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

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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

billion stallion sperm cells and all of the stud dog sperm cells were diluted to a total volume of 40 mL with Easy Mixin CST (Animal Reproduction Systems, Chino, CA, USA). Samples (1 mL) of the extended semen were cooled to a temperature of 4 °C and stored for 48 h. After incubation, the semen was evaluated for motility, and CFU counts were performed. The remainder of the extended semen was centrifuged for 15 min at 5000 rpm and the supernatant removed. The sperm pellet was resuspended in 3.5 mL of Easy Freezin LE (Animal Reproduction Systems) for the stallion and 2.0 mL for the stud dog, drawn into straws and cryopreserved. After cryopreservation, the semen was thawed in a 32–37 °C water bath for 10–15 s. The semen was evaluated for motility, and CFU counts were performed. All semen samples had motility that was within normal ranges for the procedures performed. The results are shown in the chart below. Expected CFU/mL numbers were calculated from the inoculums and were based on the expectation that centrifugation would concentrate fungal organisms. In this study, both the hyphae and conidia of *A. fumigatus* and the yeast of *C. albicans* survived cool-extension and cryopreservation. Centrifugation during the cryopreservation process concentrated the fungal organisms as well as the sperm cells.

Sample	*Candida yeast*		*Aspergillus conidia*		*Aspergillus hyphae*	
	Expected CFU (mL)	Actual CFU (mL)	Expected CFU (mL)	Actual CFU (mL)	Expected CFU (mL)	Actual CFU (mL)
Inoculum	1×10^7	0.6×10^7	0.7×10^7	1.5×10^7	Unknown	1.0×10^3
Horse RI	1×10^6	0.9×10^6	1×10^6	2.5×10^6	1×10^2	1.8×10^2
Dog RI	1×10^6	0.8×10^6	1×10^6	2.8×10^6	1×10^2	3.0×10^2
Horse CE	1.8×10^5	2.4×10^5	1×10^5	3.2×10^4	1.8×10^2	0.5×10^2
Dog CE	1.4×10^5	1.1×10^5	1×10^5	2.8×10^4	0.1×10^2	0.2×10^2
Horse CP	4.0×10^6	3.0×10^6	1.1×10^6	2.7×10^5	2.0×10^2	1.8×10^2
Dog CP	3.0×10^6	2.0×10^6	2.0×10^6	3.6×10^5	2.0×10^2	2.5×10^2

Colony forming unit count results. RI = raw inoculated, CE = cool-extended, CP = cryopreserved.

Keywords: Semen cryopreservation; Cool-extension; Equine; Canine; Fungus

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EFFECTS OF AGE AND FSH ON COLLECTION OF EQUINE OOCYTES AND DEVELOPMENTAL COMPETENCY AFTER INTRACYTOPLASMIC SPERM INJECTION

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Reduced fertility in aged mares is associated with retarded early embryonic development, potentially associated with delayed fertilization or reduced developmental competence. Intracytoplasmic sperm injections (ICSI) could reduce fertilization delays, and exogenous FSH could maximize oocyte and embryo production. Objectives were to compare oocytes collected per cycle and embryonic development after ICSI for young (4–9 years) and old (>20 years) mares, with or without FSH. Treatment with FSH (equine FSH, 6.25 mg, im, bid; Bioniche Animal Health Inc., Athens, GA, USA) began when at least one follicle >20 mm was detected during diestrus. Treated and non-treated cycles were alternated. Oocytes were collected from follicles >30 mm 20–24 h after LH (EquiPure LH™, 0.75 mg, iv; AspenBio Pharma Inc., Castle Rock, CO, USA) using transvaginal aspirations. Oocytes were matured further in TCM199 at 38.5 °C in 6% CO_2 in air. Sperm were injected 40 ± 1 h after LH, using frozen sperm from a single ejaculate. Resulting zygotes were incubated in DMEM/F12 at 38.5 °C in 5% CO_2, 5% O_2 and 90% N_2. Blastocysts considered viable (formation before 9 days and adequate quality) were transferred non-surgically into recipients 3–7 days after ovulation. Follicle and oocyte data were analyzed using two-way ANOVA for main effects of age and FSH (and their interaction); Tukey's hsd was used to separate means. Developmental data were compared