Subfertility in Stallions Associated with Spermatozoal Acrosome Dysfunction

Dickson D. Varner, DVM, MS; Steven P. Brinsko, DVM, PhD; Terry L. Blanchard, DVM, MS; Charles C. Love, DVM, PhD; Margo L. Macpherson, DVM, MS; Rebecca S. Heck, BS; and Larry Johnson, MS, PhD

Routine breeding soundness examinations do not always indicate the primary etiology for reduced fertility seen in a certain subset of subfertile or infertile stallions. Despite adequate mare management and semen evaluations which demonstrate sufficient numbers of progressively motile, morphologically normal spermatozoa in ejaculates, these stallions are unable to establish acceptable pregnancy rates in the mares to which they are bred. Stallions with this history may possess spermatozoa with acrosomes which are incapable of reacting when exposed to a potent stimulus of this event. An acrosomal responsiveness assay has value as a diagnostic aid in stallions with unexplained subfertility. Authors’ addresses: Departments of Large Animal Medicine and Surgery (Varner, Brinsko, Blanchard), Veterinary Physiology and Pharmacology (Love), and Veterinary Anatomy and Public Health (Heck, Johnson), College of Veterinary Medicine, Texas A&M University, College Station, TX 77843; Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 (Macpherson). © 2001 AAEP.

1. Introduction

For most equine breeds, selection of breeding stallions is based primarily on pedigree, athletic performance, or conformation, with little consideration given to reproductive potential. Unfortunately, after these stallions are chosen for a breeding career, many are found to be subfertile. Some stallions pass a routine breeding soundness examination, yet are unable to impregnate mares, or do so very inefficiently. Among human patients with acceptable levels of spermatozoal numbers, motility, and morphology, 15–20% produce ejaculates in which the spermatozoa fail to acrosome react within the normal range.\textsuperscript{1,2}

Mammalian spermatozoa are not immediately capable of fertilizing an oocyte following ejaculation. These cells must first undergo some final maturational changes within the reproductive tract of the female.\textsuperscript{3} The acrosome plays a pivotal role in establishing the fertilizing potential of spermatozoa. This membrane-bound organelle covers the anterior portion of the spermatozoal nucleus. A portion of the acrosomal membrane is adjacent to the overlying plasma membrane. Modification, fusion, and vesiculation of these membranes is termed the acrosome reaction. This reaction releases hydrolytic enzymes from the acrosomal matrix which facilitate spermatozoal penetration of the investments of the oocyte. Because a properly function-
ing acrosome is considered essential to fertilization, this study was conducted to test the hypothesis that acrosome dysfunction occurs in a subset of subfertile stallions, similar to that described in men.

2. Methods

Five stallions (4 Thoroughbreds and 1 Quarter Horse) with a history of subfertility or infertility (pregnancy rate less than 20% per cycle) were included in the project. Semen quality and testicular size for each of these stallions were considered satisfactory, based on the findings of a routine breeding soundness examination. Five stallions with satisfactory semen quality and known good fertility were used as control animals. All stallions were in good body condition.

Gel-free semen was collected from the stallions using an artificial vagina. The semen was immediately mixed with warmed (37°C) milk-glucose-sucrose extender containing amikacin sulfate (1 mg/ml) and potassium penicillin G (1000 U/ml) at a ratio of 1 part semen to 2 parts extender. Extended semen was incubated at 37°C for two hours. Aliquots of extended semen from each stallion were exposed to a potent stimulant of the acrosome reaction, a divalent ionophore termed A23187 (Calbiochem, La Jolla, CA; final concentration of 10 μM). Aliquots of extended semen which contained no A23187 were used as negative control samples. Following the two-hour incubation period, samples were fixed in 2% (v/v) glutaraldehyde in 0.1 M cacodylate buffer for 10 minutes, and the sperm pellets were processed for transmission electron microscopy. Sagittal views of spermatozoal heads were evaluated for presence or absence of the acrosome reaction, based on vesiculation of the outer acrosomal and plasma membranes. Data were analyzed by a two-way analysis-of-variance procedure to evaluate the effects of stallion status (fertile or subfertile) and A23187 on the incidence of acrosome-reacted spermatozoa.

3. Results

A significant fertility status × ionophore interaction was detected (p = 0.0001) for incidence of spermatozoal acrosome reactions. In samples containing no A23187, the mean percentage of acrosome-reacted spermatozoa tended to differ (p = 0.1) between fertile stallions (10.4 ± 11.6) and subfertile stallions (0.8 ± 1.8). Upon exposure of extended semen to A23187, the mean percentage of acrosome-reacted spermatozoa was markedly different (p = 0.001) between fertile stallions (84.0 ± 4.2) and subfertile stallions (5.6 ± 7.7).

4. Discussion

The acrosome reaction in stallion spermatozoa has been characterized, but the relationship of acrosomal dysfunction to fertility in stallions is poorly understood. Meyers and coworkers reported that physiological acrosome reactions in stallions are mediated by a plasma membrane progesterone receptor and that response to progesterone differs between fertile and subfertile stallions. The present study indicates that some stallions with unexplained subfertility, based on a routine breeding soundness examination, may have a defect in acrosomal structure and function. The prevalence of this condition in breeding stallions is unknown, but may parallel similar findings described for the human population.

This study revealed that transmission electron microscopy may reveal a difference in acrosomal responsiveness of spermatozoa from the fertile versus subfertile stallions. However, the difference between the two groups was magnified considerably when the spermatozoa were exposed to the calcium ionophore A23187.

Verification of the defect in suspect stallions presently requires submission of samples to a reference laboratory. Although transmission electron microscopy is considered the “gold standard” for documentation of the acrosome reaction, commercially available fluorescent probes are becoming more widely used for assessment of this event. Our laboratory has found that use of fluorescence-based techniques for detection of acrosome reactions in these subfertile stallions can lead to false positive reactions. This problem may be due to the presence of fragmented membranes without evidence of vesiculation following exposure to A23187.

In summary, use of more sophisticated techniques may be required to identify stallions with acrosomal dysfunction than those employed in routine breeding soundness examinations. The underlying cause of this dysfunction may be associated with alterations in structural membrane composition or cellular messenger systems. Studies are currently being directed at determining the underlying cause of this defect, as well as development of therapeutic strategies.

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References