Equine lactoferrin increases *in vitro* binding of polymorphonuclear neutrophils to spermatozoa

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Following insemination, spermatozoa induce a migration of polymorphonuclear neutrophils (PMNs) to the uterine lumen, which play a major role in resolution of the post-mating inflammatory response. Equine lactoferrin is an iron binding glycoprotein (~ 80 kDa) that is present in large amounts in the seminal plasma of the stallion and in the endometrium of the mare during estrus. Lactoferrin plays an important role in modulating immune and inflammatory responses, particularly as a mediator of cell-to-cell interactions. Due to its role in host defense mechanisms and its high concentration in seminal plasma, we hypothesized that lactoferrin modulates PMN-sperm interactions in the uterus. The objectives of this study were: 1) to characterize the role of lactoferrin in the post-mating inflammatory response of the uterus and 2) to characterize the expression of lactoferrin mRNA and protein in the testis and epididymis of stallions.

**Methods**

An *in vitro* binding assay between PMNs and sperm cells was performed in triplicate and was used to evaluate the immune response. PMNs were isolated from healthy mares and incubated for 30 min with dead sperm stained with propidium iodide (PI) in the presence of medium (Hank’s balanced salt solution), seminal plasma (pooled from four stallions) or purified lactoferrin. Samples were analyzed using flow cytometry, gating on PMNs by forward and side scatter (FSC-SSC) and on sperm based on the PI fluorescence signal (FL-2). Lactoferrin mRNA expression was characterized by qPCR and protein expression was characterized by immunohistochemistry and western blots from the testes and epididymides (caput, corpus and cauda) of four stallions (≥ 2-years-old). Data were tested for normality and repeated measures ANOVA was used for comparison of treatment effects.

**Results**

Both lactoferrin (62.5 ± 3.3%\(^a\)) and seminal plasma (76.5 ± 0.3%\(^a\)) treatments significantly (P<0.05) enhanced binding of PMNs to dead sperm after 30 min of incubation as compared to control medium treatment (42.9 ± 1.6%\(^b\)). Lactoferrin mRNA was highly expressed in the corpus epididymis, followed by the cauda and caput epididymis, but was absent from the testis. These results were confirmed by immunohistochemistry with prominent immunoeexpression in the luminal epithelium of the corpus epididymis, followed by cauda, caput epididymis, with no expression in the testis. Lactoferrin protein was present in tissue extracts from the cauda and corpus epididymis, but not from the caput epididymis or testis.

**Conclusions**

Lactoferrin is a seminal plasma protein that is primarily expressed and produced in the corpus and cauda of the epididymis of stallions. Seminal plasma enhances binding of PMNs to dead sperm cells *in vitro* and purified lactoferrin alone was able to reproduce this effect. These findings suggest that one of the roles of lactoferrin in the reproductive tract of the mare is to enhance cell-to-cell interactions between PMNs and nonviable sperm, which *in vivo*, might lead to the resolution of post-breeding endometritis.

**Keywords:** Lactoferrin, post-breeding endometritis, neutrophils, spermatozoa, equine