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Apical blebs on sperm storage tubule epithelial cell microvilli: Their release and interaction with resident sperm in the turkey hen oviduct

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ABSTRACT

Located at the anterior end of the turkey hen's vagina are numerous discrete tubular invaginations of the surface epithelium, collectively referred to as the sperm storage tubules (SSTs). After mating or artificial insemination, sperm ascend the vagina, enter the SSTs, and over the ensuing days and weeks, gradually exit the SSTs and are transported to the anterior end of the oviduct to fertilize a daily succession of ova. Little is known regarding the cellular and molecular mechanisms responsible for sperm subsistence in the lumen of the SST. In this study, the origin of microvillus blebs (MvBs) on the apical tips of SST epithelial cells was examined, and their possible role in sperm survival was discussed. Regardless, if sperm are present or not, transmission electron microscopy revealed two types of microvilli differentiated by the presence or absence of pleomorphic unilaminar MvBs localized to their apical tips. Although some MvBs appeared to be discharging their contents into the SST lumen, others appeared to have pinched off the microvillus stem. When SSTs contained clusters of densely packed sperm, the sperm heads of those sperm adjacent to the SST epithelial cell surface were surrounded by the microvilli. Associated with the plasmalemma of sperm throughout the SST lumina were membrane fragments and small vesicles (30–130 nm in diameter), some of which appeared to have fused with sperm. It is concluded that the MvBs are a form of shedding vesicle released from the SST epithelial cell microvilli by apocrine secretion. On the basis of observations described herein and those of other authors, it is suggested that the MvBs contribute to sustained sperm storage in the SSTs by (1) supplying metabolic substrates used by resident sperm, (2) serving as fusogenic vehicles providing exogenous macromolecules that reversibly suppress sperm functions associated with fertilization (decapacitation?) and stabilize the sperm plasmalemma, and (3) acting as transport vesicles actively transporting fluid from the SST epithelial cells to the SST lumen.

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1. Introduction

Sperm transferred into the vagina by copulation or artificial insemination has the capacity to maintain their fertilizing capacity for days to months depending on the avian species [1]. This prolonged fertile period is the result of sperm residing in discrete tubular invaginations of the

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surface epithelium lining the lumen of the uterovaginal junction (UVJ). There are about 30,000 such tubular invaginations, referred to as the sperm storage tubules (SSTs), in the UVJ mucosa of the turkey. (See Bakst [2] and Froman et al. [3] for comprehensive reviews of oviductal sperm storage in poultry.) While the female is in egg production, sperm are gradually released from the SST, ascend to the infundibulum where sperm interact with the inner perivitelline layer (IPL) enveloping the ovulated ovum, hydrolyze the IPL (polyspermy is normal in birds), and fertilize the ovum.

Unlike mammals, ejaculated chicken sperm do not require a period of capacitation to fertilize an ovum *in vitro* [4]. Furthermore, Howarth [5] reported that chicken sperm removed from the testes, epididymides, or ductus deferens and surgically transferred into the magnum portion of the oviduct resulted in fertilized eggs. However, there were no fertilized eggs if the same sperm were vaginally inseminated into the hen. More recently, Nixon et al. [6] concluded that capacitation is not a prerequisite for Japanese quail sperm to hydrolyze IPL fragments *in vitro*. These authors also noted that posttesticular maturation of Japanese quail sperm was limited to an increase in sperm motility after passage through the epididymis.

Considering the relatively short period of sperm viability after semen collection and storage *in vitro* [7], and the long duration of sperm storage in the UVJ, it has been suggested that sperm residing in the SSTs undergo a reversible suppression of metabolic activity, motility, and acrosomal enzyme activity [2,8]. In addition, the functional integrity of the sperm plasmalemma is maintained by suppression of the formation of reactive oxygen species [8], which appear to be the basis for decreased sperm viability after *in vitro* semen storage for longer than 6 hours [7]. Given the above, Bakst and Akuffo [9] alluded to the possibility that sperm within the SST lumen are in a decapacitated-like state and reach a capacitated state when released from the SSTs and enter the uterus [8].

Sperm within the SST subsist on exogenous fatty acids [3,10] and possibly other lipids derived from the SST epithelial cells [2,8]. Possible mechanisms of lipid transfer from the SST epithelial cells or its luminal contents to resident sperm were reviewed by Bakst et al. [8]. One such mechanism included the dilation of the apical tips of the SST microvilli and the shedding of microvillus vesicles into the SST lumen to interact with sperm. Several other investigators, including Burke [11], Tingari and Lake [12], and Schuppin et al. [13], observed dilation of the SST epithelial cell microvilli and similar sized vesicles in the SST lumen but did not elaborate further on possible roles of these blebs in SST function. However, Schuppin et al. [13] and Bakst et al. [8] did allude to the possibility that such blebbing was an artifact of fixation.

The contribution of shedding vesicles derived from the mammalian epididymis (epididymosomes) and prostate gland (prostasomes) in posttesticular sperm maturation has been addressed by numerous authors and reviewed by Sullivan and Saez [14]. Proteins associated with the epididymosomes and prostasomes, secreted by apocrine secretion and exocytosis, respectively, have been shown to alter sperm motility patterns, render protection to sperm against oxidative stress, modulate capacitation or decapacitation, function in ovum–sperm interaction, and modify the cholesterol:phospholipid ratio in the sperm plasmalemma. Prostasomes in particular, with their high cholesterol content, have been proposed to fuse with sperm and inhibit capacitation and the acrosome reaction [15].

Resident sperm no doubt interact with secretions, including macromolecules, from the SST epithelium, and such interactions contribute to prolonged sperm storage in the hen. Considering the multiple roles of shedding vesicles secreted by the mammalian epididymis and prostate gland in posttesticular sperm maturation, the following study examines the origin and secretion of SST epithelial cell microvillus blebs (MvBs) and their possible role in prolonged sperm survival in the hen's oviduct.

2. Materials and methods

The care, use, and procedures involving animals in this study were approved by the Beltsville Area Animal Care and Use Committee. A total of six Large White commercial turkey hens in egg production were used in this study. All hens were kept in single cages in an environmentally controlled house, exposed to a photoperiod of 14 hours of light and 10 hours of dark, and provided water *ad libitum*. Standard corn-based commercial breeder diets were provided to the hens (17% protein) and toms (14% protein). Hens were inseminated weekly with 300 million sperm for 7 or more weeks before tissue collection. At 42, 46, and 51 weeks of age, two hens were euthanized by cervical dislocation 24 hours after their last insemination, and the uterus and vagina were removed as one piece. Removal of the connective tissue enveloping the uterus and the coiled vagina exposed the UVJ, the site of the SSTs. The contiguous segments were then straightened and cut from the vagina through the uterus to expose the longitudinal mucosal folds of the vagina and UVJ [16]. To isolate UVJ folds (about 1.5–2 mm in both height and width) containing SSTs, an individual fold was grasped with fine forceps and then cut longitudinally through the lamina propria. Isolated folds about 10 mm long were then transferred to a Petri dish containing fixative and the SSTs were localized by stereomicroscopy (Nikon SMZ1500). Once localized, individual folds with SSTs were trimmed to about 2 to 3 mm in length, transferred to fresh fixative, and used for transmission electron microscopy (TEM). After samples for TEM were kept in fixative, additional unfixed UVJ folds containing SSTs were isolated as described previously, and a squash preparation was prepared and immediately examined by differential interference contrast microscopy (Zeiss Axioskop with the QICam digital camera using the NIS-Elements Software package).

Samples were fixed overnight at room temperature in either 2% glutaraldehyde in 0.1-M cacodylate buffer or 3.0% glutaraldehyde and 2.0% paraformaldehyde in 0.1-M cacodylate buffer. After fixation, the mucosal folds were washed in buffer and further trimmed to 3- to 4-mm pieces containing SSTs. All trimmed specimens were postfixed in 2% osmium tetroxide buffered in 0.1-M cacodylate. Samples were dehydrated in ethanol and embedded in Spurr Low-Viscosity Embedding Media (Polyscience). Thin sections