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Equine endometrial cytology and bacteriology: Effectiveness for predicting live foaling rates

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ABSTRACT

Endometritis is an important cause of sub-fertility in mares. The critical indicator of reproductive success and financial return for commercial studs is live foaling rate. Endometrial bacteriology and/or cytology are used to diagnose endometritis and thus identify mares at risk of early embryonic death. However, mares with endometritis may conceive but then abort in late gestation. The aims of this study were to establish, as part of a standard breeding examination (1) whether a threshold percentage of uterine polymorphonuclear neutrophils (PMNs) exists above which a significant reduction in live foaling rate is evident; (2) the relationship of a positive bacteriology result to live foaling rate, and (3) the relationship of a combination of positive cytology and bacteriology result to live foaling rate.

Guarded endometrial swabs (n = 2660) were collected from 1621 Thoroughbred mares on 17 commercial stud farms by five veterinarians during a single breeding season. All mares were included regardless of age, history or parity. Cytological and bacteriological analyses were performed on each swab and subsequent live foaling rates recoded. Data were analysed by comparing 0%, ⩾1%, ⩾2%, ⩾5% or ⩾25% PMNs of total cells counted, or categories of bacterial growth to live foaling rates, using Pearson's chi-squared test. A threshold value of ⩾1% PMNs, culture of a single bacterial isolate and a combination of both these parameters were associated with significantly reduced live foaling rates. Positive cytology alone, positive bacterial culture alone, or combined positive cytology and bacteriology were equally indicative of the likelihood of a mare producing a live foal.

Introduction

In the United Kingdom in 2007 the aggregate value of public Thoroughbred sales was £302 million[1] (British Horseracing Authority, 2009) making the equine breeding industry a significant contributor to national and international economics. However, mares, particularly Thoroughbreds, exhibit poor reproductive efficiency (Sullivan et al., 1975; Morris and Allen, 2002). According to Thoroughbred Studbook Weatherbys, in 2011, of 7390 Thoroughbred mares bred in the UK, only 62.5% produced a live foal.[2]

Uterine inflammation (endometritis) is judged to be the most important gynaecological condition of horses (Traub-Dargatz et al., 1991; Troedsson, 1999; Card, 2005) and is the most common cause of embryonic loss before 35 days in normally cycling mares (LeBlanc, 2003). Endometritis occurs when foreign molecules, often spermatozoa and/or bacteria, are introduced into the reproductive tract, specifically at mating and/or due to general opportunistic bacterial contamination (Watson, 2000; Riddle et al., 2007; LeBlanc, 2008). Opportunistic organisms typically include Gram positive (e.g. *Streptococcus zooepidemicus*; *S. zooepidemicus*) and Gram negative (e.g. *Escherichia coli*; *E. coli*) bacteria (Riddle et al., 2007; LeBlanc, 2008).

The inflammatory response to uterine challenge is characterised by an influx of polymorphonuclear neutrophils (PMNs), resulting in uterine luminal fluid and endometrial secretion of luteolytic prostaglandin (PG) $F_{2\alpha}$, which are incompatible with pregnancy (Watson et al., 1987; Pycock and Newcombe, 1996).

Uterine cytology and bacteriology are commonly employed in commercial practice as part of the pre-breeding examination in order to diagnose endometritis and its infectious or define non-infectious status and so indicate the likelihood of pregnancy success (Riddle et al., 2007; LeBlanc, 2010). However, there are differences in the guidelines for interpreting cytology (Card, 2005). Various cytological parameters are considered indicative of endometritis, for example: ⩾10% PMNs observed in a high power field (HPF) (Baranski et al., 2003); >0.5% PMNs of total cells counted (Ricketts and Mackintosh, 1987); 2–10% PMNs of total cells (Crickman and Pugh, 1986).

It has been reported that the use of bacteriology alone or in combination with cytology does not necessarily improve success

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[1] £1 = approx. $1.55, €1.19 at 4 May 2013.

[2] See: www.weatherbys.co.uk (accessed 4 May 2013).

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in diagnosing endometritis, although it does allow the identification of any causal agent (Riddle et al., 2007; LeBlanc, 2010). Several investigators have related pre-defined cytological or bacteriological characteristics to pregnancy rate although the parameters used have varied. Riddle et al. (2007) determined that the 28 day pregnancy rate was 7–36% when a micro-organism was cultured and/or a positive cytology (>2 PMNs per field) was observed, which was significantly lower than in mares negative for both examinations (60%).

Baranski et al. (2003) examined mares during foal heat and observed a pregnancy rate (date of pregnancy diagnosis not specified) of 18.2% in mares with positive cytology ($\geqslant$10 PMN per HPF) and bacteriology, and in mares where either cytology or bacteriology was positive pregnancy rates were 18–23%, compared to 54% in negative animals. Most recently no correlation of positive cytology (using a variety of assessment criteria) or bacteriology was reported to be associated with 70 day pregnancy rate (Nielsen et al., 2012).

No work has been reported to examine whether there is a critical threshold of PMNs detected in relation to pregnancy outcome. Furthermore, early pregnancy rates used as an indicator of success do not reflect the most critical measure of reproductive efficiency and financial return, namely the production of a live foal (Carrick and O'Meara, 2010). Mares with endometritis may conceive but experience bacterial placentitis and abortion as late as 120 days of gestation (Asbury, 1983; LeBlanc, 2003).

The aims of this study were to determine whether, as part of a standard breeding examination, a threshold percentage of uterine PMNs could be determined above which a significant reduction in live foaling rate is evident. We also examined the relationship of positive bacteriology to live foaling rate and the relationship of a combination of positive cytology and bacteriology results to live foaling rate.

Materials and methods

Animals and sample collection

A total of 2660 guarded uterine endometrial swabs were collected from 1621 Thoroughbred mares on 17 commercial stud farms by five veterinarians from the same practice in the Upper Hunter Valley region of New South Wales in Australia (latitude 32°5′S, longitude 150°52′E). The swabs were collected as part of routine management and thus represented a random population of mares encountered in practice, and were all analysed at the same in-house practice laboratory. Maiden, foaling, barren and problem mares were included and all had endometrial swabs collected for cytological and bacteriological analysis prior to mating.

Mares were bred on subsequent oestrus periods by natural cover, normally within 12 h of ovulation. Generally, animals were not bred on foal heat, with the exception of some when the end of the breeding season was approaching. Mares found to have a positive bacteriology were treated with intra-uterine antibiotics, Intervet Neomycin–Penicillin, every 24 h from the time of diagnosis until 48 h post cover. Several mares had multiple swabs collected throughout the season which were included in the study. Immediately after collection the swabs were placed in a charcoal free AMIES transport medium and transferred within a few hours to the laboratory.

Cytology

Each swab was rolled onto a frosted microscope slide and left to air dry. A commercial Romanowsky-type stain kit (Fronine, Thermo Fisher Scientific) was used and slides were prepared according to manufacturer instructions. Specimens were evaluated under a light microscope at ×40 and under oil at ×100 magnification. Seventy-seven (2.5%) of samples were hypocellular samples and were discarded. A minimum of 100 cells in ten fields were counted and percentage PMNs of the total cells observed was determined.

Bacteriology

Swabs were plated on horse blood agar (HBA; Oxoid Australia) and incubated for 48 h at 36.7 °C. The plates were checked at 24 and 48 h for bacterial growth. Bacterial growth was classified as: no growth; mixed flora (two or more organisms); or single isolate (one bacterial isolate only). Where bacterial growth was detected Gram positive or Gram negative organisms were identified using Gram stain smears (Thermo Fisher).

Live foaling rate

Live foaling rate was determined from the Australian and New Zealand stud books.

Data analysis

All swabs taken from mares within the season along with the first and the last swab of the season prior to mating were analysed. All swabs were included in order to ascertain whether a swab taken at any time of the season was indicative of problems in addition to the more obvious first and last swab.

When assessed for cytology (investigation 1) samples were assigned to groups according to % PMNs of total cells counted: 0 (all swabs $n = 2487$, first swab $n = 1514$, last swab $n = 1529$); $\geqslant$1% (all swabs $n = 173$, first swab $n = 106$, last swab $n = 91$), $\geqslant$2% (all swabs $n = 125$, first swab $n = 73$, last swab $n = 64$); $\geqslant$5% (all swabs $n = 79$, first swab $n = 50$, last swab $n = 29$), and $\geqslant$25% (all swabs $n = 35$, first swab $n = 23$, last swabs $n = 18$). Foaling rates for each classification were compared to 0% PMN. Data were analysed for all swabs, the first swab of the season and for the last swab collected from the mare prior to the final mating of the season. The lower threshold values (0%, $\geqslant$1%, $\geqslant$2%, $\geqslant$5% PMNs) were chosen to encompass typical parameters reported by existing literature (Ball et al., 1988; Overbeck et al., 2011). The upper value ($\geqslant$25% PMNs) was chosen to insure the widest range of possible thresholds was considered. An individual sample may have appeared in more than one PMN group, for example, a sample that yielded 10% PMNs appeared in both $\geqslant$2% and $\geqslant$5% PMN groups but not in the $\geqslant$25% PMN group.

When assessed for bacteriology (investigation 2a) foaling rates were compared for each classification (no growth, mixed flora, single isolate). Data were analysed for all swabs, first and last swab collected from the mare prior to the final mating of the season.

Investigation 2b considered the positive bacteriology samples observed in all swabs, first swab and the last mare swab of the season and were divided into Gram positive and Gram negative groups and compared to foaling rate.

Investigation 3 combined cytology and bacteriology where foaling rates were compared for samples that exhibited positive cytology only ($\geqslant$1% PMNs), a single isolate only (positive bacteriology), or a paired single isolate culture result with positive cytology. This was done for all swabs, first and final swabs of the season.

Pearson's chi-square (χ^2) analysis was used throughout to determine if significant differences existed between foaling rates. Significance was assumed where $P < 0.05$. Once parameters were determined for cytology (investigation 1), bacteriology (investigation 2a) and combined cytology and bacteriology (investigation 3), the sensitivity, specificity, negative predictive value and positive predictive value were calculated for each, using live foaling rate as the best standard. The sensitivity was the proportion of mares that had a positive diagnosis of all mares that did not produce a live foal (true positive). The specificity was the proportion of mares that had a negative diagnosis of all the mares that produced a live foal (true negative). The negative predictive value was the proportion of mares that were truly negative (negative diagnosis and produced a live foal) of all negatively diagnosed animals. The positive predicted value was the proportion of mares that were truly positive (positive diagnosis and did not produce a live foal) of all positively diagnosed animals.

Results

Investigation 1

Of all the samples that were collected ($n = 2660$), 2487 (93.5%) had no PMNs present and were associated with a foaling rate of 66.9%. In comparison to these PMN negative samples, those that exhibited $\geqslant$1% PMN had a significantly reduced foaling rate (group $\geqslant$1% PMNs, $P < 0.05$; $\geqslant$2% PMNs, $P < 0.01$; $\geqslant$5% PMNs, $P < 0.05$) with the exception of $\geqslant$25% PMNs (Fig. 1).

For the first swab of the season ($n = 1621$), 1514 (93.4%) mares had no PMNs present, related to a foaling rate of 74%. In comparison to these PMN negative samples, swabs that exhibited $\geqslant$2% PMN had a significantly reduced foaling rate ($P < 0.05$) and those with $\geqslant$5% had a tendency for a reduced foaling rate ($P < 0.07$). No effect was observed for the $\geqslant$1% and $\geqslant$25% PMNs (Fig. 2).

For the final swab of the season ($n = 1621$), 1529 (94.3%) mares had no PMNs present, related to a foaling rate of 74%. In comparison to these PMN negative samples, swabs that exhibited $\geqslant$1% and