indicating the cells were alive during the assays. We added lectins or antibodies to block specifically a glycan on oviduct cells and then added sperm to determine if sperm binding was affected. For example, the lectin Sambucus Nigra agglutinin (SNA), which blocks 6-sialylated glycans, reduced the ability of oviduct cells to bind sperm by about 60%. In contrast, Maackia Amurensis lectin II (MAL-II), a lectin that blocks 3-sialylated glycans, had no effect (Figure 2). Similarly, antibodies to Le^X or bi-SiaLN reduced sperm binding to oviduct cells.

In addition, we also added specific soluble oviduct glycans or structural analogs to occupy receptors on sperm. Both $suLe^X$ and bi-SiaLN glycans reduced sperm binding to oviduct cells by about 60% whereas $suLe^A$ and non-sialylated lactosamine had no effect [27,28]. In total, these experiments demonstrated that the 2 glycan motifs, $suLe^X$ and 6-sialylated structures, were necessary for sperm binding to oviduct cells.

Because sperm bound to specific oviduct glycans, those glycans might affect sperm in some of the same ways that binding to oviduct cells affects sperm, such as increasing sperm lifespan, probably one of the most consequential effects on sperm. When sperm were allowed to bind $suLe^X$ or 6-sialylated glycans in capacitating medium, sperm lifespan was increased, compared to free-swimming sperm or sperm bound to fibronectin-coated beads (Figure 3). Sperm viability was 2-3 fold higher among sperm bound to immobilized oviduct glycans compared to free-swimming sperm. This is consistent with the model that sperm lifespan is extended by binding to glycans in the isthmus, as the sperm reservoir is formed.

It is not clear how sperm lifespan is extended by binding to oviduct glycans. Initial experiments investigated sperm intracellular free Ca^{2+}. Ca^{2+} is central to many events in cells, especially sperm. It is important for motility, capacitation, and the acrosome reaction. Suppressing the gradual increase in intracellular free Ca^{2+}suppresses the final maturation of sperm. So we tested if binding to glycans affected sperm intracellular Ca^2. Sperm were with a Ca^{2+} probe, Fluo-4, and various glycans were added to sperm.

Fluo-4 fluorescence, indicating Ca^{2+} concentration, increased during sperm capacitation. But that increase was reduced by bi-SiaLN and $suLe^X$ glycans. So, suppression of the Ca^{2+} increase and perhaps other sperm functions may be the way that sperm lifespan is prolonged.

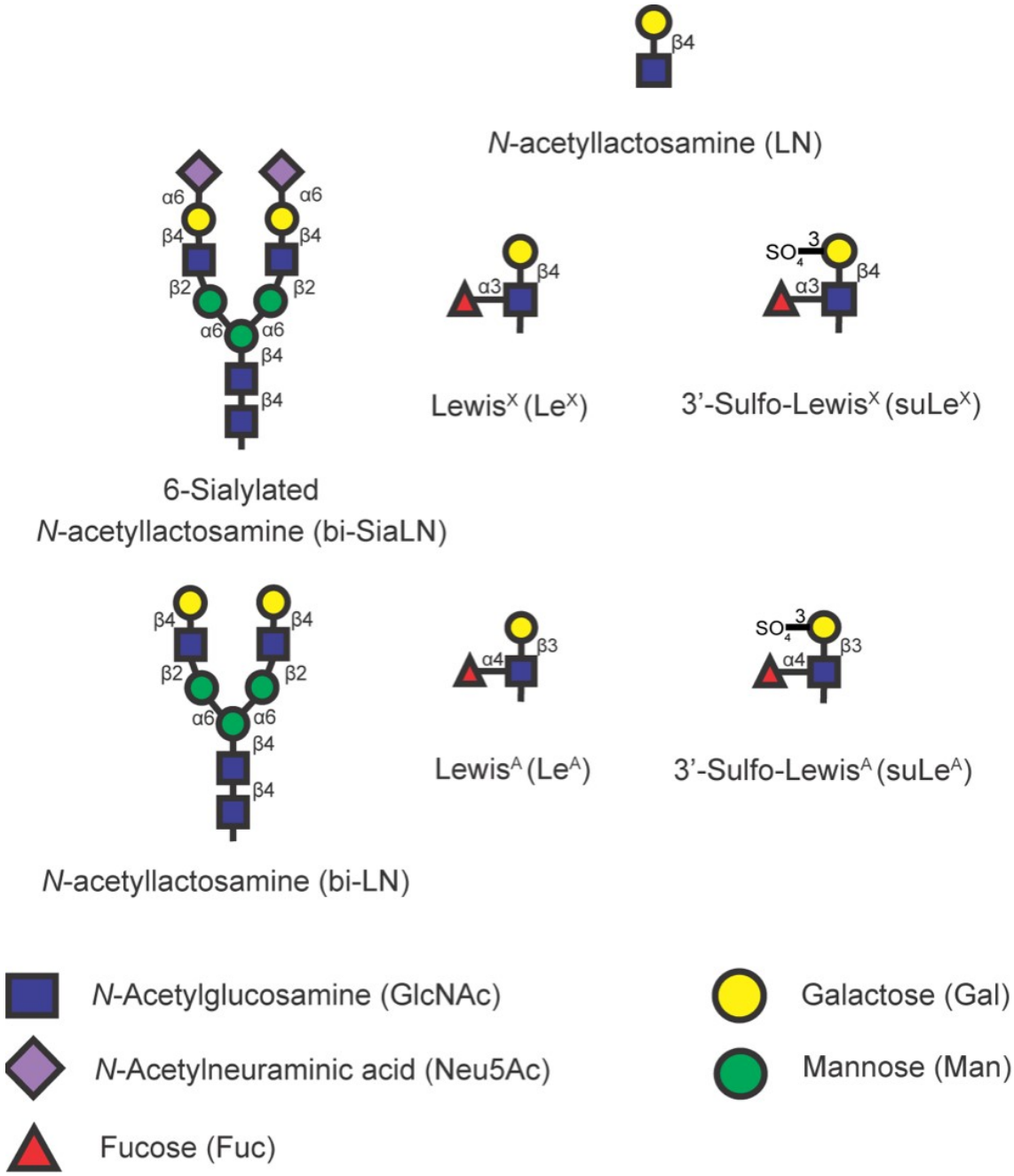

Figure 1. Glycan structures that bind porcine sperm and related structures that do not. Screening hundreds of glycans using an array demonstrated that 3'-sulfo-Lewis X trisaccharide and biantennary 6-sialylated structures bound porcine sperm but the related 3'-sulfo-Lewis A or biantennary non-sialylated structures did not. The key showing how each monosaccharide is represented in the figure is at the bottom. Linkages between monosaccharides are included for the glycans.

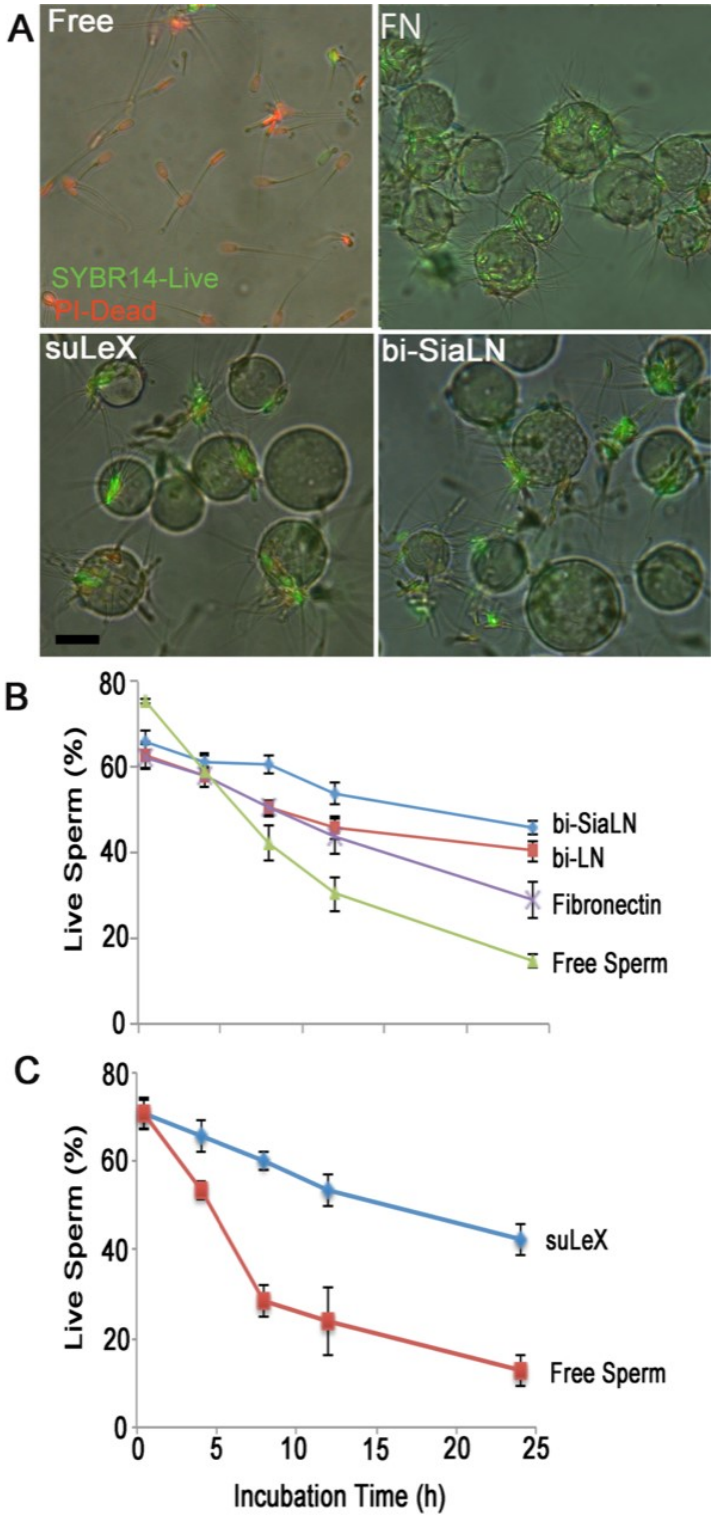

Figure 2. Sperm bound to oviduct glycans immobilized on beads have a prolonged lifespan**.** Sperm were allowed to bind to suLeX or bi-SiaLN glycans attached to beads and incubated for up to 24 hr. Sperm viability was assessed by SYBR14 (live) and propidium iodide (dead) staining. A) Most free sperm were dead, but sperm bound to oviduct glycans or fibronectin were mostly live. B) Time course of live sperm that were bound to bi-SiaLN, non-sialylated biantennary glycan, fibronectin, or were free. Viability was highest among sperm bound to bi-SiaLN, but all sperm bound to beads had higher viability than free sperm. C) Time course of live sperm that were bound to $suLe^X$ or were free. Sperm bound to $suLe^X$ on beads had higher viability than free sperm.

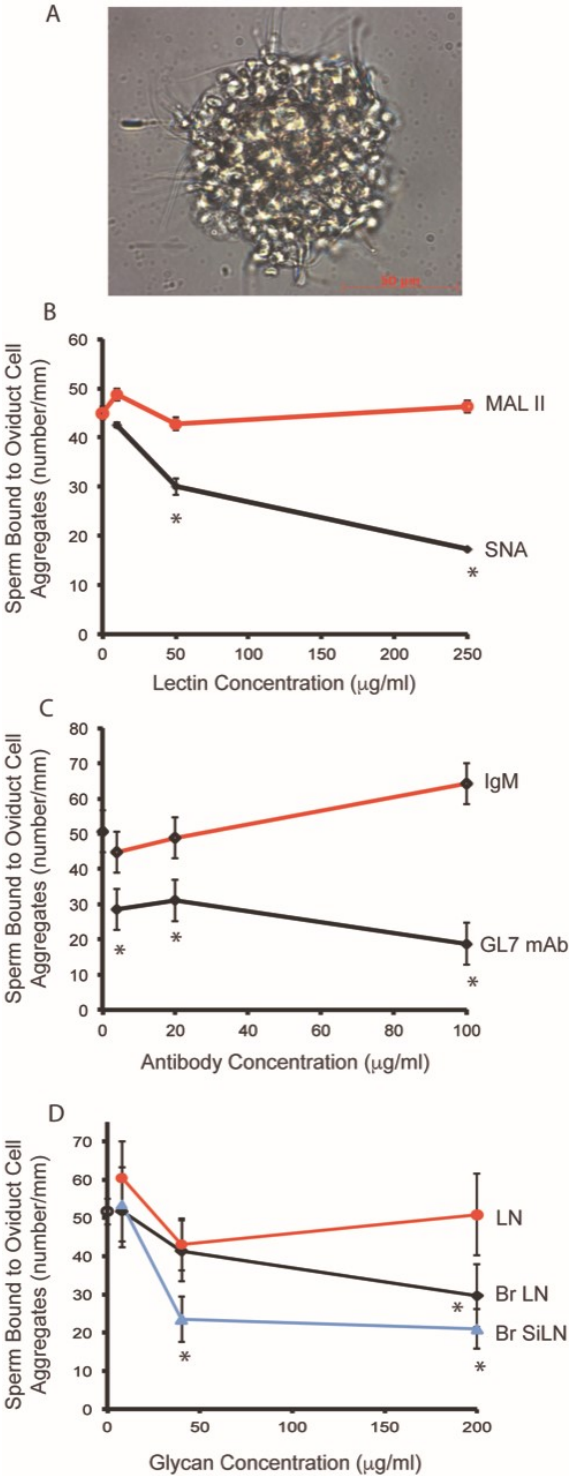

Figure 3. Blocking oviduct glycans or putative receptors on sperm reduced sperm binding to oviduct cells. Oviduct cells were collected and allowed to aggregate. A) Sperm bound to an aggregate of oviduct cells. B) Simbucus nigra agglutinin (SNA), a lectin that binds 6-sialylated structures blocked the ability of oviduct cells to bind sperm. In contrast, Maackia Amurensis lectin II (MAL-II), a lectin that binds 3-sialylated structures had no effect. C) A monoclonal antibody (GL7) to sialylated structures reduced sperm binding to oviduct cells but control IgM had no effect. D) Sperm were preincubated with varying concentrations of biantennary, 6-sialylated lactosamine glycans (Br SiLN), biantennary lactosamine glycans (Br LN), or the disaccharide lactosamine (LN), and then challenged with oviduct cell aggregates. Asterisks indicate concentrations at which SNA, GL7, or glycans were different from controls.

Sperm Release from the Oviduct Epithelium

Mechanism of Sperm Release

To fertilize oocytes, bound sperm must be released from the isthmic epithelium and move to the ampulla to fertilize oocytes. There is evidence for both a gradual unregulated sperm release but also, superimposed on that, a release that is activated by some signal, the source of which is uncertain. One hypothesis is that basal unregulated sperm release is accounted for by the release of sperm as they complete capacitation, a "pre-programmed" sperm detachment. The second type of release, signal-induced release, has been proposed and investigated by many. There are reports that progesterone can activate sperm detachment, can promote sperm chemotaxis, increase intracellular Ca^{2+} and induce hyperactivated motility. Intracellular Ca^{2+} and hyperactivated motility can be increased in human sperm by progesterone activation of a sperm-specific channel called CatSper. We have investigated the role of progesterone and CatSper in sperm detachment from the isthmus.

First, we determined if progesterone could stimulate sperm release from oviduct cell aggregates in vitro. A concentration of 80 nM progesterone-induced release within 20 min of about 50% of the sperm that had been first bound to oviduct cells. The same concentration of progesterone also promoted sperm release from suLeX-beads within 20 min. Thus, progesterone was sufficient to release sperm from oviduct cells and immobilized oviduct glycans. Sperm release was not affected by RU-486, an inhibitor of the genomic progesterone receptor. Binding to oviduct cells and oviduct glycans suppressed intracellular Ca^{2+} so we considered whether progesterone would induce sperm release by overriding the Ca^{2+}-suppressing effects. We found that progesterone increased intracellular Ca^{2+} at 30 min of incubation, which coincided with the time of sperm release from oviduct cells and glycan-coated beads.

In human sperm, progesterone binds to a protein called ABHD2. ABHD2 is inactive without progesterone and the CatSper channel is closed. But when progesterone binds to ABHD2, it cleaves 2-arachidonoylglycerol, which before cleavage blocks CatSper. When 2-arachidonoylglycerol is cleaved and the CatSper channel opens, extracellular Ca^{2+} moves inside sperm and the sperm motility pattern changes from basal to hyperactivated motility. We tested methyl arachidonyl fluorophosphonate (MAFP), an inhibitor of ABHD2, and observed that it diminished sperm release from immobilized oviduct glycans in response to progesterone [40]. Similarly, NNC 55-0396, a CatSper channel inhibitor, also reduced sperm release from oviduct cells and immobilized oviduct glycans. These results indicated that the progesterone-CatSper-Ca^{2+} system is important for sperm release.

Because activation of CatSper leads to hyperactivated motility, we proposed that hyperactivated motility was an integral part of sperm release. When porcine sperm become hyperactivated, their beating becomes more asymmetrical generating more force. Previous work using bovine sperm showed that hyperactivation was a component of sperm release. So to determine if hyperactivation was sufficient to release sperm from oviduct glycans, we used a pharmacological approach to force sperm that were not capacitated to hyperactivate to determine if hyperactivation was sufficient to release sperm. To avoid unrelated side effects, we investigated several compounds that induce hyperactivation in different ways and they induced the release of uncapacitated sperm. We found that progesterone, as well as other activators of CatSper (4-aminopyridine and procaine) and a cell-permeable cAMP analog (cBIMPS) induced release of about half of the sperm, compared to vehicle control. So hyperactivation is sufficient to release a subset of sperm from immobilized oviduct glycans even if the sperm were not capacitated. It's not clear whether the sperm that remained bound did not hyperactivate or if they needed additional factors to release from oviduct glycans. But it is interesting to consider that, from a biological standpoint, heterogeneous sperm release to fertilize both early and later ovulating oocytes might provide higher fertility than synchronized sperm release.

Sperm release might also be promoted by the degradation of oviduct glycan receptors. Although receptors for $suLe^X$ and bi-SiaLN have not been identified conclusively, there are studies demonstrating the importance of sperm protein degradation by the ubiquitin-proteasomal system (UPS) for a variety of processes. We used inhibitors of the UPS to determine if protein degradation was required for progesterone-induced sperm release from oviduct glycans. We allowed sperm to bind to $suLe^X$ or bi-SiaLN glycan-coated beads and then induced sperm release with progesterone. Progesterone stimulated the release of sperm from either glycan. If we inhibited the ubiquitin proteasomal system with one inhibitor, MG132, it blocked the release of about half of the sperm. If we added a second inhibitor, CLBL, if blocked release of more sperm. Finally, if we used a cocktail of 3 inhibitors, MG132, CLBL, and epoxomicin, nearly all progesterone-induced sperm release was eliminated. Thus, progesterone-

induced sperm release required the function of the ubiquitin proteasomal system.

Conclusions

Sperm deposited in the female reproductive tract must pass through a series of barriers before reaching the site of fertilization in the ampulla. Only a very few sperm make the complete journey to fertilize oocytes. The oviduct must coordinate sperm storage and release with the availability of ovulated cumulus-oocyte complexes. To accomplish this, the oviduct produces peristaltic waves of smooth muscle contraction that move oviduct fluid and sperm. It has complex folds forming walls that sperm swim against and fluid waves that provide direction to sperm progress by rheotaxis. The porcine oviduct also has two specific glycan motifs that retain sperm in the isthmus forming a reservoir that suppresses the normal increase in intracellular Ca^{2+} and prolongs sperm lifespan. The stored sperm can be released by progesterone acting through CatSper channels to overcome the suppression of intracellular Ca^{2+}. Although sperm binding and release appear to be a dynamic process, progesterone action promotes an overall net sperm release that allows sperm to complete the journey to the site of fertilization. Understanding this complex process will ultimately lead to improved sperm storage and release by the oviduct and higher fertility.

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