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Dynamics of Bovine Sperm Interaction with Epithelium Differ Between Oviductal Isthmus and Ampulla[1]

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ABSTRACT

In mammals, many sperm that reach the oviduct are held in a reservoir by binding to epithelium. To leave the reservoir, sperm detach from the epithelium; however, they may bind and detach again as they ascend into the ampulla toward oocytes. In order to elucidate the nature of binding interactions along the oviduct, we compared the effects of bursts of strong fluid flow (as would be caused by oviductal contractions), heparin, and hyperactivation on detachment of bovine sperm bound in vitro to epithelium on intact folds of isthmic and ampullar mucosa. Intact folds of oviductal mucosa were used to represent the strong attachments of epithelial cells to each other and to underlying connective tissue that exist in vivo. Effects of heparin on binding were tested because heparin binds to the Binder of SPerm (BSP) proteins that attach sperm to oviductal epithelium. Sperm bound by their heads to beating cilia on both isthmic and ampullar epithelia and could not be detached by strong bursts of fluid flow. Addition of heparin immediately detached sperm from isthmic epithelium but not ampullar epithelium. Addition of 4-aminopyridine immediately stimulated hyperactivation of sperm but did not detach them from isthmic or ampullar epithelium unless added with heparin. These observations indicate that the nature of binding of sperm to ampullar epithelium differs from that of binding to isthmic epithelium; specifically, sperm bound to isthmic epithelium can be detached by heparin alone, while sperm bound to ampullar epithelium requires both heparin and hyperactivation to detach from the epithelium.

fallopian tube, fertilization, heparin, oviduct, spermatozoa

INTRODUCTION

In numerous mammalian species, many sperm that reach the oviduct bind to epithelial cells in the caudal isthmus and are thereby held in a storage reservoir. Near the time of ovulation, sperm begin to detach from the epithelium and then migrate toward the site of fertilization in the oviductal ampulla reviewed in [1]. During this migration, mouse sperm have been observed repeatedly attaching to and detaching from the epithelium [2]; however, the exact mechanisms for detaching sperm from oviductal epithelium as they move out of the reservoir and toward oocytes are incompletely understood.

In bovine sperm (species: *Bos taurus*), binding to epithelium is mediated by three members of the Binder of SPerm (BSP) family of proteins that coat the surface of sperm, primarily in the acrosomal region of the head [3, 4]. Oviductal receptors for the BSP proteins on sperm have been tentatively identified to be four members of the annexin (ANXA) family of proteins, namely, ANXA1, ANXA2, ANXA4, and ANXA5 [5].

There is evidence that sperm capacitation plays a role in detaching sperm from oviductal epithelium. Capacitation is a maturational process that prepares sperm to undergo the acrosome reaction and fertilize eggs [6]. When capacitated bovine sperm were added to oviductal epithelium in vitro, significantly fewer sperm bound to the epithelium than when uncapacitated sperm were added [7]. In those experiments, capacitation had been stimulated by heparin [8], which could have prevented attachment by interacting with the BSP proteins on the surface of sperm [9], because these proteins each contain two heparin-binding sites [10, 11]. Heparin has been shown to release sperm bound to cultured oviductal epithelium in vitro [12].

In addition to heparin stimulation, another mechanism that has been implicated in detaching sperm is motility hyperactivation, which sperm undergo while in the oviduct [13–15]. Hyperactivated sperm swim vigorously using a flagellar beating pattern characterized by asymmetrical, high-amplitude bending (Fig. 1; Supplemental Movie S1 [Supplemental Data are available online at www.biolreprod.org] [16]). Hyperactivation has been observed in mouse sperm detaching from epithelium in oviducts retrieved from mated females during the periovulatory period [2, 17]. According to mathematical modeling, hyperactivation could provide the force necessary to detach sperm from oviductal epithelium [18].

Finally, sperm detachment might be facilitated by contractions of the smooth muscle layer of the oviduct wall. The contractions create strong bursts of fluid flow in the oviductal lumen that could break the attachment of sperm to epithelium, especially if other factors reduce the strength of the binding. Chang and Suarez [2] reported that some mouse sperm bound to ampullar epithelium detached immediately after a contraction of the wall of the oviduct.

We developed a new methodological system that enabled us to test the roles of heparin, hyperactivation, and bursts of fluid flow in detaching bovine sperm from oviductal epithelium. This system was designed to mimic the cellular relationships

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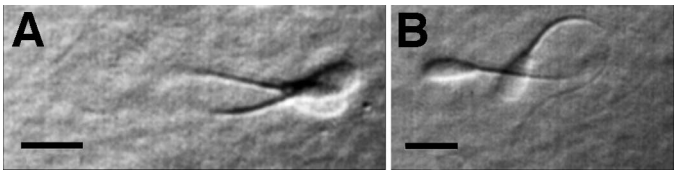

FIG. 1. Swimming patterns of activated (progressive, nonhyperactivated (**A**) and hyperactivated (**B**) bovine sperm. Note the highly asymmetrical flagellar beating pattern of the hyperactivated sperm. Each panel shows a pair of merged differential contrast images taken of the maximum bend achieved on each side of the flagellum. Images are taken from Supplemental Movie S1. Bar = 10 μm.

and biophysical properties of the oviductal epithelium in vivo by using epithelium in folds of mucosa dissected from the oviductal lining. The epithelial cells in these mucosal folds were firmly anchored on underlying connective tissue and in tight association with their neighbors. In this system, the mucosal folds were held in place by a fine plastic mesh in a chamber on a warmed microscope stage, which allowed for continuous observation of a given area, even in the presence of bursts of fluid flow.

Sperm have different functional interactions with isthmic and ampullar oviductal epithelium, namely, storage interactions in the lower isthmus and movement toward oocytes in the lower ampulla. Consequently, the nature of the binding interactions and the factors that detach sperm could differ between the isthmus and ampulla. Therefore, we utilized our system to compare the effects of heparin, hyperactivation, and fluid flow on sperm detachment from epithelium of the two regions.

MATERIALS AND METHODS

Ethics Statement

Animals were not used in these experiments. Semen was provided by Genex Cooperative, Inc., and oviducts were obtained from an abattoir (Cargill Meat Solutions).

Reagents and Media

Bovine serum albumin (BSA, fraction V) and HEPES-free acid were purchased from Calbiochem Corporation. All other chemicals were from Sigma-Aldrich unless otherwise specified. A modified Tyrode-balanced salt solution, Tyrode albumin lactate pyruvate (TALP [8]), was used for washing and diluting sperm and for incubating oviductal mucosal folds with sperm. TALP consisted of 99 mM NaCl, 3.1 mM KCl, 25 mM $NaHCO_3$, 0.39 mM NaH_2PO_4, 10 mM HEPES-free acid, 25.4 mM sodium lactate, 2 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.11 mg/ml sodium pyruvate, 5 mg/ml gentamycin, and 6 mg/ml BSA at pH 7.45 and 300 mOsm/kg. TALP was equilibrated at 38.5°C with 5% CO_2 in humidified air before use. PBS was used as a transport medium for oviducts and consisted of 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, and 154 mM NaCl, pH 7.4, supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin (HyClone; Thermo Scientific).

Sperm Processing

Plastic straws of frozen semen of *B. taurus*, diluted in egg yolk extender (50×10^6 sperm per straw), were generously provided by Genex Cooperative, Inc., and stored in liquid nitrogen. The semen had been processed for cryopreservation as described by Senger et al. [19], except that the antibiotics used by Senger et al. [19] had been changed to a combination of gentamicin, tylosin, lincomycin, and spectinomycin [20]. For each experiment, three straws from one of four fertile Holstein bulls were thawed in 37°C water for 30 sec. Then live sperm were separated from seminal plasma, extender, and dead sperm by density gradient centrifugation ($300 \times g$, 10 min) through two layers (40%/80%) of BoviPure diluted in BoviDilute according to the manufacturer's recommendations (Nidacon International). The sperm pellet was then washed in 3 ml TALP ($300 \times g$, 5 min). Roughly 100×10^6 sperm were resuspended in 1 ml TALP medium, and motility was assessed at 38.5°C using bright-field microscopy.

Oviductal Epithelium Preparation

Oviducts from nonpregnant cows were kindly donated by Cargill Meat Solutions. The oviducts were collected shortly after slaughter and brought to the laboratory barely submerged in ice-cold PBS. At the laboratory, the oviducts were rinsed with ice-cold PBS, and the tissue was kept moist throughout the dissection procedures. First, the mesosalpinx was trimmed off, and the isthmic and ampullar segments of the oviduct were separated and placed into two separate Petri dishes containing PBS at 4°C until use. In preliminary experiments, we found that storage of ampullar segments in cold PBS preserved the sperm binding capability of ampullar epithelial cells for more than 24 h, so they were stored for up to 18 h before use. Isthmic segments were used within 8 h of slaughter.

To prepare mucosal folds of ampullar epithelium, the ampulla was cut open longitudinally with Cohan-Vannas spring scissors and pinned onto a layer of paraffin in a glass Petri dish, with the mucosal surface exposed but moistened with PBS. Small pieces (hereafter referred to as ''folds'') were cut from the primary mucosal folds (Fig. 2A) using iris scissors (Fine Science Tools). The folds were transferred to 60 × 15-mm tissue culture dishes (BD Falcon) containing cold TALP and were kept at 4°C until use within 2 h.

The isthmic epithelium was much more fragile than ampullar epithelium; therefore, isthmuses were not pinned down during dissection. Instead, each isthmus was placed on a plastic cutting board and kept moist by adding fresh, cold TALP throughout the procedure. Any remaining surrounding tissue was trimmed off carefully, and a #11 scalpel was used to cut two successive 2-cm segments proximal to the utero-tubal junction. The segments were opened by cutting the wall longitudinally using iris scissors. Then primary mucosal folds, including all of the underlying tissue, were isolated using the scalpel to make longitudinal cuts through the oviductal wall. The folds were kept in TALP medium at 4°C and were used within 2 h.

Testing Effects of Heparin, 4-AP, and Bursts of Fluid Flow on Sperm Detachment from Oviductal Epithelium

Epithelial folds were transferred to a well of a 12-well cell suspension plate (Greiner Bio-One) containing 400 μl of warm, pre-equilibrated TALP and kept in a CO_2 incubator (38.5°C, 5% CO_2 in humidified air) while sperm were prepared as described above. Then 150 μl of sperm suspension (~15 million sperm cells) were added to the well. The well plate was incubated for 30 min in the CO_2 incubator to allow sperm to bind to the epithelium. After incubation, the folds with attached sperm were gently washed to remove nonbound sperm by sequentially dipping the folds in three wells filled with fresh, 38.5°C TALP. The folds were then placed in an RC-26GLP Open Diamond Bath Imaging Chamber (Warner Instruments) with a floor comprised of a 24 × 50-mm coverslip and coupled to a P-1 chamber platform (Warner Instruments). The chamber contained ~500 μl of 38.5°C, pre-equilibrated TALP and was placed on a temperature-controlled glass plate (OkoLab) on top of a heated microscope stage, both controlled at 38.5°C, on an inverted Zeiss Axiovert 35 microscope. The folds were held in place with a plastic mesh and a stainless-steel anchor (Fig. 2B). The crest of a fold that showed an intact layer of epithelium with vigorously beating cilia was focused on for each experiment. Sperm bound to the crest of the fold were digitally recorded for 1 min at 19 frames/sec using a 20× phase contrast objective and a Neo sCMOS high-definition digital camera (Andor) controlled by NIS Elements 4.0 imaging software (Nikon). Then the fluid in the chamber was carefully replaced by fresh 38.5°C TALP or TALP containing 4 mM 4-aminopyridine (4-AP; Tocris Bioscience), 100 μg/ml heparin (Sigma-Aldrich H3393; heparin sodium salt from porcine intestinal mucosa, 132 USP units/mg [21, 22]), or both reagents. Fluid was added using a 10-ml syringe attached to a polyethylene tube (PE-160, ID 1.14 mm, Warner Instruments), which was inserted into a portal in the chamber, and the fluid was removed by means of a second syringe with its corresponding polyethylene tube attached to a stainless-steel suction tube (Fig. 2B). This fluid replacement procedure was repeated three times to ensure that final concentrations of additives were identical among replicate treatments. To evaluate the effect of bursts of fluid flow, the syringe was used to introduce ~100 μl TALP into the chamber in <1 sec, thus creating a short burst of very rapid flow. The same area of the fold that had been recorded before addition of treatment was recorded again 10 min after beginning the treatment. Each experimental condition was paired with a control of TALP alone. Controls were run immediately after each treatment. The digital videos were reviewed to characterize sperm behavior and count numbers of sperm bound to a length of 300 μm along the crest of the fold. Counting was done by a person who was

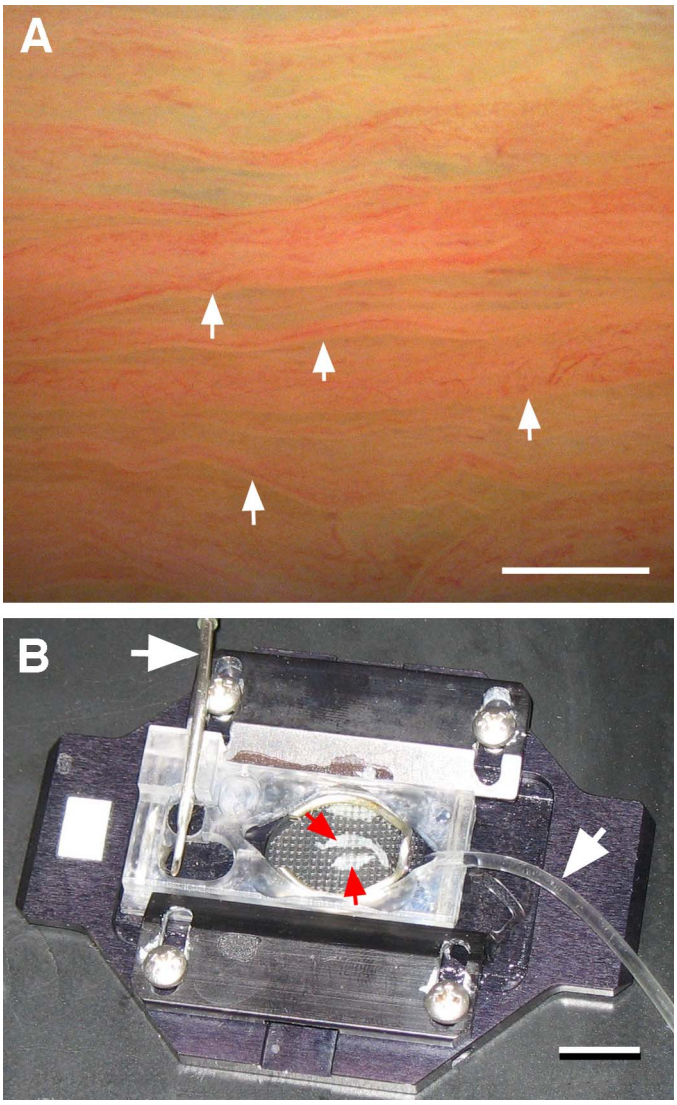

FIG. 2. Preparation of folds of oviductal epithelium. **A**) Primary folds of mucosa (arrows) run longitudinally through the length of the oviduct. Bar = 1 mm. **B**) Laminar flow chamber containing mucosal folds (red arrows). The folds are anchored by a plastic mesh held in place with a metal spring. White arrows indicate the inflow (right) and outflow (left) tubing. Bar = 1 cm.

blind to the experimental condition. The Nikon software was used to mark each sperm on the video as it was counted.

A single batch of frozen semen from each of at least four bulls was used for each type of experiment. The percentage of sperm remaining bound after treatment or control was determined by dividing the number of sperm bound to a 300-μm length of the crest of each fold 10 min after starting the treatment by the number of sperm bound to the same segment before treatment. The percentage of sperm remaining bound after each treatment was compared with that remaining bound after the matched control using a matched pair analysis, paired by bull, on JMP (JMP Statistical Discovery Software; SAS Institute, Inc.). We established strict criteria for inclusion of samples; that is, only samples in which at least 50 sperm/300 μm were counted in the videos taken before treatment were included. Additionally, in control conditions, we required that more than 80% of the initial numbers of sperm should still be bound after 10 min. Data are shown as mean ± SEM, and P values of <0.05 were considered significant.

To measure the current created by the cilia and attached sperm, we selected two control videos where loose debris could be seen moving over the epithelial surface. The movements of debris particles were traced using the manual tracking plugin in ImageJ (NIH) software, and the speed of the particle was determined using MATLAB software (MathWorks, Inc.).

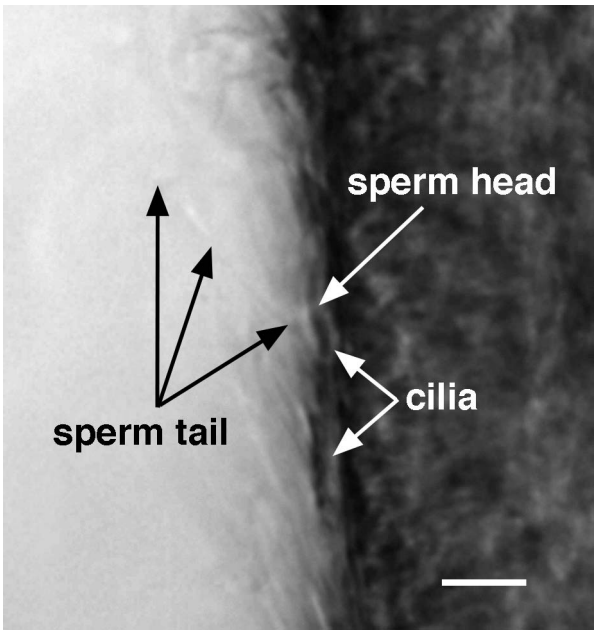

FIG. 3. Several sperm attached to cilia on epithelium (image from Supplemental Movie S2). The length of one of the sperm tails is indicated by black arrows. One of the sperm heads, with a dark acrosomal region and light postacrosomal region, is indicated by a white arrow. The cilia, which appear as a pale layer between the sperm heads and the black apical surface of the epithelium, are also indicated by white arrows. Bar = 20 μm.

Testing Effects of Heparin and Hyperactivation on Free-Swimming Sperm

The effects of heparin and 4-AP on swimming patterns were tested on free-swimming sperm. Frozen/thawed sperm were prepared as described above, and sperm concentration in 38.5°C TALP was adjusted to 12×10^6 sperm/ml. Then aliquots of sperm suspensions were mixed 1:1 with a 38.5°C TALP solution of 8 mM 4-AP, 200 μg/ml heparin, or both, or TALP alone as control. A 10-μl droplet of sperm suspension was placed on a prewarmed 75 × 25-mm slide and covered with a prewarmed 50 × 24-mm coverslip; this created an 8.3-μm-deep chamber that ensured that most of the sperm head and tail would remain in focus. Sperm swimming patterns were recorded for 3 min, beginning 30 sec after addition of treatment, using a 20× phase objective with the warm stage and imaging equipment described above. The digital videos were reviewed to evaluate sperm swimming patterns.

Each experimental condition was paired with a control that was performed immediately afterward. Replicate tests were performed using straws from single batches of samples from each of four bulls. At least 200 sperm were analyzed per sample. Sperm were identified as either activated, hyperactivated, agglutinated, or immotile. Hyperactivation was defined as asymmetrical flagellar beating that produced circular or figure-eight swimming patterns [23] and is illustrated in Figure 1. Morphologically abnormal sperm were not counted. Data were analyzed on JMP using ANOVA and Tukey post hoc comparisons. Data are shown as mean ± SEM, and family-wise P-values of <0.05 were considered significant.

RESULTS

Heparin and 4-AP Affected Sperm Detachment from Isthmic and Ampullar Epithelium Differently

Close inspection of videos revealed sperm bound by their heads to the carpet of cilia on the oviductal epithelium rather than to the surface of the epithelium at the base of the cilia (Fig. 3; Supplemental Movie S2). The flagella of bound sperm beat vigorously for over an hour in the chamber. Ciliary and flagellar beating generated a visible current near the surface of the epithelium. Bits of debris swept by the current were tracked

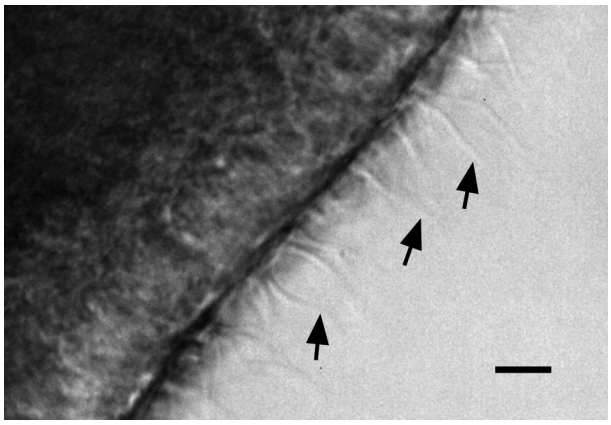

FIG. 4. Bursts of strong fluid flow did not detach sperm from epithelium. Arrows indicate the flagella of some of the attached sperm. Image taken from Supplemental Movie S3. Bar = 20 μm.

on videos moving at a speed of 117 ± 5 μm/sec (n = 44 tracks from two folds).

Bursts of strong fluid flow, produced by syringe, changed the orientation of sperm flagellar beating. After the bursts ended, however, sperm regained their original orientation. Sperm were not observed detaching from the epithelium during or after a burst of flow (Fig. 4; Supplemental Movie S3). The number of sperm attached to ampullar folds remained unchanged even after 10 min of continuously applying bursts of flow strong enough to cause the fold to move.

Addition of heparin to sperm bound to isthmic and ampullar folds resulted in head-to-head agglutination of some of the bound sperm (Fig. 5; Supplemental Movie S4). Heparin elicited sperm detachment from isthmic folds: the percentages of sperm remaining bound after 10 min were significantly lower than those of sperm remaining bound after 10 min under control conditions (Fig. 6). In contrast, heparin did not elicit detachment of sperm bound to ampullar folds (Fig. 6).

Addition of 4-AP increased the bend amplitude and asymmetry of the flagella of bound sperm, thereby hyperactivating the sperm; however, it did not elicit sperm detachment from either isthmic or ampullar folds in the absence of heparin (Fig. 6). When both 4-AP and heparin were added, all of the sperm appeared to hyperactivate, and the sperm were seen to pivot vigorously around the point of contact between the head and cilia. Pivoting sperm detached rapidly from both isthmic and ampullar oviductal folds (Fig. 7; Supplemental Movie S5).

Preincubation of sperm with 4-AP or heparin (Fig. 8; Supplemental Movie S6) for 10 min before adding them to folds did not prevent binding from occurring. Preincubation of folds with heparin for 10 min prior to adding untreated sperm also failed to prevent sperm binding (not shown). Only when both sperm and folds were preincubated with heparin for 10 min prior to coincubation was sperm attachment lower than when heparin was not present despite the fact that the final concentration of heparin was the same in all cases (Fig. 8).

Free-Swimming Sperm Were Hyperactivated by 4-AP, but Heparin Had No Effect on Motility

In the absence of folds, addition of 4 mM 4-AP rapidly elicited hyperactivation in almost all of the motile sperm (Figs. 1 and 9). Addition of heparin rapidly induced to head-to-head agglutination of free-swimming sperm but did not elicit hyperactivation. Since the sperm swimming patterns can be

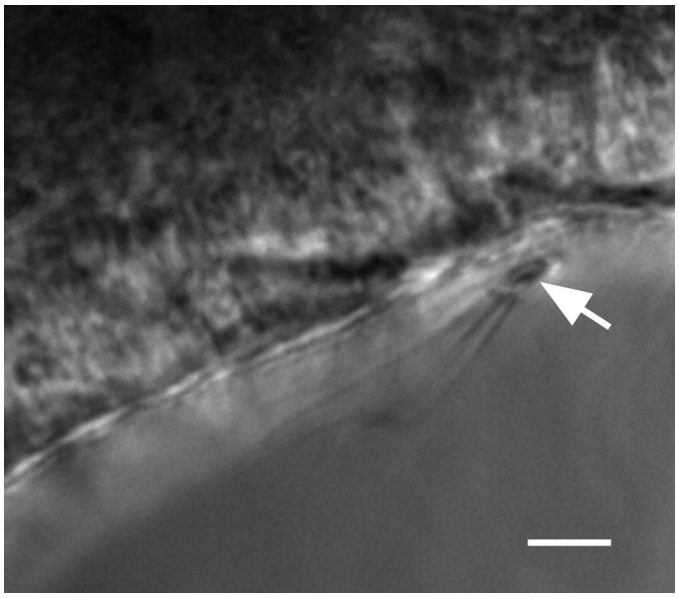

FIG. 5. Heparin elicited sperm agglutination. Arrow indicates agglutinated heads of four sperm bound to isthmic epithelium 10 min after addition of heparin. Image taken from Supplemental Movie S4. Bar = 20 μm.

affected by their agglutination, agglutinated sperm were excluded from further evaluations. When only nonagglutinated sperm were evaluated, the rates of hyperactivation in the presence of 4-AP plus heparin were similar to those in 4-AP alone. The percentage of hyperactivated sperm in 4-AP ± heparin was significantly higher than in heparin alone or in control conditions (Fig. 9).

DISCUSSION

Using a new in vitro system that more closely replicates the histological structure and cellular relationships of the oviductal lining than previously used systems of explants [24] and monolayer cultures of epithelium [25], we assessed whether bursts of strong fluid flow, hyperactivation, or heparin were

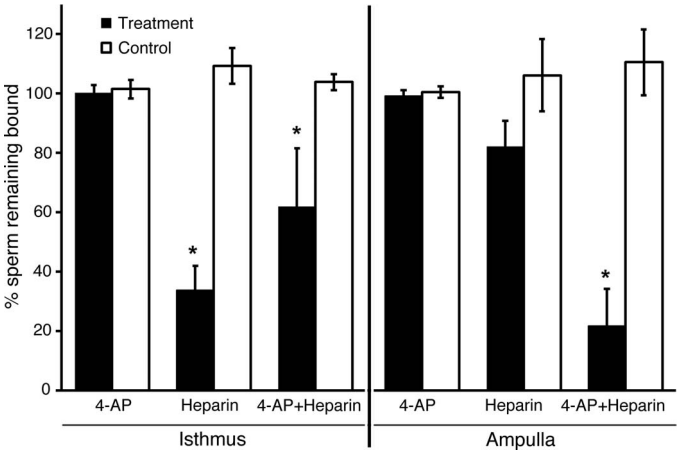

FIG. 6. Heparin and 4-AP affected sperm detachment differently in epithelia from the isthmus and ampulla. Whereas heparin alone produced sperm detachment from isthmic epithelium, stimulation of hyperactivation by 4-AP was additionally required to detach sperm from ampullar epithelium (means ± SEM for four replicates; $^*P < 0.05$ when treatment is compared with its paired control).

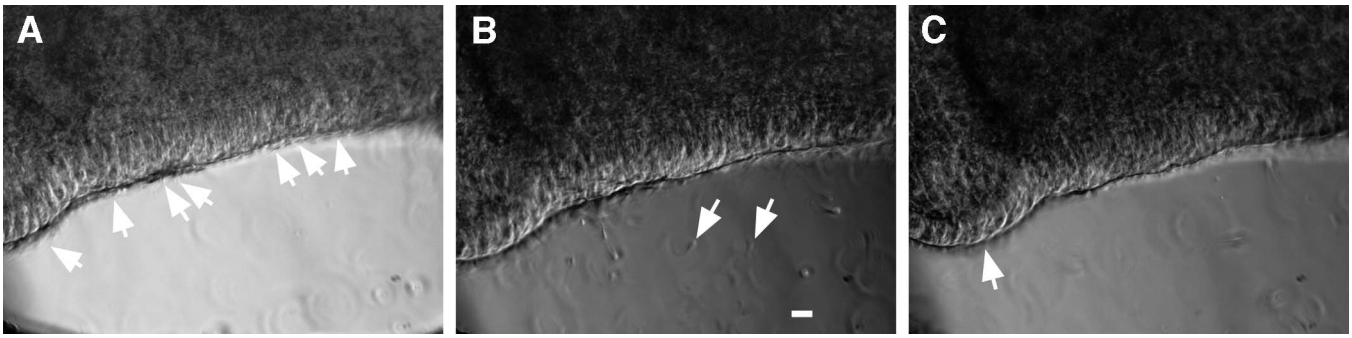

FIG. 7. Treatment of bound sperm with heparin and 4-AP stimulated sperm detachment from ampullar epithelium. **A**) Sperm (white arrows) attached to the cilia before treatment. **B**) One min after treatment, sperm displayed hyperactivation and detached from epithelium (white arrows). **C**) Ten min after treatment, very few sperm (white arrow) remained bound to the epithelium (images taken from Supplemental Movie S5). Bar = 20 μm.

necessary and sufficient to elicit detachment of bovine sperm from oviductal epithelium in the lower isthmus and ampulla. The system allowed us to treat bound sperm with bursts of fluid flow and rapid additions of heparin and 4-AP while maintaining the same area of tissue under observation so that behavior of the same sperm could be observed before and after treatment.

Importantly, whereas heparin alone was sufficient to trigger detachment of sperm from isthmic epithelium, stimulation of hyperactivation by 4-AP was additionally required to detach sperm from ampullar epithelium. As sperm bound to ampullar epithelium began to hyperactivate in the presence of heparin, they twisted about their longitudinal axes; this twisting could have produced forces that were effective for tearing sperm off of the ampullar cilia. The additional requirement of hyperactivation indicates that the binding interaction is stronger in the ampulla than in the isthmus or that the effect of heparin is weaker due to a different density or arrangement of receptors on the ampullar epithelium than on isthmic epithelium.

The mucosal folds of epithelium used for this study were densely carpeted by cilia, which, along with the sperm flagellar beating, produced a strong linear flow. The beating flagella of bound sperm were oriented in the same direction as the cilia. The current created by the rapid beating of cilia and sperm flagella swept away debris but did not detach sperm. Furthermore (and unexpectedly), the strong bursts of fluid flow that we mechanically applied to bound sperm failed to produce detachment of sperm from either isthmic or ampullar epithelium. We had created these bursts of flow in order to mimic the effects of contractions of the smooth muscle in the wall of the oviduct, which have been associated with sperm detachment, at least in rodents and humans [2, 26–28].

Heparin was required for sperm detachment in both the isthmus and the ampulla. A likely explanation for the ability of heparin to induce detachment is that it specifically interferes with the interaction between BSP proteins on sperm and receptors on oviductal epithelium. Each BSP protein (BSP1, BSP3, BSP5) alone significantly enhances bovine sperm attachment to oviductal epithelium [4], and each BSP protein contains two heparin-binding sites [4, 10]. Oviductal annexins, which have been implicated as receptors for sperm [5], also possess heparin-binding sites [29–31]. By interacting with its binding sites on BSPs and/or annexins, heparin could disrupt interactions between sperm and oviductal epithelium.

Surprisingly, pretreatment of sperm or oviductal epithelium with heparin did not prevent sperm binding when only one of the binding partners was pretreated. We observed a reduction in binding only when both sperm and epithelium had been pretreated with heparin, even though the final amount of heparin was not higher than when either was pretreated. The preincubation of heparin with both sperm ligands and oviductal receptors may have been required in order to provide sufficient time for heparin to interact with all of its binding sites on sperm (BSPs) and on the oviduct (annexins or other receptors) before receptors and ligands came into contact. Heparin is a long, flexible molecule comprised of negatively charged, repeating disaccharide units that interact electrostatically with positively charged binding sites [32]. Each heparin molecule is able to

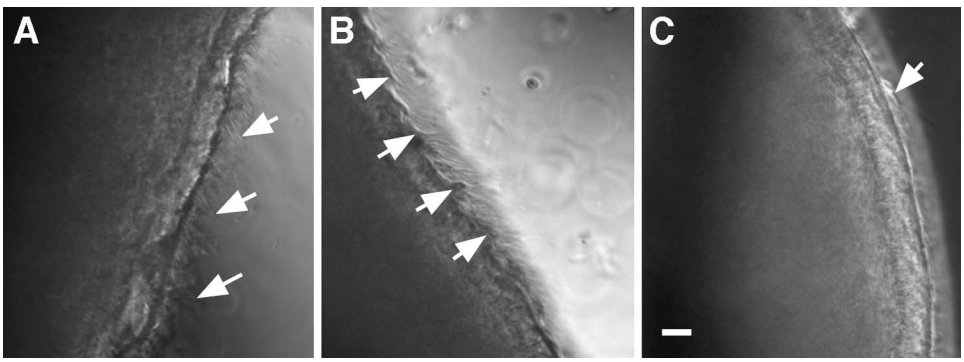

FIG. 8. Pretreatment of sperm with 4-AP to induce hyperactivation or with heparin did not prevent attachment to oviductal epithelium. **A**) Hyperactivated sperm attached to epithelium. Arrows indicate some of the bound sperm. **B**) Sperm pretreated with heparin were able to bind in high numbers. Arrows indicate groups of bound, agglutinated sperm. **C**) In contrast, when both sperm and oviductal epithelium were pretreated with heparin for 10 min, few sperm bound to epithelium. Arrow indicates two bound sperm. Images taken from Supplemental Movie S6. Bar = 20 μm.

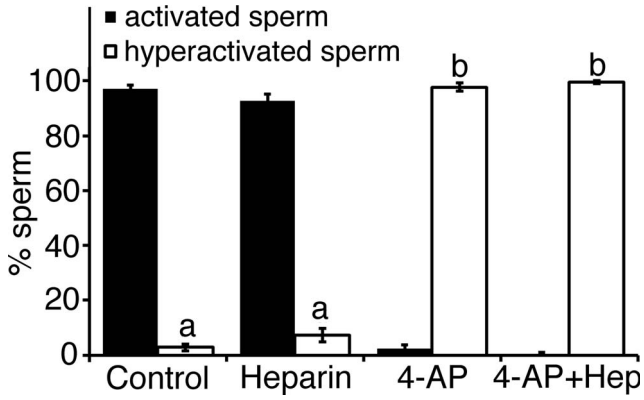

FIG. 9. Effects of 4-AP and heparin on sperm swimming patterns in the absence of epithelium. Heparin alone did not hyperactive sperm, whereas 4-AP elicited hyperactivation in nearly all sperm regardless of the presence of heparin (means ± SEM for three replicate experiments; different letters denote family-wise $P < 0.05$).

bind to several molecules of BSPs [32]. The putative oviductal receptors, annexins [5], are also reported to bind heparin [31, 33].

Our observations of bovine sperm detachment from oviductal epithelium differ in some respects from observations we reported earlier for mouse sperm. When viewed through the walls of intact oviducts removed from mated females shortly after ovulation, only hyperactivated mouse sperm were seen to detach from epithelium along the length of the oviduct [2, 17, 34]; however, mouse sperm in ampulla remained bound to epithelium for longer intervals than sperm bound in the isthmus [2]. So, like bovine sperm, there was some indication that mouse sperm bound more strongly in the ampulla than in the isthmus; however, unlike bovine sperm, strong fluid flow appeared to detach mouse sperm. Nevertheless, the strong contractions seen in the mouse ampulla not only may have created a strong fluid flow but also may have caused epithelial cells or cumulus to physically scrape sperm off of the epithelium. Also, very few mouse sperm were ever seen in the ampulla outside of the cumulus, so our observations could not be confirmed by statistical analysis.

In conclusion, we developed a new system to observe the interaction between sperm and oviductal epithelium in conditions that more closely resemble the cell and tissue relationships of the oviductal lining in vivo. In this system, the epithelial cells remained tightly associated with one another and firmly anchored to underlying connective tissue. This preserved a dense carpet of beating cilia on the surface, thereby maintaining biophysical properties of oviductal epithelium in vivo, including a directed current created by the coordinated beating of the cilia and aligned beating of the flagella of bound sperm. Sperm bound directly to the cilia and could not be detached by normal ciliary beating or strong bursts of fluid flow. Our results indicate, for the first time, that the conditions required for sperm detachment in the ampulla are different than those in the isthmus in that heparin alone can detach sperm from isthmic epithelium, while initiation of hyperactivation is additionally required to detach sperm from ampullar epithelium.

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REFERENCES

1. Suarez SS. Regulation of sperm storage and movement in the mammalian oviduct. Int J Dev Biol 2008; 52:455–462.
2. Chang H, Suarez SS. Unexpected flagellar movement patterns and epithelial binding behavior of mouse sperm in the oviduct. Biol Reprod 2012; 86:140.
3. Gwathmey TM, Ignotz GG, Suarez SS. PDC-109 (BSP-A1/A2) promotes bull sperm binding to oviductal epithelium in vitro and may be involved in forming the oviductal sperm reservoir. Biol Reprod 2003; 69:809–815.
4. Gwathmey TM, Ignotz GG, Mueller JL, Manjunath P, Suarez SS. Bovine seminal plasma proteins PDC-109, BSP-A3, and BSP-30-kDa share functional roles in storing sperm in the oviduct. Biol Reprod 2006; 75: 501–507.
5. Ignotz GG, Cho MY, Suarez SS. Annexins are candidate oviductal receptors for bovine sperm surface proteins and thus may serve to hold bovine sperm in the oviductal reservoir. Biol Reprod 2007; 77:906–913.
6. Smith TT, Yanagimachi R. Capacitation status of hamster spermatozoa in the oviduct at various times after mating. J Reprod Fertil 1989; 86: 255–261.
7. Lefebvre R, Suarez SS. Effect of capacitation on bull sperm binding to homologous oviductal epithelium. Biol Reprod 1996; 54:575–582.
8. Parrish JJ, Susko-Parrish J, Winer MA, First NL. Capacitation of bovine sperm by heparin. Biol Reprod 1988; 38:1171–1180.
9. Thérien I, Bleau G, Manjunath P. Phosphatidylcholine-binding proteins of bovine seminal plasma modulate capacitation of spermatozoa by heparin. Biol Reprod 1995; 52:1372–1379.
10. Calvete JJ, Campanero-Rhodes MA, Raida M, Sanz L. Characterisation of the conformational and quaternary structure-dependent heparin-binding region of bovine seminal plasma protein PDC-109. FEBS Lett 1999; 444: 260–264.
11. Chandonnet L, Roberts KD, Chapdelaine A, Manjunath P. Identification of heparin-binding proteins in bovine seminal plasma. Mol Reprod Dev 1990; 26:313–318.
12. Gualtieri R, Mollo V, Braun S, Barbato V, Fiorentino I, Talevi R. Bovine oviductal monolayers cultured under three-dimension conditions secrete factors able to release spermatozoa adhering to the tubal reservoir in vitro. Theriogenology 2013; 79:429–435.
13. Suarez SS, Ho HC. Hyperactivated motility in sperm. Reprod Domest Anim 2003; 38:119–124.
14. Chang H, Suarez SS. Two distinct Ca(2+) signaling pathways modulate sperm flagellar beating patterns in mice. Biol Reprod 2011; 85:296–305.
15. Hunter RH. Components of oviduct physiology in eutherian mammals. Biol Rev Camb Philos Soc 2012; 87:244–255.
16. Ho HC, Suarez SS. An inositol 1,4,5-trisphosphate receptor-gated intracellular Ca(2+) store is involved in regulating sperm hyperactivated motility. Biol Reprod 2001; 65:1606–1615.
17. DeMott RP, Suarez SS. Hyperactivated sperm progress in the mouse oviduct. Biol Reprod 1992; 46:779–785.
18. Simons J, Olson S, Cortez R, Fauci L. The dynamics of sperm detachment from epithelium in a coupled fluid-biochemical model of hyperactivated motility. J Theor Biol 2014; 354:81–94.
19. Senger PL, Mitchell JR, Almquist JO. Influence of cooling rates and extenders upon post-thaw viability of bovine spermatozoa packaged in .25- and .5-ml French straws. J Anim Sci 1983; 56:1261–1268.
20. Shin SJ, Lein DH, Patten VH, Ruhnke HL. A new antibiotic combination for frozen bovine semen 1. Control of mycoplasmas, ureaplasmas, *Campylobacter fetus* subsp. *venerealis* and *Haemophilus somnus*. Theriogenology 1988; 29:577–591.
21. Fukui Y, Sonoyama T, Mochizuki H, Ono H. Effects of heparin dosage and sperm capacitation time on in vitro fertilization and cleavage of bovine oocytes matured in vitro. Theriogenology 1990; 34:579–591.
22. Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 1995; 44:915–923.
23. Marquez B, Suarez SS. Different signaling pathways in bovine sperm regulate capacitation and hyperactivation. Biol Reprod 2004; 70(6): 1626–1633.
24. Lefebvre R, Chenoweth PJ, Drost M, LeClear CT, MacCubbin M, Dutton JT, Suarez SS. Characterization of the oviductal sperm reservoir in cattle. Biol Reprod 1995; 53:1066–1074.
25. Gualtieri R, Mollo V, Barbato V, Talevi R. Ability of sulfated glycoconjugates and disulfide-reductants to release bovine epididymal sperm bound to the oviductal epithelium in vitro. Theriogenology 2010; 73:1037–1043.
26. Battalia DE, Yanagimachi R. Enhanced and co-ordinated movement of the hamster oviduct during the periovulatory period. J Reprod Fertil 1979; 56: 515–520.

27. Eisenbach M, Giojalas LC. Sperm guidance in mammals—an unpaved road to the egg. Nat Rev Mol Cell Biol 2006; 7:276–285.
28. Wildt L, Kissler S, Licht P, Becker W. Sperm transport in the human female genital tract and its modulation by oxytocin as assessed by hysterosalpingoscintigraphy, hysterotonography, electrohysterography and Doppler sonography. Hum Reprod Update 1998; 4:655–666.
29. Horlacher T, Noti C, de Paz JL, Bindschädler P, Hecht ML, Smith DF, Fukuda MN, Seeberger PH. Characterization of annexin A1 glycan binding reveals binding to highly sulfated glycans with preference for highly sulfated heparan sulfate and heparin. Biochemistry 2011; 50: 2650–2659.
30. Shao C, Zhang F, Kemp MM, Linhardt RJ, Waisman DM, Head JF, Seaton BA. Crystallographic analysis of calcium-dependent heparin binding to annexin A2. J Biol Chem 2006; 281:31689–31695.
31. Ishitsuka R, Kojima K, Utsumi H, Ogawa H, Matsumoto I. Glycosaminoglycan binding properties of annexin IV, V, and VI. J Biol Chem 1998; 273:9935–9941.
32. Sankhala RS, Damai RS, Anbazhagan V, Kumar CS, Bulusu G, Swamy MJ. Biophysical investigations on the interaction of the major bovine seminal plasma protein, PDC-109, with heparin. J Phys Chem B 2011; 115(44):12954–12962.
33. Shao C, Zhang F, Kemp MM, Linhardt RJ, Waisman DM, Head JF, Seaton BA. Crystallographic analysis of calcium-dependent heparin binding to annexin A2. J Biol Chem 2006; 281:31689–31695.
34. Ho K, Wolff CA, Suarez SS. CatSper-null mutant spermatozoa are unable to ascend beyond the oviductal reservoir. Reprod Fertil Dev 2009; 21: 345–350.