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Sperm-bound antisperm antibodies prevent capacitation of bovine spermatozoa

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ABSTRACT

It was hypothesized here that sperm-bound antisperm antibodies (ASAs) impair the ability of bovine spermatozoa to undergo capacitation, bind to the zona pellucida, and complete the acrosome reaction. The effect of ASA binding on these functions was evaluated in frozen/thawed spermatozoa from four bulls before and after induction of ASAs. Ejaculates were divided into ASA negative (<10% immunoglobulin [Ig]G- and IgA-bound spermatozoa) or ASA positive (≥20% IgG and/or IgA-bound spermatozoa). The percentage of capacitated (Merocyanine 540 positive) live spermatozoa in response to heparin was lower in ASA-positive than ASA-negative ejaculates ($P < 0.0001$). Treatment with heparin resulted in a higher percentage of capacitated spermatozoa compared with control treatments in ASA-negative but not ASA-positive ejaculates. The percentage of capacitated spermatozoa after heparin treatment was negatively correlated with IgA ($P = 0.02$, $R^2 = -0.48$) but not IgG binding. Sperm binding to the zona pellucida was lower in IgA-positive (six spermatozoa/oocyte; 3–10 spermatozoa/oocyte) than IgA-negative ejaculates (seven spermatozoa/oocyte; 4–13 spermatozoa/oocyte) ($P = 0.019$). Zona binding was negatively correlated with the percentage of IgA-bound spermatozoa ($P = 0.04$; $R^2 = -0.24$) but not IgG-bound spermatozoa. The percentage of acrosome-reacted spermatozoa was higher in calcium ionophore A23187-treated than control aliquots in both ASA-negative and ASA-positive ejaculates ($P < 0.0001$). However, the percentage of acrosome-reacted spermatozoa did not differ between ASA-positive and ASA-negative samples, and no correlation was identified with IgG or IgA binding. It was concluded that sperm-bound IgA affected the ability of bovine spermatozoa to undergo capacitation. ASAs inhibited the changes in plasma membrane fluidity associated with capacitation and binding of spermatozoa to the zona pellucida.

1. Introduction

The ability to undergo capacitation is a critical component of a spermatozoon's fertilizing ability. The functional changes occurring during capacitation confer spermatozoa the ability to bind to the zona pellucida, undergo the acrosome reaction, and fuse with the oocyte. Sperm capacitation involves removal of proteins coating the sperm plasma membrane, release of sterols from the plasma membrane, increase in membrane fluidity, migration of proteins on the surface of the plasma membrane, appearance of a protein-free areas, and thinning of the plasma membrane [1–3]. Membrane re-arrangement results in exposure of receptors and ligands that mediate sperm–zona binding and zona-induced acrosome reaction. The acrosome reaction is a calcium-dependent event that

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results in the release of acrosomal contents and exposure of molecules from the inner acrosomal membrane. The acrosome reaction may be induced by soluble factors like progesterone from the cumulus cells in the vicinity of the oocyte [4] or by direct interaction with zona pellucida proteins on binding to the zona pellucida [5]. The ability of acrosome-reacted spermatozoa to bind to the zona pellucida seems to vary with species [6]. Nevertheless, sperm capacitation is required for induction of the acrosome reaction and binding to the zona pellucida, regardless of the order in which these events occur [6,7]. Furthermore, only acrosome-reacted spermatozoa are able to complete penetration of the zona pellucida and fuse with the oocyte's plasmalemma [1]. Therefore, both capacitation and acrosome reaction are mandatory for fertilization. Updated reviews of the signaling and molecular mechanisms of sperm capacitation, acrosome reaction, and sperm–zona interaction were published elsewhere [1–3,6]. The ability of spermatozoa to capacitate, bind to the zona pellucida, and undergo the acrosome reaction *in vitro* are correlated with *in vivo* fertility in bulls [8–11].

In humans, antisperm antibodies (ASAs) prevented sperm capacitation, sperm–zona binding, and the acrosome reaction [12–16]. Human ASAs prevented the loss cholesterol from the sperm plasma membrane and the increase of membrane fluidity associated with capacitation [12,16], thereby affecting exposure and phosphorylation of surface proteins [15]. As a result, progesterone induction of the acrosome reaction was inhibited by ASAs [15]. To the authors' knowledge, the effect of sperm-bound ASAs on the ability of bovine spermatozoa to undergo capacitation, zona binding, and the acrosome reaction has not been critically studied. Presence of naturally occurring sperm-bound immunoglobulin (Ig)G and IgA was recently reported in beef bulls [17]. The high percentage of ASA-bound spermatozoa detected in ASA-positive bulls could significantly reduce the number of competent spermatozoa in an insemination dose, thereby reducing fertility. In a previous study, it was reported that sperm-bound ASAs decreased the ability of spermatozoa to bind to oviductal epithelial cells [18], which possibly impairs the ability of spermatozoa to form an oviductal reservoir [18]. To further understand the mechanisms of immune-mediated infertility in cattle, this study was designed to determine the effect of sperm-bound IgG and IgA on the ability of bovine spermatozoa to capacitate, bind to the zona pellucida and undergo the acrosome reaction. It was hypothesized that experimentally induced sperm-bound IgG and IgA impairs these functions.

2. Materials and methods

2.1. Experimental animals

This is the second in a series of studies aimed at understanding the effect of ASAs on sperm function in bulls. The animals and general protocols have been described in detail elsewhere [18]. Briefly, four *Bos taurus* bulls of Aberdeen Angus breed or cross between 12 and 24 months of age were included in the study. The bulls were housed and fed in accordance with animal care guidelines of Kansas State University's Institutional Animal Care and Use Committee. The study and the experimental methods were approved by the Kansas State University's Institutional Animal Care and Use Committee (protocol no. 3183).

Bulls underwent a complete breeding soundness examination. Scrotal circumference was determined with a scrotal measuring tape. The scrotal contents, prepuce, and penis were evaluated for the presence of pathology by visual inspection and palpation. The accessory sex glands were palpated per rectum to identify abnormalities. Semen was collected using electroejaculation. Sperm motility was evaluated using a computer-assisted semen analyzer (IVOS; Hamilton Thorn Research, Beverly, MA, USA). Hancock stain was used for morphologic assessment. One hundred spermatozoa were classified on the basis of their morphologic abnormalities using light microscopy under oil immersion at $\times$ 100. Bulls were classified as satisfactory breeders if they met all of following requirements [19]: no gross genital pathology, $\geq$30% individual sperm motility, $\geq$70% morphologically normal spermatozoa, and minimum scrotal circumference of 30 cm at $\leq$15 months of age, 31 cm at 15 to 18 months, 32 cm at 18 to 21 months, 33 cm at 21 to 24 months, and 34 cm at $>$24 months. Bulls not meeting at least one of these criteria were classified as nonsatisfactory breeders [19]. These minimum values are the guidelines recommended by the Society for Theriogenology to classify a bull as a satisfactory potential breeder.

Only bulls classified as satisfactory potential breeders, and with less than 10% IgG- and IgA-bound spermatozoa, were used in the study. Three ejaculates were first collected and cryopreserved every 48 hours from each bull. These ejaculates served as the ASA-negative control group [17]. Bulls were then immunized with autologous spermatozoa to induce formation of ASAs. A response was considered positive when the percentage of IgG- or IgA-bound spermatozoa was $\geq$20% [18,20]. Once a positive response was detected, three more ejaculates were collected and cryopreserved every 48 hours. These ejaculates served as the ASA-positive treatment group. The bulls were used as their own controls to minimize the influence of individual variation on the results. Although IgA binding was not compared here between fresh and frozen samples, a previous study showed that cryopreservation does not change the percentage of ASA-bound spermatozoa compared with fresh semen [21].

2.2. Immunization of bulls and ASA binding

Washed ejaculated spermatozoa, 1×10^9, were diluted to 1 mL in Dulbecco's phosphate-buffered saline (DPBS; Invitrogen, Grand Island, NY, USA). One milliliter of Freund's complete adjuvant (Sigma–Aldrich, St. Louis, MO, USA) was then added. Each bull was immunized with 2 mL of inoculum containing 1×10^9 autologous spermatozoa. The inoculum was administered intramuscularly in the neck in four different aliquots of 0.5 mL each. Booster immunizations were administered every 21 days until an acceptable response was detected ($\geq$20% IgG- or IgA-bound spermatozoa). Semen was processed in the same way as for primary immunizations but Freund's incomplete adjuvant (Sigma–Aldrich) was used instead of Freund's complete adjuvant [20]. Semen collections began 21 days after the last immunization.