Nitric oxide’s dose-dependent inhibition of uterine contractility: a potential mechanism underlying persistent breeding-induced endometritis in the mare

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Persistent breeding-induced endometritis (PBIE) is a major cause of equine infertility. Mares susceptible to PBIE have increased uterine nitric oxide (NO) concentrations and decreased uterine contractility. Nitric oxide may have a role in the development of PBIE in mares through an inhibitory effect on uterine contractility. The objectives of this study were to test the effect of NO on uterine contractility in-vitro and to evaluate whether this effect varied between the longitudinal and circular muscle layers of the uterus. It was hypothesized that NO would have a dose-dependent inhibitory effect on spontaneous uterine contractility irrespective of the muscle layer. Reproductive tracts were collected postmortem from eight non-pregnant mares (age 4 to 19 years; body weight 405 to 530 kg). Transrectal examination of the reproductive tract was performed before euthanasia to evaluate stage of the estrous cycle and presence of any apparent abnormality. After euthanasia, one uterine tissue sample was collected for histological evaluation and four full-thickness uterine tissue strips (10–12 mm × 2 mm), two parallel to each muscle layer, were excised for in-vitro contractility evaluation. Strips were suspended in tissue chambers containing Krebs–Henseleit solution, with continuous aeration (95% O2–5% CO2; pH 7.4) at 37°C. After equilibration, spontaneous contractility was recorded (pre-treatment) and strips excised in each direction were randomly allocated to each of two groups: 1) SNAP (S-nitroso-N-acetylpenicillamine, an NO donor); or 2) NAP (N-acetyl-D-penicillamine, vehicle and time-matched control). These were treated at 15 min intervals with increasing concentrations (10^-7 M to 10^-3 M) of SNAP and NAP, respectively. Contractility data were recorded throughout the experiment. Data were log transformed and analyzed for main effects and appropriate interactions using a linear mixed-effects model (PROC MIXED, SAS) with repeated measures. Significance was set at P<0.05 with a Bonferroni correction for multiple comparisons. An interaction effect of group-by-concentration was observed (P<0.0001). The mean contractility after treatment with 10^-3 M and 10^-3 M SNAP were significantly lower than the pre-treatment contractility and the mean contractility after treatment with lower SNAP concentrations. In contrast, contractility did not change significantly in the NAP treated controls. The main effect of muscle layer and its interactions with group, concentration, and stage of estrous cycle were not significant. Other secondary findings included significant main effects of stage of the estrous cycle (increased contractility in estrus compared to diestrus), uterine histology grade (decreased contractility in grade IIB compared to grade I) and age (decreased contractility in mares aged > 8 years compared to mares aged ≤ 8 years). In conclusion, results of this study indicate that NO has a dose-dependent inhibitory effect on spontaneous uterine contractility irrespective of the muscle layer in the mare. The presence of increased NO concentrations in the uteri of mares susceptible to PBIE coupled with our findings that NO decreases uterine contractility constitutes a potential mechanism underlying development of PBIE in the mare.

Keywords: Equine, nitric oxide, uterine contractility, breeding-induced endometritis