Competitive session

HEAT SHOCKED SPERMATOZOA INDUCE FUNCTIONAL AND STRUCTURAL DAMAGE TO PMNS

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Phagocytosis by polymorphonuclear neutrophils (PMNs) is an important mechanism of sperm elimination from the mare's reproductive tract. Whereas viable spermatozoa need to be protected from PMN-phagocytosis, dead and damaged spermatozoa should be eliminated from the uterus soon after ejaculation or AI. We have recently identified a specific seminal plasma protein (SPPα) that selectively protects viable spermatozoa from PMN-binding and phagocytosis. We also found that heat shocked spermatozoa were readily bound and phagocytosed by PMNs in the presence of seminal plasma. However, heat shocked spermatozoa appeared resistant to PMN-binding and phagocytosis in the absence of seminal plasma. We hypothesized that spermatozoa release substances that cause functional and/or structural changes to PMNs in the absence of seminal plasma. Three stallions were collected three times each, and spermatozoa were washed and reconstituted in a commercial semen extender (EquiPro, Minitube of America, Verona, WI, USA). Samples were stored at room temperature for 1 h (viable) or heat shocked at 45 °C (non-viable) for 1 h. This heat shock protocol has been validated to induce changes to equine spermatozoa consistent with cell death. Aliquots were divided into six equal fractions, washed three times each, centrifuged to separate spermatozoa from supernatant, and reconstituted into one of the following: (1) viable sperm + supernatant from non-viable sperm; (2) viable sperm + supernatant from viable sperm; (3) viable sperm + extender; (4) non-viable sperm + supernatant from non-viable sperm; (5) non-viable sperm + supernatant from viable sperm; (6) non-viable sperm + extender. PMN's were isolated from peripheral blood from a healthy mare, and samples were subjected to in vitro assays for PMN binding, stained and examined for PMN-sperm binding and viability of PMN's. PMN-sperm binding was expressed as the percentage of PMN's (mean ± S.E.M.) that bound at least one sperm cell. PMN viability was expressed as the percentage of PMN's (mean ± S.E.M.) that were ruptured or degranulated. Differences between treatment groups were determined by randomized complete block ANOVA and Bonferroni's comparison tests. Viable sperm + extender (66.2 ± 2.1), viable sperm + supernatant from viable sperm (64.9 ± 2.1) and non-viable sperm + supernatant from viable sperm (61.0 ± 2.1), had superior PMN-binding compared to viable sperm + supernatant from non-viable sperm (43.5 ± 2.1), non-viable sperm + supernatant from non-viable sperm (39.1 ± 2.1) and non-viable sperm + extender (34.3 ± 2.1; $P < 0.05$). PMN-damage was less common in viable sperm + supernatant from viable sperm (17.2 ± 2.9), viable sperm + extender (19.6 ± 2.9) and non-viable sperm + supernatant from viable sperm (21.4 ± 2.9) compared to non-viable sperm + extender (64.8 ± 2.9), non-viable sperm + supernatant from non-viable sperm (70.6 ± 2.9) and live sperm + supernatant from non-viable sperm (80.6 ± 2.9; $P < 0.05$). In conclusion, we inferred that dead spermatozoa secreted substances that induced damage to PMNs. Previous studies have shown that seminal plasma protects PMNs from this effect, maintaining their capacity to phagocytose dead spermatozoa in the uterus.

Keywords: PMN's; Spermatozoa; Uterus; Inflammation; Immunology

DOI: 10.1016/j.theriogenology.2007.05.031

SURVIVABILITY OF *Candida albicans* AND *Aspergillus fumigatus* IN COOL EXTENDED AND CRYOPRESERVED EQUINE AND CANINE SEMEN

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Fungal infection is an uncommon but important disease in reproduction that can lead to decreased fertility and abortion. This study was performed to determine if semen experimentally infected with two common fungal organisms can survive the processes of 48 h cool-extension (CE) and cryopreservation (CP). *Candida albicans* yeast and *Aspergillus fumigatus* conidia were added to raw semen (RI, raw inoculated) at a concentration of 1×10^6 colony forming units per milliliter (CFU/mL). *A. fumigatus* hyphae were added to raw semen at a 10-fold dilution of the inoculum. Two