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Ability of sulfated glycoconjugates and disulfide-reductants to release bovine epididymal sperm bound to the oviductal epithelium in vitro

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Abstract

In Bos taurus, at ejaculation, epididymal sperm acquire a number of proteins secreted in the seminal plasma that increase their ability to interact with the female reproductive tract. Sperm-oviduct interaction comprises a transient sperm adhesion to the isthmus, the lower portion of the oviduct, followed by sperm release around ovulation. Oviductal fluid molecules, such as sulfated glycoconjugates and disulfide-reductants, are able to release bovine ejaculated sperm bound to the oviductal epithelium in vitro through the reduction of sperm surface protein disulfides to sulfhydryls. To understand whether the sperm molecules sensitive to releasing signals are already exposed on the surface of epididymal sperm, we studied the ability of cauda epididymal sperm to adhere to the oviductal epithelium and to be released by sulfated glycoconjugates and the disulfide-reductant penicillamine. Surface protein sulfhydryls in cauda epididymal sperm were analyzed in the initial suspension, in sperm bound to the in vitro–cultured oviductal epithelium, and in released sperm. Results showed that epididymal sperm are able to bind the oviductal epithelium in vitro, although at a lower extent than frozen-thawed ejaculated sperm; the interaction is mediated by oviductal cell microvilli that closely bind to the plasma membrane of the sperm head rostral region, as previously shown for ejaculated sperm. The sulfated glycoconjugates heparin, fucoidan, and dextran sulfate, as well as the disulfide-reductant penicillamine, are all powerful inducers of sperm release. The level of sulfhydryls in sperm surface proteins was (1) high in the initial sperm suspension; (2) low in bound sperm; (3) markedly increased in sperm released by heparin or by penicillamine. In conclusion, epididymal sperm are already able to bind the oviductal epithelium and to respond to the inducers of release through the reduction of sperm surface protein disulfides to sulfhydryls.

Keywords: Adhesion; Bovine; Epididymal sperm; Oviduct; Protein sulfhydryls; Release

1. Introduction

In mammals, spermatozoa transiently adhere to the epithelial cells lining the caudal isthmus, the lower region of the oviduct, and this interaction extends the fertile life of sperm in the female reproductive tract. The adhesion between sperm and oviductal cell plasma membranes is ensured by molecules exposed on the sperm rostral surface capable of binding to carbohydrates on the oviductal cell surface species-specifically [1–7].

In cattle, the ability to interact with the oviduct has been reported to develop at ejaculation; that is, when epididymal sperm adsorb on their surface a number of

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proteins secreted in the seminal plasma (BSPs) [8,9]. In particular, epididymal sperm have been reported to have a low oviduct-binding ability, whereas incubation with purified BSPs significantly increases binding to the oviduct in vitro [8,9].

Sulfated glycoconjugates [10] and disulfide-reductants [11] have been shown to powerfully induce the release of ejaculated sperm adhering to the in vitro–cultured oviductal epithelium. Both release inducers were shown to act on sperm [10,11] through the reduction of sperm surface protein disulfides (SS) to sulfhydryls (SH) [12] and to trigger capacitation-related changes [11,13]. It has been hypothesized that the action of releasing signals directly or indirectly modifies the sperm molecules involved in the adhesion, causing a loss of the sperm affinity for the oviductal epithelium [14]. To understand whether the sperm molecules sensitive to releasing signals are already exposed on the surface of epididymal sperm or are adsorbed from the seminal plasma at ejaculation, we studied the ability of cauda epididymal sperm to adhere to the oviductal epithelium and to be released by sulfated glycoconjugates and the disulfide-reductant penicillamine. Data showed that cauda epididymal sperm already expose adhesion molecules that bind the oviduct in vitro, and sperm releasing signals are able to disrupt such interaction through the reduction of sperm surface protein SS to SH.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA; fraction V), penicillamine, heparin (sodium salt, purified from porcine intestinal mucosa; H3393), fucoidan, dextran sulfate, Hoechst 33342, Protease Inhibitor Cocktail (P2714), and Medium 199 (M4530) were from Sigma Chemical Company (Milan, Italy); 3-(*N*-Maleimidylpropionyl)-biocytin (MPB), fetal calf serum (FCS), gentamicin, amphotericin B, HEPES, and sodium bicarbonate were from Invitrogen (Milan, Italy); glutaraldehyde, osmium tetroxide, and sodium cacodylate were from TAAB Laboratories (Rome, Italy). Reagents and buffers for SDS-PAGE, including the molecular-weight Precision Plus Protein Standards, were from Bio-Rad (Milan, Italy). ImmunoPure Standard ABC Staining Kit and BCA Protein Assay Kit were from Pierce (Milan, Italy). Nitrocellulose (0.45-μm pore size; PROTRAN) was from Whatman (Dassel, Germany). Reagents and water for preparation of salines and culture media were all cell culture tested.

2.2. Preparation of oviductal epithelial cells

Bos taurus oviducts were collected at the time of slaughter and transported to the laboratory in Dulbecco's phosphate-buffered saline (PBS) supplemented with 50 μg/mL gentamicin at 4 °C. Laminae of epithelial cells were recovered from oviducts of single animals by squeezing and were cultured at 39 °C, 5% CO_2 in air, 95% humidity, in M199 supplemented with 50 μg/mL gentamicin, 1 μg/mL amphotericin B, and 10% FCS. Bovine oviductal epithelial cells were used either as swimming-everted vesicles, referred to as explants, within 24 h of culture or as confluent monolayers. Monolayers of bovine oviductal epithelial cells were grown on gelatin-coated glass coverslips as previously described and used within 24 h after attainment of cell confluence [15]. Within each experiment, oviductal monolayers or explants from a single individual were washed three times in Tyrode's albumin lactate pyruvate medium (TALP) [16], modified as described in Ref. 17, and left in this medium until sperm addition (0.5 to 1 h).

2.3. Sperm preparation

Frozen bovine semen (0.5-mL straws; approximately 40×10^6 spermatozoa per straw; motility after thawing ≥70%), obtained from Semen Italy (San Giuliano Saliceta, Modena, Italy), was used in all experiments. Straws were thawed in a water bath at 38 °C for 30 sec, and 0.5 mL semen was either labeled with 10 μg/mL Hoechst 33342 for 5 min at 39 °C, 5% CO_2 in air, or directly washed in 10 mL and then in 5 mL TALP with or without BSA (BSA-free TALP) by centrifugation at $170 \times g$ for 10 min and 5 min, respectively. After resuspension in fresh medium, recovered sperm were assessed for concentration and percentage motility using a hemocytometer placed on a microscope stage heated to 39 °C by the computer-aided sperm-analysis system Sperm Class Analyzer (Microptic s.l., Barcelona, Spain).

Testes with epididymides and vasa deferentia attached were obtained from a local slaughterhouse and were brought to the laboratory within 1 h. Cauda spermatozoa were obtained through retrograde perfusion. Briefly, 5 mL TALP with or without BSA were infused into the lumen of the deferens with a 25-gauge needle and recovered by a cut in the cauda epididymis. Sperm suspensions with a motility ≥80% were processed as described above.

2.4. Sperm-oviduct binding assays

Ejaculated and cauda epididymal sperm suspensions recovered after centrifugation in TALP were added to