Dose-dependent inhibition of uterine contractility by nitric oxide: A potential mechanism underlying persistent breeding-induced endometritis in the mare

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ABSTRACT

Nitric oxide (NO) may have a role in persistent breeding-induced endometritis in mares through an inhibitory effect on uterine contractility. The objectives of this study were to test the effect of NO on spontaneous uterine contractility in-vitro and to evaluate whether this effect varied between the longitudinal and circular muscle layers of the uterus. Reproductive tracts were collected from eight euthanized non-pregnant mares (age 4 – 19 years; body weight 405 – 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 was recorded throughout the experiment. An interaction effect of group-by-concentration was observed (P < 0.0001). The mean contractility after treatment with 10−4 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 effect of treatment on uterine contractility was not influenced by age or weight of the mare, stage of estrous cycle, uterine histology grade, or muscle layer. Secondary findings included significant main effects of stage of 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.

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1. Introduction

A mild transient endometritis which occurs after breeding in mares is a normal, physiological response and does not warrant any treatment [1]. In contrast, persistent breeding-induced endometritis (PBIE), where inflammation and intra-uterine fluid retention persist, has a significant negative impact on fertility. Considered as
a major reproductive problem in the mare [2], PBIE has been re-
ported in about 15% of 746 estrous cycles in a Thoroughbred broodmare population [3] and in as many as 43% of 552 estrous cycles in a mixed population of mares [4]. In the latter study, mares with PBIE had a lower pregnancy rate compared to normal mares (49% versus 62%). It has been suggested that the reduced pregnancy rate in mares with PBIE could result either from a direct negative effect on embryonic survival or indirectly from premature luteol-
ysis due to increased prostaglandin F2alpha (PGF2α) effect on embryonic survival or indirectly from premature luteol-
ysis. The only documented evidence that NO may have an inhib-
itory effect on spontaneous uterine contractility in the mare is the reported in ability of myometrial tissue in vitro to respond to electrical stimulus in the presence of NO [22]. To our knowledge, the effect of different NO concentrations on spontaneous uterine contractility in the mare has not been investigated.

The objectives of this study were to test the effect of NO on spontaneous 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 contrac-
tility irrespective of the muscle layer.

2. Materials and methods

All animal procedures in this study were conducted in accor-
dance with the guidelines of the University of Guelph Animal Care Committee and conformed to the recommendations of the Cana-
dian Council on Animal Care. The method of collection and pro-
cessing of uterine tissue samples and the basic protocol for measuring uterine contractility in this study were similar to those used previously by Hirshbrunner et al. [23] for investigating sponta-
neous uterine contractility in the mare.

2.1. Collection and transportation of uterine tissue

Reproductive tracts were collected from clinically healthy non-
pregnant light breed mares (N = 8) within 30 min of euthanasia using a standard protocol involving an intravenous overdose of pentobarbital sodium (Euthansol, Merck Animal Health Intervet Canada Corp, Kirkland, QC, Canada) at the Ontario Veterinary Col-
lege, University of Guelph. The mares ranged in age from 4 to 19 years and in body weight from 405 to 530 kg. Transrectal palpation and ultrasonography of the reproductive tract were performed prior to euthanasia to evaluate stage of the estrous cycle and presence of any apparent abnormality. A mare was considered to be in estrus if she had a relaxed cervix, uterine edema, at least one follicle with diameter greater than or equal to 35 mm and no corpus luteum (CL) and in diestrus if she had a firm cervix, tonic uterus, no uterine edema and a CL. After euthanasia, the reproductive tract was visually examined for any gross abnormality and a tissue sample was collected from the base of the right uterine horn for histological evaluation. An 8–9 cm long, full thickness circumfer-
ental segment close to the base of the right uterine horn was excised and transported within 15 min to the muscle contractility laboratory in a flask containing Krebs-Henseleit solution (KHS) consisting of (in mM): NaCl, 118; KCl, 4.75; CaCl2, 2.54; MgSO4, 1.18; NaHCO3, 24.8; KH2PO4, 1.18; Glucose, 10. All chemicals used in the preparation of KHS were purchased from Fisher Scientific, Waltham, MA, USA. The solution was kept at room temperature and pre-aerated with 95% O2–5% CO2 mixture to reach a pH of 7.3–7.4.

2.2. Preparation of uterine tissue strips and experimental protocol

The circumferential uterine segment was incised along its lon-
gitudinal axis and pinned flat in a dissecting dish containing Krebs-
Henseleit solution continuously aerated with 95% O2–5% CO2. Full thickness uterine tissue strips of about 10–12 mm length and 2 mm width were dissected, using a custom designed scalpel with two parallel blades. Two strips were excised parallel to the direction of longitudinal muscle fibers and two strips were excised parallel to the circular muscle fibers. Each strip was tied at the ends with 5.0 gauge suture silk and suspended in an individual organ bath containing 10 mL of warm (37°C) Krebs-Henseleit solution continu-
ously aerated with 95% O2–5% CO2. The strips were attached to a fixed point at one end and an isotonic force transducer (model FT03, Grass Medical Instruments, Quincy, MA, USA) at the other.
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