

EVALUATION AND DIAGNOSIS OF ACROSOME FUNCTION / DYSFUNCTION IN THE STALLION

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An efficient and accurate assay to evaluate sperm acrosomal status and responsiveness would have an application for breeding stallions. The goal of this study was to determine if flow cytometric analysis of stallion sperm stained with FITC-PSA after exposure to the calcium ionophore, A23187, could provide a clinical diagnosis of acrosomal dysfunction. **Objectives:** 1) Assess acrosome response rate (ARR) (intact to reacted) following exposure to A23187, between stallions with normal fertility and unexplained subfertility. 2) Determine differences in the ARR between fresh and cool-stored stallion sperm. **Materials and Methods:** Obj. 1) Ejaculates from fertile (n=16) and subfertile (n=4) stallions were extended to 25×10^6 sperm/mL in a skim milk-based extender. Obj. 2) Two ejaculates from 3 fertile stallions were evaluated immediately after collection and after 24 hours of cooled storage in an EquitainerTM. Sperm Preparation: Aliquots (1mL) treated with no ionophore (control) or 10 μ M A23187 were incubated at 37°C in an atmosphere of 95% air and 5% CO₂ for 0, 1, 2, and 3h. Following incubation, samples were fixed with 2% paraformaldehyde for 10 minutes at room temperature; then permeabilized with 95% ethanol at -20°C for 10 minutes. Samples were then resuspended in 20% fetal bovine serum in DPBS, labeled with FITC-PSA for 10 minutes, washed twice with DPBS, then analyzed by flow cytometry (FACScan, Becton-Dickinson). **Results:** For fertile stallions, the percentage of acrosome intact (%AI) sperm was higher ($p < 0.01$) in control samples than A23187 samples, at incubation times 1, 2, and 3h (Control-59, 56, and 51% vs. A23187- 46, 29, and 23%, respectively), but not at Time 0. For subfertile stallions, %AI was not affected by ionophore treatment ($P > 0.05$) or incubation period ($P > 0.05$). Regardless of incubation time or treatment, %AI averaged ~6% higher ($P < 0.001$) in fresh semen than in cool-stored semen. **Discussion:** The results indicate a difference in ARR of sperm between fertile and subfertile stallions, even though sperm motility and morphologic parameters were similar between groups. Subfertile stallion sperm exhibited little response (<11% acrosome reacted-AR) after 3h of A23187 exposure, while the fertile stallions demonstrated a substantial response (36% AR) as soon as 1h after ionophore contact. In addition, sperm stored for 24h in an EquitainerTM exhibited a small (~6%) but significant decrease in the %AI. Storage conditions may, therefore affect acrosomal integrity and contribute to reduced fertility when cooled-semen is used. With this assay, flow cytometry provided efficient and objective results for depicting acrosomal response of stallion spermatozoa.

Keywords: stallion, sperm, acrosome reaction, A23187, flow cytometry, examination