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Minireview

The Oviductal Sperm Reservoir in Mammals: Mechanisms of Formation

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In large mammals, millions of sperm are necessary to fertilize only a few oocytes (reviewed in [1, 2]). Artificial insemination has been used to increase reproductive efficiency, but still, thousands of excess sperm must be used to ensure fertilization. For example, carefully regulated insemination of cows with semen from highly fertile bulls requires a few million sperm to achieve pregnancy or nonreturn rates over 60% [3–5]. Of the millions of sperm normally ejaculated in natural mating, only thousands reach the isthmus of the oviduct, and there, most are held back in a reservoir. Only a few reach the ampulla at the time of fertilization (reviewed in [1, 6]).

The oviductal reservoir for sperm may serve a number of functions. First, it may prevent polyspermic fertilization, by allowing only a few sperm at a time to reach the oocyte in the ampulla. Sperm numbers have been artificially increased at the site of fertilization in the pig by surgical insemination directly into the oviduct [7, 8], by resecting the oviduct to bypass the reservoir [9], and by administering progesterone into the muscularis [10, 11]. In all of these cases, the incidence of polyspermy increased. Second, the oviductal reservoir may maintain the fertility of sperm between the onset of estrus and fertilization. Bull sperm fertility and motility are maintained longer in vitro if the sperm are incubated with oviductal epithelium [12, 13]. Third, the processes of capacitation and motility hyperactivation may be regulated within the reservoir. Capacitation is defined herein as a set of changes in the sperm plasma membrane that enables sperm to undergo the acrosome reaction. Hyperactivation is an increase in flagellar beat amplitude and asymmetry that is observed in sperm recovered from the ampulla of the oviduct near the time of ovulation. Capacitation of bull sperm is enhanced by incubation in medium conditioned by oviductal epithelium [14] or in oviduct fluid [15]. Hyperactivation of human sperm may be enhanced by incubations with cultured oviductal epithelium [16]. Knowledge of how the oviductal reservoir modulates sperm transport and capacitation could be applied to increasing the reproductive efficiency of cattle and other valuable species.

There is strong evidence from multiple species that the oviductal reservoir is created by binding of sperm to oviductal epithelium. Motile sperm have been observed to bind to the apical surface of the oviductal epithelium in cattle [17] (Fig. 1), mice [18], hamsters [19], pigs [20], and horses [21]. The trapping action of the sperm-binding moieties may be enhanced by the narrow lumen of the uterotubal junction and isthmus. The bovine lumen is particularly tortuous and narrow in the uterotubal junction [22, 23]. There are large and small folds in the mucosa, some of which create grooves that end blindly. There is also a multilayered vascular plexus in the wall that resembles erectile tissue and could serve to reduce the lumen [22]. The wall of the junction and lower isthmus contains a thick muscular layer that could further compress the lumen, the action being accentuated by muscle in the infundibulocornual ligament that could act to increase the flexure of the sigmoidal lumen [24]. The narrowness of the lumen is especially apparent in frozen sections, because tissue does not shrink as it does during preparation of paraffin-embedded sections [23]. Thus, sperm entrapment in the reservoir may be due to a number of factors that enhance the trapping effectiveness of adhesive molecules on the epithelium, particularly by increasing contact of sperm with the mucosal surface.

Little is known about sperm release from the epithelium for fertilization. Changes in the hormonal state of oviductal epithelium related to impending ovulation do not appear to affect the number of binding sites for sperm [17, 20, 21]; however, the state of the sperm does affect binding. Capacitation involves changes in the plasma membrane over the sperm head and therefore may lead to sperm release by eliminating or modifying binding molecules on the head. Hyperactivation may provide the force necessary for overcoming the attraction between sperm and oviductal epithelium. Smith and Yanagimachi [19] reported that hamster sperm that had undergone both capacitation and hyperactivation in vitro did not bind to epithelium when infused into hamster oviducts. While using transillumination to study motile sperm within oviducts removed from mated mice, we noted that only hyperactivated sperm detached from epithelium [25]. Also, when bull sperm were capacitated by treatment with heparin in vitro, their binding to explants of oviductal epithelium was reduced [26]. In this case, hyperactivation was not observed. Therefore, we propose that changes in the sperm head surface are responsible for loss of binding affinity, although the pull produced by hyperactivation may enhance the ability of sperm to release themselves. While the binding sites present on the epithelium may not be reduced in number or affinity, epithelial secretions initiated by signals of impending ovulation could enhance sperm capacitation, thereby effecting sperm release

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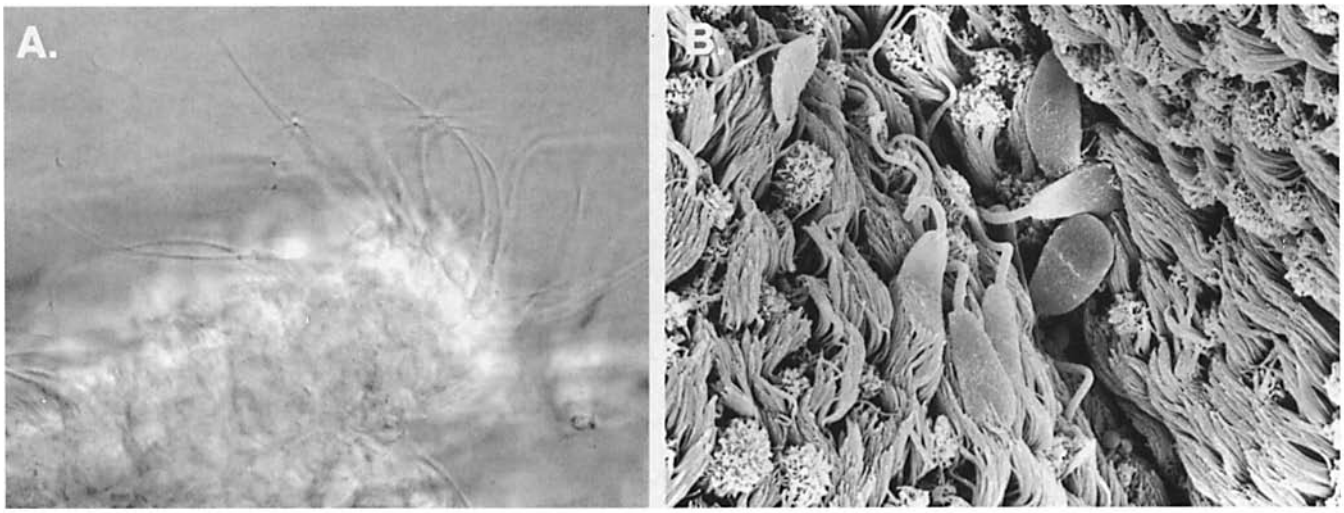

FIG. 1. **A**) Bull sperm binding to explants of bovine oviductal epithelium in vitro (differential interference contrast optics, ×700; protocol described in [17]). **B**) Scanning electron micrograph of bull sperm attached to bovine oviductal epithelium after infusion in vivo (×1700; adapted from Lefebvre et al., Biol Reprod 1995; 53:1071, Figure 4C [17]).

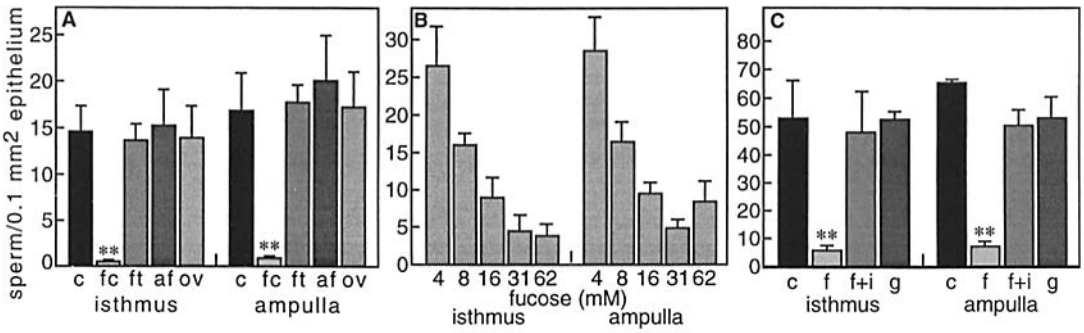

FIG. 2. Fucose inhibits bull sperm binding to explants of oviductal epithelium taken from estrous heifers. **A**) Glycoproteins and polysaccharides expressing a variety of terminal sugar residues were tested for the capacity to competitively inhibit binding of bull sperm to oviductal explants. Only fucoidan, which is composed of fucose and fucose-sulfate, significantly reduced the density of bound sperm. c, Sperm-TALP control; fc, fucoidan; ft, fetuin; af, asialofetuin; ov, ovalbumin. Mean ± SEM sperm/0.1 mm² of epithelium, n = 5 replicates. Density of sperm binding did not differ between isthmic and ampullar epithelium, $p > 0.05$. **Fucoidan reduced binding, $p < 0.001$. **B**) Next, fucose was tested at various doses for its capacity to inhibit bull sperm binding to explants, and it did so in a dose-dependent manner. Mean ± SEM sperm/0.1 mm² of oviductal epithelium; n = 5 replicates. **C**) Finally, explants pretreated with 0.1 U/ml fucosidase for 5 h at 39°C bound significantly fewer sperm than those pretreated with fucosidase plus its specific inhibitor, deoxyfuconojirimycin, or with 5 U/ml galactosidase. c, Sperm-TALP control; f, fucosidase; f+i, fucosidase + inhibitor; g, galactosidase. Mean ± SEM sperm/0.1 mm² of epithelium; n = 3 replicates. Density of sperm binding did not differ between isthmic and ampullar epithelium, $p > 0.05$. **Fucosidase pretreatment reducing sperm binding, $p < 0.001$. Adapted from Lefebvre et al., Biol Reprod 1997; 56;1200–1201, Figures 1, 3, and 4 [30].

[16]. Soluble oviductal factors do enhance capacitation of bull sperm [14, 15]. So, sperm release is brought about by changes within the sperm, although these changes could be initiated by signals from the oviduct.

Sperm binding to oviductal epithelium appears to involve carbohydrate recognition. The first evidence for this came from our studies with fetuin and its terminal sugar sialic acid, which were found to inhibit binding of hamster sperm to the epithelium [27]. Colloidal gold-labeled fetuin bound to the heads of fresh epididymal hamster sperm but not to sperm that had been incubated under capacitating conditions until they became hyperactivated. Fetuin also bound to certain glycoprotein bands on Western blots of membrane extracts from fresh hamster sperm, and the bands were reduced in extracts from sperm incubated under capacitating conditions. These data indicate that there is a lectin on the heads of uncapacitated hamster sperm that binds fetuin and is responsible for attachment of sperm to the epithelium. We also investigated binding of stallion sperm to epithelium and found that asialofetuin and its ter-

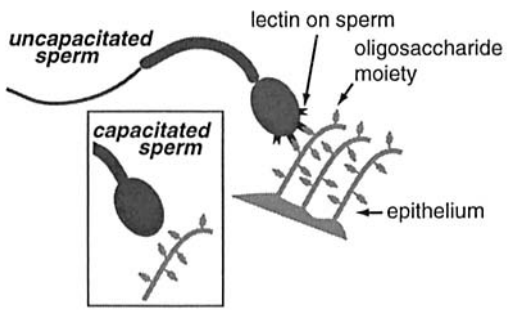

FIG. 3. Diagram of the proposed scheme for formation of the sperm reservoir and the eventual release of sperm for fertilization. Uncapacitated sperm bind to carbohydrate moieties on glycoproteins or glycolipids on the surface of the oviductal epithelium via a lectin-like molecule. This molecule is lost or modified during capacitation, allowing the sperm to release.

minal sugar, galactose, blocked sperm binding [28]. Dobrinski and coworkers were able to isolate a galactose-binding protein from the plasma membrane of stallion sperm using galactose affinity chromatography [29]. In cattle, bull sperm binding to oviductal epithelium was determined to be specifically blocked by fucose [30]. Pretreatment of epithelium with fucosidase, but not galactosidase, reduced binding [30] (Fig. 2). Thus, carbohydrate involvement in sperm binding to epithelium appears to be a widespread phenomenon, although the particular carbohydrate moiety that constitutes the binding site varies according to species. In each of the three species studied so far, a different sugar maximally inhibited binding. These species differences may not seem so unusual when one considers that a single amino acid residue can determine the ligand specificity of a lectin [31, 32] and that closely related animal lectins have different carbohydrate specificities [33].

Other forms of heterotypic binding between cells involve carbohydrate recognition. Examples are the selectins, which mediate leukocyte binding to endothelium [34], and glycolipid ligands on ciliated respiratory cells, which are recognized by mycoplasmas [35]. Selectins mediate temporary binding between the two cell types, just as binding between sperm and epithelium is temporary. Carbohydrate recognition is also implicated in sperm-zona binding (reviewed in [36]) and sperm-Sertoli cell binding [37]. During the course of evolution, lectins with different specificities could have arisen to regulate sperm attachment to these different surfaces.

In summary, the formation of a sperm reservoir in the oviductal isthmus appears to be regulated by carbohydrate recognition between sperm and the oviductal epithelium. The narrowness of the lumen of the isthmus, and perhaps the mucus within the lumen, may enhance sperm binding by slowing their progress and increasing contact with the epithelial surface. There may be a lectin on the surface of sperm that is responsible for binding and that is lost or modified during capacitation, thereby allowing sperm to be released (Fig. 3). Hyperactivation may provide the force to pull sperm away from their attachment sites.

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