The objective of this study was to determine the levels of Toll-like receptor 4 (TLR-4) tissue expression in the equine endometrium, and the relative levels of TLR-4 tissue expression in mares resistant and susceptible to endometritis. All mares were evaluated for susceptibility to endometritis using the standard *Streptococcus equi* subspecies *zooepidemicus* (*Strep*) challenge model. Briefly, clean mares were infected with 10^5 colony forming units of *Strep* on the day of ovulation then evaluated for uterine inflammation (uterine culture and cytology) at 72 hours. Mares with no infection/inflammation at 72 hours were considered resistant (R, n=5); those with significant uterine bacterial growth and/or more than 5% neutrophils on cytology were considered susceptible (S, n=4). At the 72-hour post-challenge evaluation a uterine biopsy was also taken. After treatment of all infected mares (uterine lavage and antibiotics as needed), each mare received at least one untreated cycle and then in a subsequent uninfected estrous cycle an endometrial biopsy was performed within 24 hours of ovulation. Endometrial tissues were frozen in liquid nitrogen and stored until further processing. Endometrial tissues were thawed at room temperature, lysed, and mRNA was extracted (QIAshredder, RNasey Mini kit, Qiagen, Inc., Mississauga, ON, Canada). The mRNA was processed into cDNA and real-time polymerase chain reaction (RT-PCR) was performed for each sample in duplicate (QuantiTect Reverse Transcription Kit, Qiagen; Brilliant SYBR Green QPCR Master Mix, VWR International, LLC, Edmonton, AB, Canada). The primers for TLR-4 and glyceraldehyde-3-phosphate dehydrogenase (normalizing gene, GAPDH) had been previously validated (custom primers, Invitrogen Canada, Inc., Burlington ON, Canada)\(^1\). The mRNA concentrations were measured by evaluating the TLR-4 PCR cycle threshold (Ct) then standardized to the GAPDH Ct, resulting in the adjusted Ct (\(\Delta\)Ct). The difference between the post-*Strep* level and the estrous level of mRNA was calculated (\(\Delta\Delta\)Ct). The fold-change in the mRNA level was calculated using the equation \(2^{-\Delta\Delta\text{Ct}}\). The paired t-test was used for analysis of differences of the \(\Delta\text{Ct}\) at estrus and post-*Strep* in the mares by category and as a single group. A two-sample t-test was used to compare the \(\Delta\Delta\text{Ct}\) and the fold-change differences between R and S mares (Stata 10.0, StataCorp, College Station, TX, USA). No differences were found between R and S mares in terms of the \(\Delta\text{Ct}\), \(\Delta\Delta\text{Ct}\), or \(2^{-\Delta\Delta\text{Ct}}\). Values of the \(\Delta\text{Ct}\) of the combined groups of mares post-*Strep* (2.4 ± 0.5, mean ± SD) tended to be increased relative to values at estrus (1.5 ± 1.8) (\(P < 0.09\)). These findings suggest a different level of TLR-4 tissue expression in the endometrium of mares in estrus compared to post *Strep* infection. No differences in TLR-4 tissue expression were found between R and S mares that could account for their differing responses to intrauterine infection with *Strep*. The lack of significant differences may be due to the small sample size in this study. This is the first report of TLR-4 tissue expression in the equine endometrium demonstrated through real-time PCR.

**Keywords:** Toll-like receptor-4, endometritis, real-time PCR, RNA, mare
Reference