Effects of lactoferrin on stallion sperm survival and function in vitro
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Lactoferrin is an iron-binding glycoprotein found in many biological secretions including blood, tears, milk and saliva. Our laboratory demonstrated that lactoferrin reduces post-breeding uterine inflammation in mares by decreasing expression of pro-inflammatory cytokines. Therefore, lactoferrin could potentially be incorporated into commercially available semen extenders to modulate the uterine inflammation post-breeding. Our hypothesis was that addition of lactoferrin to semen extender would not be detrimental to sperm survival and function. Our objective was to determine the effects of lactoferrin on stallion sperm motility and viability during 48 h of storage at 5°C. Four ejaculates from each of four stallions, on a regular collection schedule, were collected, diluted 1:1 (v:v) with skim milk-based extender without antibiotics (EZ-Mixin® BF, Animal Reproduction Systems, Chino, CA), and centrifuged at 400 x g for 12 min to remove seminal plasma. Sperm were then resuspended in the same extender containing 0 (control), 10, 20, and 30 mg/mL of lactoferrin and stored at 5°C for 48 h. Total (TM) and progressive (PM) motility were evaluated subjectively and by Computer-Assisted Semen Analysis (CASA; Ceros®, Hamilton Thorn Biosciences, Beverly, MA) at 0, 24 and 48 h of storage. Plasma membrane integrity (i.e. viability) and growth of microorganisms were evaluated at 24 h of incubation. Sperm viability was determined by exclusion of propidium iodide stain using an automated cell counter (NucleoCounter®, ChemoMetec A/S, Denmark). Individual microorganism growth was scored from 0 to 5 (0 = no growth; 5 = heavy growth) according to the number of colonies observed after 48 h of aerobic incubation. When multiple organisms were isolated from a sample, the sum of the growth scores for each microorganism was used as the sample’s score. Data were analyzed by ANOVA for repeated measures and significance was set at P<0.05. Data expressed as mean ± SD. TM and PM were similar between control and all lactoferrin groups at times 0 and 24 h of storage. However, a significant decrease in TM and PM was observed at 48 h of storage when lactoferrin was present at 20 mg/ml (TM = 47±17%; PM = 24±14%) and 30 mg/ml (TM = 44±17%; PM =22±12%), compared to control (TM = 61±20%; PM = 38±18%). The percentage of sperm with intact plasma membrane after 24 h of storage was similar between all groups. Growth of microorganisms was significantly reduced when lactoferrin was present at 20 mg/ml (5±3) and 30 mg/ml (4±3) compared to control (12±5). In conclusion, lactoferrin did not affect sperm motility and viability during the first 24 h of storage and was effective in reducing the growth of microorganisms. Potentially, addition of lactoferrin to semen extenders at concentrations <20 mg/ml may allow modulation of uterine inflammation post-breeding, particularly in mares susceptible to mating-induced endometritis. Future studies in our laboratory will focus on the effects of lactoferrin on fertility of mares susceptible to endometritis.

Keywords: Lactoferrin, stallion, semen, spermatozoa

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