

A SELECTIVE ROLE OF SEMINAL PLASMA IN PMN-BINDING AND PHAGOCYTOSIS OF LIVE AND NON-VIABLE EQUINE SPERMATOZOA

A.L. Desvouses^{1,2,3}, C. Dow^{1,2,3}, W.C. Buhi^{1,2,3}, J. Hayna^{1,2,3}, M.H.T. Troedsson^{1,2,3}

¹Department of Animal Science, University of Florida, Gainesville, FL 32510, USA,

²Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL 32510, USA, ³Department of Obstetrics and Gynecology³, University of Florida, Gainesville, FL 32510, USA

Breeding-induced endometritis is characterized by an influx of polymorphonuclear neutrophils (PMNs) into the uterine lumen. Excess spermatozoa bind to PMNs and become phagocytosed. Seminal plasma suppressed PMN-binding and phagocytosis of spermatozoa, but it is not known if these effects are selective for live sperm. We hypothesized that seminal plasma protects viable, but not dead sperm from PMN-binding and phagocytosis. Three stallions were collected three times each, and the spermatozoa were washed and reconstituted in a commercial semen extender (EquiPro, Minitube of America, Verona, WI). Aliquots were stored at room temperature for 1 h (live), or snap frozen at -80°C for 1 h and thawed at room temperature (non-viable sperm). The aliquots were divided into four equal fractions, and one of the following treatments were added to each fraction: (1) seminal plasma, (2) semen extender, (3) ammonium sulfate precipitated seminal plasma proteins with protease inhibitor (SPP⁺), or (4) ammonium sulfate precipitated seminal plasma proteins without protease inhibitor (SPP⁻). PMNs were isolated from peripheral blood from a healthy mare, and samples were subjected to in vitro assays for PMN binding and phagocytosis. Results from PMN binding assays were expressed as the percentage of PMNs (mean $\pm$ S.E.M.) with at least one spermatozoon bound; phagocytosis assays were expressed as the percentage of PMNs that had phagocytosed at least one spermatozoon. Seminal plasma (40 ± 3.1) and SPP⁺ (42 ± 3.1) suppressed PMN-binding of live sperm compared to semen extender alone (73 ± 3.1 ; $p < 0.0001$). Similarly, seminal plasma (35 ± 3.0) and SPP⁺ (35 ± 3.0) suppressed phagocytosis of live sperm compared to semen extender alone (73 ± 3.0 ; $p < 0.0001$). This effect was also observed, but to a lesser degree in SPP⁻ treated samples ($p < 0.05$). Binding to PMNs was lower for non-viable spermatozoa (38 ± 3.1) compared to live spermatozoa (73 ± 3.1) in the absence of seminal plasma. Phagocytosis of non-viable (24 ± 3.0) spermatozoa was also lower than live sperm (73 ± 3.0) in the absence of seminal plasma ($p < 0.0001$). The addition of seminal plasma increased PMN-binding and phagocytosis (71 ± 3.1 and 58 ± 3.0 , respectively) of non-viable spermatozoa ($p < 0.05$). The addition of SPP⁻ to samples also increased PMN-binding and phagocytosis of non-viable spermatozoa (50 ± 3.1 and 46 ± 3.0 , respectively; $p < 0.05$), but to a lesser degree than for seminal plasma treated samples ($p < 0.05$). The addition of protease inhibitors removed this effect from SPP. This is the first report that seminal plasma selectively protects live spermatozoa from PMN-binding and phagocytosis and promotes PMN-binding and phagocytosis of non-viable spermatozoa; perhaps more than one protein is involved.

Keywords: PMNs; Spermatozoa; Seminal plasma; Uterus; Inflammation