The association between the endometrial biopsy grade and selected epidemiological and reproductive variables in a population of subfertile mares

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Summary

Background: Endometrosis, a multifactorial disease, is an important cause of subfertility in the mare and its management is reliant on histological classification.

Objectives: This study aimed to determine the association between increased endometrial biopsy grade with firstly, a mare's breed specifically either thoroughbred or non-thoroughbred, age and barren status and secondly, a clinical history indicating one or more of acute endometritis, positive bacterial culture, previous dystocia and uterine fluid retention.

Study design: Retrospective case series

Methods: This retrospective study used historical reproductive data (2000-2015) from a population of 41 sub-fertile mares of various breeds, but mostly thoroughbreds (30/41; 73%). Biopsies were interpreted by a single, experienced evaluator using the Kenney-Doig classification (1986).

Results: Endometrial biopsy grade was positively correlated with age (ρ = 0.606; P= 0.001) but not associated with other evaluated variables (P>0.05).

Main limitations: Use of a small, non-randomly selected sub-fertile mare population.

Conclusions: Mare age was an important predictor of her reproductive performance and performing an endometrial biopsy is therefore indicated in older mares.

Keywords: mare; biopsy; endometrosis; reproductive performance.

Clinical relevance

- Older mares had a higher endometrial biopsy grade suggesting a reduced chance of successfully carrying a pregnancy to term.
- Age should be an indication for obtaining an endometrial biopsy during a breeding soundness evaluation.
- This study failed to support an association between a mare's endometrial biopsy grade and a history including barren status ≥2 years, uterine intraluminal fluid retention, breed allocation (thoroughbred or non-thoroughbred), acute endometritis, a positive bacterial culture, or previous dystocia.

Introduction

Reduced reproductive performance in the mare has been associated with economic and genetic losses to the horse industry (Bosh, Powell, Neibergs, et al. 2009; Tibary and Pearson 2015). A prospective cohort study reported that only 83% of pregnancies produced a live foal (Rose et al. 2018). In the

thoroughbred, the overall rate of pregnancy failure contributes to a major loss to this industry (Morris and Allen 2002; Todd et al. 2020) and the incidence of pregnancy loss remains largely unchanged over the past few years despite increased application of reproductive therapeutics (Rose et al. 2018). Endometrosis or degenerative endometrial fibrosis with glandular changes is an important cause of subfertility in the mare (Brinsko and Blanchard 2011; Tibary and Pearson 2015; Tibary and Ruiz 2019). Endometrial biopsy is considered the gold standard for diagnosis of endometrial pathology and is indicated in any non-pregnant mare where uterine disease is suspected (Ricketts 1975; Snider, Sepoy, and Holyoak 2011; Schlafer 2007). Endometrial biopsy has been a principal procedure in the assessment of equine uterine health and histopathology has remained the mainstay of evaluating uterine pathology and assessing uterine health (Snider, Sepoy, and Holyoak 2011). The endometrial biopsy is a safe and effective means of predicting a mare's prognosis for foaling (Van Camp 1988). In the hands of a reasonably experienced operator, this procedure has few complications and side effects (Van Camp 1988) and there is no apparent negative effects on reproductive performance (Kenney 1978). Endometrial biopsy is an established minor surgical procedure that is modestly invasive, easy and a cheap diagnostic technique, with the most common sequelae being slight bleeding at the biopsy site (Sertich 1996; Snider, Sepoy, and Holyoak 2011; Joana et al. 2007). This rapid technique is frequently included in the examination for breeding soundness evaluation and additionally is capable of estimating the mare's ability to carry a foal to term (Snider, Sepoy, and Holyoak 2011). However,

endometrial biopsy does require a variable turnaround time of at least several days and is dependent on access to an appropriate laboratory and a suitably qualified specialist with expertise in clinical equine reproduction. Reports investigating the link between endometrial biopsy findings and epidemiological and reproductive variables are scarce (Ricketts and Alonso 1991).

Subfertility is defined as any form of reduced fertility with a prolonged period of unwanted non-conception (Gnoth et al. 2005). Subfertility is associated with mare-level factors including breed (Mahon and Cunningham 1982; Todd et al. 2020), age (Grüninger et al. 1998; Morris and Allen 2002; Todd et al. 2020), barren status (Leishman, Miller, and Doig 1982; Morris and Allen 2002), uterine fluid retention (Woodward et al. 2012), acute bacterial endometritis (LeBlanc and Causey 2009; Riddle et al. 2007; Tibary and Ruiz 2018), endometrial fibrosis (Grüninger et al. 1998; Tibary and Ruiz 2018; Brinsko and Blanchard 2011), previous dystocia (Morley and Townsend 1997) and biopsy grade (Brinsko and Blanchard 2011). Endometritis and endometrosis have been reported to be the most common uterine pathologies causing mare subfertility or infertility (Tibary and Ruiz 2018). Mares aged 11-years or more show increased endometrosis and reduced fertility (Schoon et al. 2000) and it has been estimated that 30% of broodmares over the age of 18 years are affected (Alvarenga and Carmo 2009). In a population of 2246 mares, the overall incidence of pregnancy loss was 6.4% (between day 15-42) and 1.6% (between day 43-65), a further 1.3% of pregnancies were lost thereafter and

4.5% by birth that included stillbirths (Rose et al. 2018). A retrospective study of thoroughbred mares reported that both age and reproductive status significantly affected the per cycle pregnancy rate and incidence of pregnancy loss (Morris and Allen 2002). Similarly, foaling rate declined with increasing mare age in another population of thoroughbred mares (Todd et al. 2020). Age is reportedly associated with the presence and incidence of endometrial fibrosis and angiopathies, both of which are contributors to subfertility in mares (Grüninger et al. 1998). An association of both biopsy grade and age with fluid retention and of age alone with biopsy grade has been reported (Woodward et al. 2012). Years of barrenness is correlated with the number of fibrotic layers found around the endometrial glands (Leishman, Miller, and Doig 1982; Morris and Allen 2002; Kenney and Doig 1986).

This retrospective case study utilized historical sets of reproductive data from a sub-fertile mare population. The aim was to improve our understanding of two important, albeit incompletely defined aspects of endometrial biopsies in the mare. These are identifying risk factors for endometrial pathology and supporting the inclusion of an endometrial biopsy in a breeding soundness examination. The hypotheses were firstly that the selected demographic variables of breed defined as thoroughbred or non-thoroughbred, age and barren status increased the endometrial biopsy grade. Secondly, a clinical history including at least one of acute endometritis, positive bacterial culture, previous dystocia and uterine fluid retention will be associated with an

increase in endometrial biopsy grade. Evaluating these hypotheses would help define the utility of an endometrial biopsy during a breeding soundness examination.

Materials and methods

Study population

The source material originated from mares that underwent an endometrial biopsy procedure during 2000-2015. All samples were obtained and submitted at various stages within the physiological breeding season relevant to the Northern or Southern hemispheres. The purpose of the submission was to provide an additional diagnostic tool to investigate an apparent subfertility of varying duration and to evaluate the endometrial integrity of an affected broodmare. The data consisted of individually identified endometrial biopsy sections stored in customized containers together with their digitally archived reports. The study population included mares (n=41) regarded as sub-fertile that had prompted a clinical decision to obtain and submit an endometrial biopsy for interpretation. Submissions were predominantly sourced from thoroughbred mares (75%; 30/41). The common clinical practice of obtaining biopsy samples in thoroughbred mares has been speculatively linked to the pioneering work of Kenney during the late 1970s. In United Kingdom equine practice, biopsy samples are most commonly obtained from mares who repeatedly fail to conceive during the breeding season or from mares during routine post-season barren examination (Ricketts and Barrelet 1997; Snider, Sepoy, and Holyoak 2011;

Rossdale and Ricketts 1980). Different veterinarians practicing in either South Africa or Saudi Arabia obtained the biopsies included in this study. Biopsies were placed in sealed labelled plastic containers filled with 10% buffered formalin for transfer to the Faculty of Veterinary Science, University of Pretoria in South Africa. Staff of the Pathology Section sectioned and prepared all submitted biopsies. Each tissue section was stained with hematoxylin and eosin and mounted on an individual glass slide labelled with a printed unique identity code before transfer to the Section of Reproduction. All interpretations and reporting were by a single, experienced evaluator with specialist registration in Theriogenology. Mares without matching biopsy reports and biopsy specimens were excluded.

Variable selection

Seven variables were selected based on their reported association with subfertility:

- Breed; specifically thoroughbred or non-thoroughbred (Mahon and Cunningham 1982; Todd et al. 2020)
- age (Grüninger et al. 1998; Morris and Allen 2002);
- barren status ≥2 years (Leishman, Miller, and Doig 1982; Morris and Allen 2002);
- uterine fluid retention (Woodward et al. 2012);
- acute endometritis (LeBlanc and Causey 2009; Riddle et al. 2007; Tibary and Ruiz 2018);

- positive bacterial culture (LeBlanc and Causey 2009; Riddle et al. 2007; Tibary and Ruiz 2018); and
- previous dystocia (Morley and Townsend 1997).

Breed, of which the population was categorized as either thoroughbred (n=30; 30/41; 73%) or non-thoroughbred mares consisting of all other breeds combined (n=11; 11/41; 27%).

The study population included 26 mares with known age (26/41; 63%) that was divided into groups with younger mares ≤7 years-old (n=3; 3/26; 12%); mares 8-15 years-old (n=12; 12/26; 46%) and mares ≥16-years-old (n=11; 11/26; 42%). The median age of the mares was 15 years old; ranging from 5 to 24 years. Additionally, these 26 mares were dichotomised based on the median age of 15-years-old for further statistical analysis. Specifically, 14 of the 26 mares were 15 years of age or younger and 12 mares were older than 15 years of age.

A barren mare was defined as having been cyclic and bred but without subsequently producing a foal. Thirteen (n= 13; 13/41; 32%) of the study mares were recorded as being barren for ≥2 successive years.

Presence or absence of intra-uterine fluid on clinical examination was recorded for nine (9/41; 22%) mares with fluid retention in seven (7/9; 78%) and no fluid retention in two (2/9;22%) mares, respectively.

The presence of polymorphonuclear cells (PMNs) on a biopsy section was used to define acute inflammation (endometritis). The presence or absence of PMNs was recorded in 20 (20/41; 48%) mares in the study population with signs of acute endometritis present in 11/20 (55%) mares and no signs of endometritis in 9/20 (45%) mares.

Bacterial culture results were derived from the information accompanying the sample submissions. These results were obtained concurrently with the endometrial biopsy from endometrial swabs submitted for culture to various laboratories. This information was available from 22/41 (53%) mares and with a positive bacterial culture recorded in a sub-population of 12/22 (55%) and a negative bacterial culture recorded in 10/22 (45%) mares, respectively.

A history of dystocia was recorded in four (4/41; 10%) mares.

The biopsy reports

The individual identity label on each section was used to locate the corresponding report from a digitally stored archive. Reports followed a standardized template (Appendix 1) with a header that identified the mare, country and place of origin, owner details, submitting veterinarian and date of sampling. Any information derived from the brief clinical and breeding history submitted by the referring veterinarian was included. The biopsy was interpreted and reported following a standardized protocol. The histological

evaluation was performed from the level of the luminal epithelium to the myometrium with the pertinent features derived from those described by Kenney and Doig (1986). Each mare was assigned a biopsy grade using the Kenney and Doig classification. The resulting report included a recommended clinical management based on both the histological interpretation and the available information derived from the clinical history accompanying the submitted biopsy (Appendix 1, 2, 3, 4 & 5).

Histological evaluation of the endometrial biopsy

Each section was evaluated using light microscopy. The categorical grade assigned was based on a histological evaluation of the endometrium's integrity, which is associated with the ability of a mare to carry a pregnancy to term (Ricketts 1975; Kenney and Doig 1986). The four classification categories were grades I, IIA, IIB or III. A grade was determined by consideration and assessment of multiple factors. These included the degree and type of inflammation (acute and chronic), glandular nesting, peri-glandular fibrosis and different cell layer integrity and vascular and lymphatic changes (Kenney and Doig 1986; Ricketts 1975). Clinical information describing cyclical status at the time of biopsy can be useful for interpretation. The dynamic nature of the endometrium responding to the cyclical physiological changes associated with ovarian steroid hormones has an important influence on the observed histological picture (Kenney 1978). Sampling during diestrus will avoid the estrogenic effects including hyperemia

and edema of tissues that might obscure details of the endometrial architecture (Kenney 1978).

A general comment was recorded on the section's quality, normal architecture, and tissue types before making detailed observations on the different cell layers and cellular components. Chronic endometrial inflammation with resultant fibrosis, including the degree of severity and extent of distribution was an important finding. This permanent change, if widespread, negatively affects future reproductive performance in the mare (Kenney and Doig 1986; Tibary and Ruiz 2018).

An automated fluorescence microscope, Olympus® BX63 with an Olympus® DP72 camera (Olympus, Tokyo, Japan), was used to obtain photographs of representative sections that were obtained from multiple mares with different endometrial biopsy grades to illustrate the categorization.

Grade I was assigned to a mare with >70% chance of carrying a pregnancy to term based on the histological evaluation. The endometrium had none or slight pathological changes such as mild fibrosis or acute inflammation (Kenney and Doig 1986). Good glandular density was present and easily identifiable epithelial cells without histological signs associated with apoptosis were visible. The endometrium of **mare 1** was assigned a biopsy grade I with good glandular density and no obvious endometrial architectural abnormalities being observed (Figure 1).

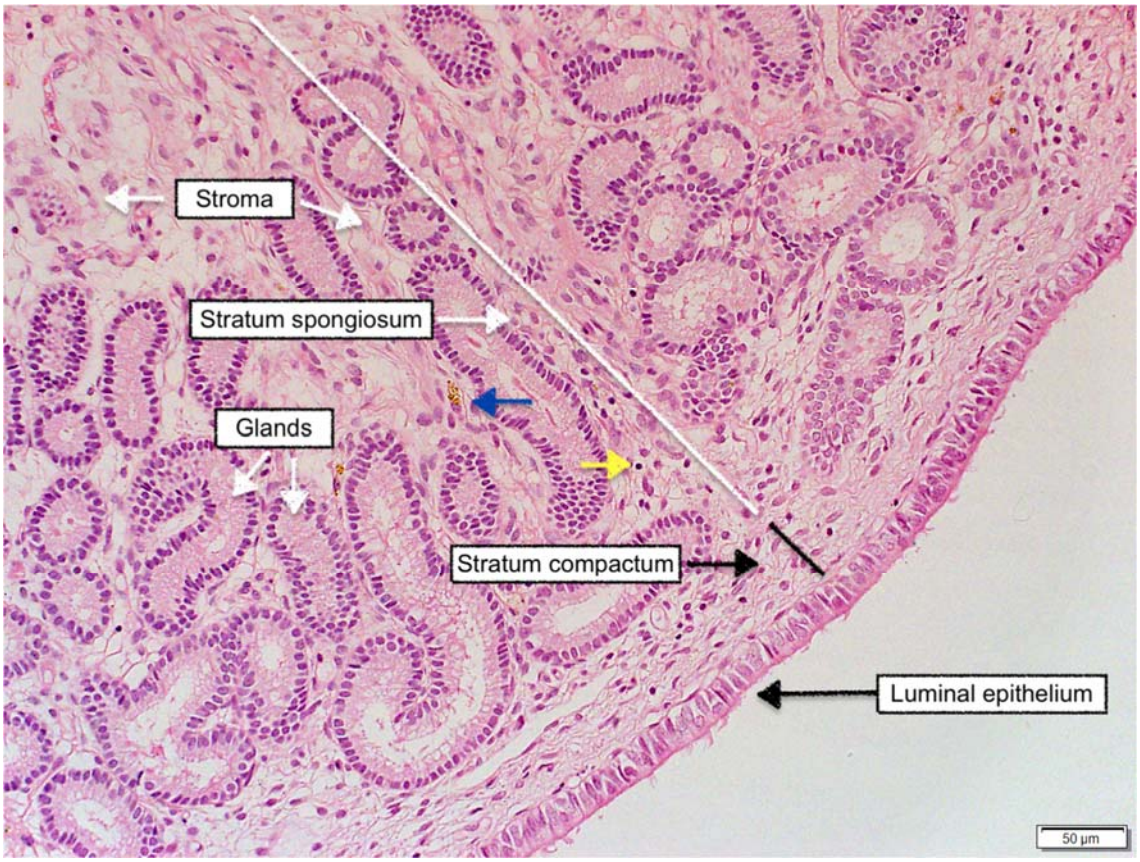

Figure 1. Biopsy section (mare 1) categorised as grade I exhibiting normal endometrial architecture. The blue arrow indicates haemosiderin in the stroma of the stratum spongiosum. The yellow arrow indicates a lymphocyte, contributing to the diffuse mild lymphocytic infiltrate. H&E stain. Obj × 20

Grade IIA refers to a mare with a 50-70% chance of carrying a pregnancy to term. Mild fibrosis with mild to moderate inflammatory changes with or without peri-glandular nesting may be present. A biopsy grade IIA was assigned to **mare 14** diagnosed with a diffuse mild subacute endometritis (Figure 2).

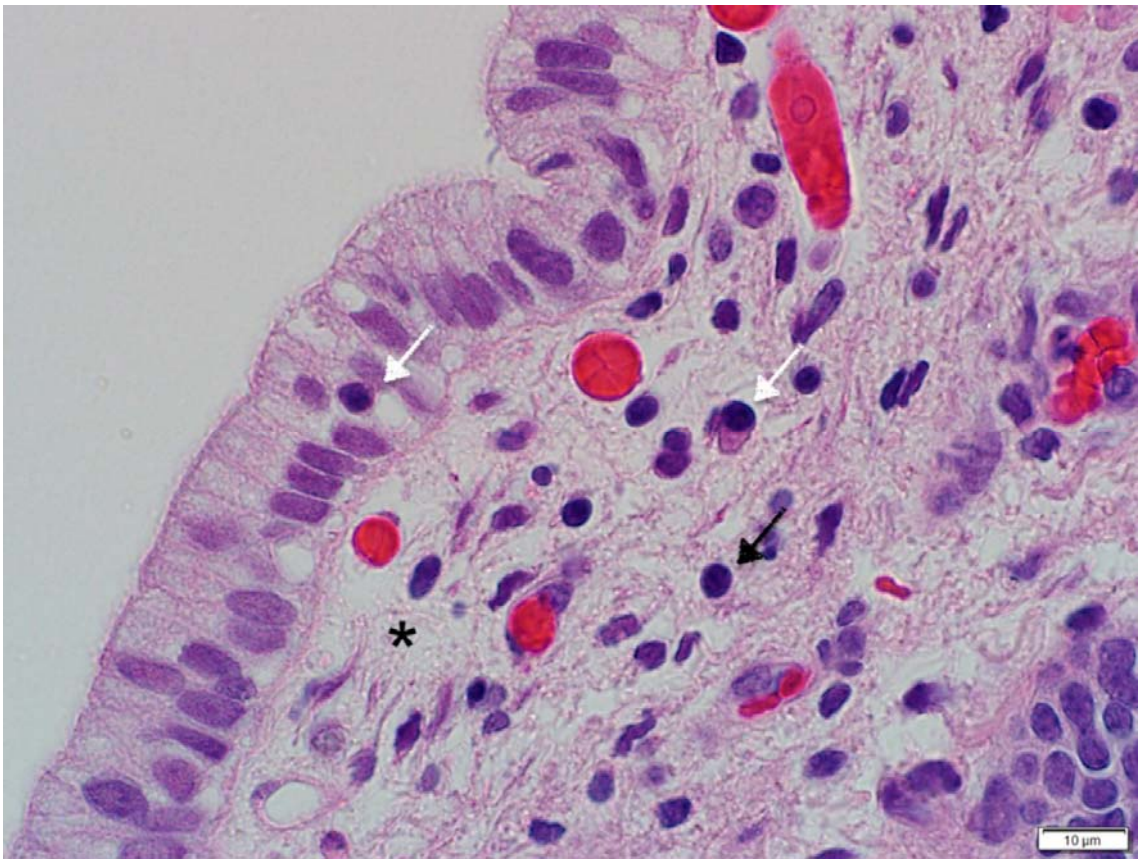

Figure 2. Biopsy section (mare 14) categorised as grade IIA exhibiting a diffuse mild lymphoplasmocytic infiltrate in the stratum compactum, the white arrows indicate plasma cells and the black arrow a lymphocyte. The asterisk (*) indicates mild diffuse oedema in the stratum compactum. H&E stain. Obj × 100

Grade IIB refers to a mare with a 30-50% chance of carrying a pregnancy to term. Moderate fibrosis with diffuse, moderately severe inflammation and widespread fibrosis of individual gland branches, uniformly distributed and with an average of two to four fibrotic glandular nests per 5.5 mm of linear field (Kenney and Doig 1986). Apoptosis of glandular cells can be present occasionally. This apoptotic fibrosis is illustrated by both **mare 3** (Figures 3) and **mare 4** (Figure 4). **Mare 3** additionally showed glandular nesting and

intraluminal exudate, whereas **mare 4** had mild acute endometritis with mild to moderate endometrosis.

Figure 3. Biopsy section (mare 3) categorised as grade IIB exhibiting glandular nesting with scattered mild peri-glandular fibrosis. The arrow shows glandular intraluminal purulent exudate. H&E stain. Obj × 40

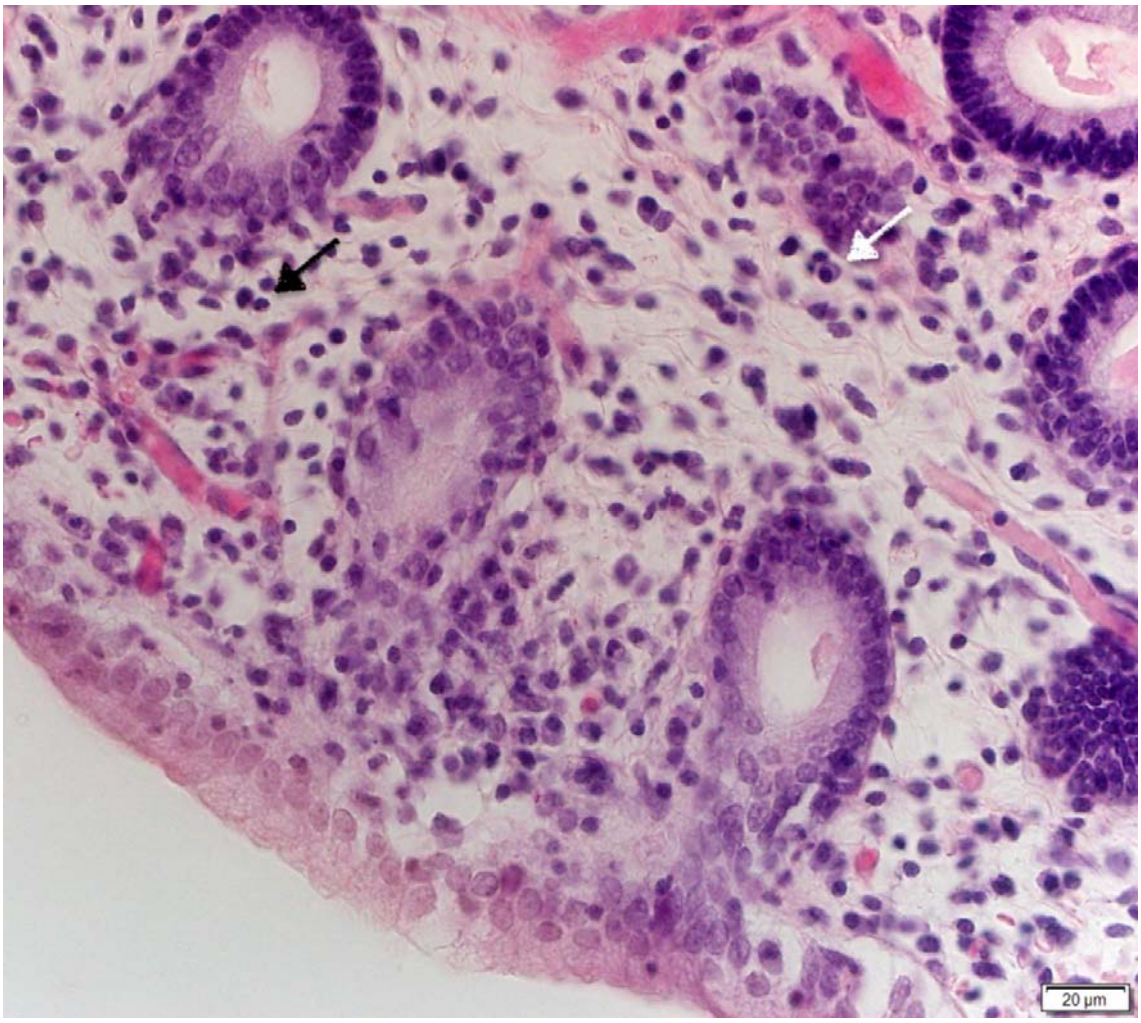

Figure 4. Biopsy section (mare 4) categorised as grade IIB exhibiting a round cell and neutrophil infiltrate and peri-glandular fibrosis. The black arrow shows a neutrophil and the white arrow a plasma cell. H&E stain. Obj × 60

Grade III refers to a mare with <30% chance of carrying a pregnancy to term. Diffuse, severe and uniformly widespread inflammation with fibrosis affecting > five gland branches in a 5.5 mm linear field (Kenney and Doig 1986) and apoptosis affecting glandular epithelial cells is frequently observed. Figure 5 illustrates sections obtained from **mare 12** categorized as Grade III.

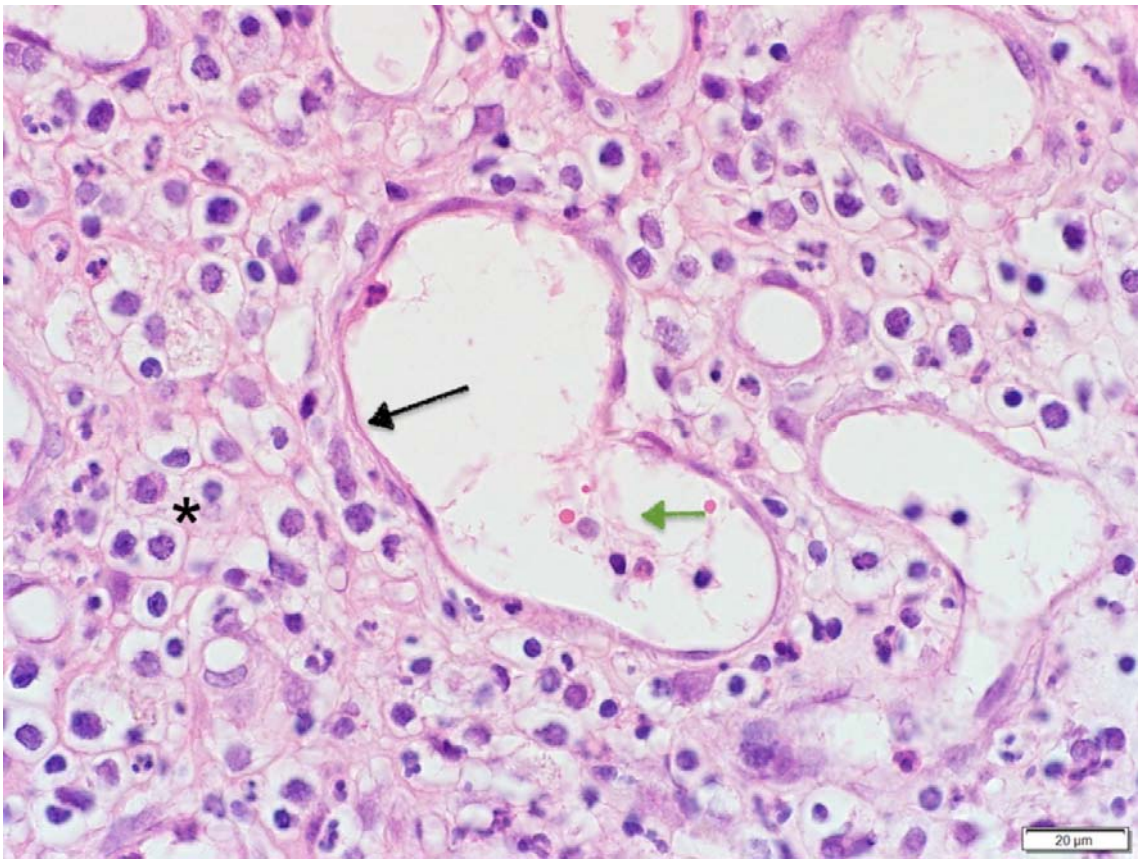

Figure 5. Biopsy section (mare 12) categorised as grade III exhibiting glandular necrosis and absence of viable glands in the stratum spongiosum (*). The black arrow indicates the loss of glandular epithelium. Note the severe dilation of this gland. The green arrow indicates an exudate. H&E stain. Obj × 60

Data analysis

Data were entered into an Excel® spreadsheet. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS version 26). The association between age and biopsy grade was estimated using Spearman's rho correlation coefficient. The biopsy grade was compared between defined groups (either present variable/absent variable) for the

categorical variables using Mann-Whitney U tests under the null hypothesis that the distribution of biopsy grades was equal.

Results

The study population of 41 mares were resident in either South Africa or Saudi Arabia and biased towards thoroughbreds (n=30; 73%), with 15 each from South Africa and Saudi Arabia. The additional four known breeds were Arabian (n=4) with two each from South Africa and Saudi Arabia, warmblood (n=2), Friesian (n=2), American saddlebred (n=2) and one of unknown breed.

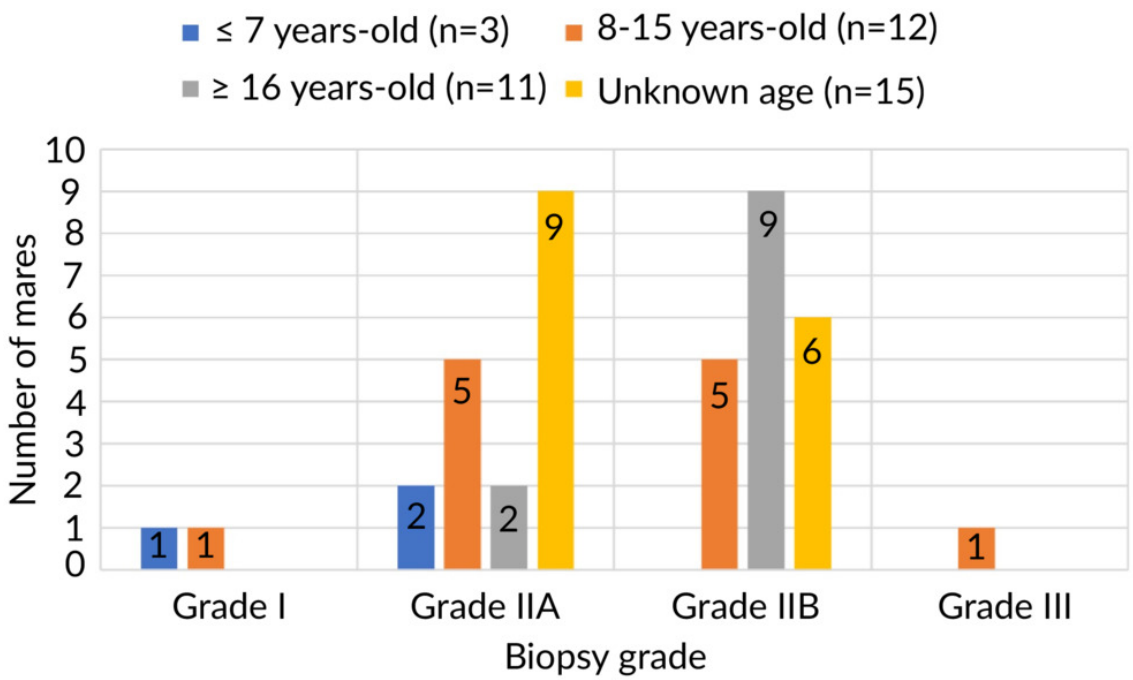

Figure 6. The distribution of different age groups within each biopsy categorical grade shown across the population of mares

Endometrial biopsy grades assigned to this population of mares regarded as sub-fertile were category I (2; 5%), category IIA (18; 44%), category IIB (20;

49%) and category III (1; 2%) (Figure 6). This population had a median biopsy categorical grade of IIA.

The median biopsy grade for thoroughbreds was IIB with an absolute range of I to IIB and similar to non-thoroughbred breeds that also had a median biopsy grade of IIB and an absolute range of I to III (Table 1). The was no difference in biopsy grade between these groups (P =0.942).

Table 1: A summary of results with the association between the endometrial biopsy grade and selected epidemiological and reproductive variables in a population of 41 sub-fertile mares.

Variable	n	Yes		No		p-value
Thoroughbred breed	41	30	IIB (I to IIB)	11	IIB (I to III)	0.942
Barren mare	41	13	IIB (I to IIB)	28	IIA (I to III)	0.772
Uterine fluid retention	9	7	IIB (IIA to IIB)	2	(I; IIB)	0.500
Acute endometritis	20	11	IIB (I to III)	9	IIB(IIA to IIB)	1.000
Positive bacterial culture	22	12	IIA (IIA to III)	10	IIA (I to IIB)	0.254
History of dystocia	41	4	IIB (IIA to IIB)	37	IIA (I to III)	0.406
Age <15 years old	26	12	IIA (I to III)	12	IIB (IIA to IIB)	0.026

Mares less than 15 years of age (n=12) had a median (absolute range) biopsy grade of IIA (I to III) compared to IIB (IIA to IIB) for older mares (n=12) with a p-value of P= 0.026. Furthermore, mare age and her assigned biopsy grade were also positively correlated (ρ= 0.605; P =0.001).

The median biopsy grade for barren mares (n=13) was IIB with a range of grade I to IIB compared to a median of IIA with a range of I to III for mares with no history of being barren (n=28). There was no significant difference in

biopsy grades between a mare barren for ≥2 years and other mares (P = 0.772).

The distribution of the biopsy grades between mares with reported history of fluid retention (n=7) was not different to those without fluid retention (n=2) (P = 0.500). The median biopsy grade for mares with recorded fluid retention status was IIB with a range of grade IIA to IIB compared to grades of I and IIB for the two mares without a recorded history of fluid retention.

The distribution of the biopsy grades between mares with histological evidence of acute endometritis (n=11) did not differ to mares without these signs (n=9) (P =1). The median biopsy grade for mares with recorded presence of PMNs was category IIB with a range of grade I to III whereas the median was IIB with a range of IIA to IIB for mares without PMNs detected.

There was no difference in the distribution of the biopsy grades between mares with a recorded history of a positive bacterial culture (n=12) to those with a negative bacterial culture (n=10) (P = 0.254). The median biopsy grade for mares with a positive bacterial culture was category IIA with a range of grade IIA to III compared to a median of IIA with a range of I to IIB for mares without a recorded history of a positive bacterial culture.

There was no difference in the distribution of the biopsy grades between mares with (n=4) or without (n=37) a recorded history of a dystocia (P =

0.406). The median biopsy grade for mares with recorded dystocia was grade IIB with a range of grade IIA to IIB compared to a median of IIA with a range of I to III for mares with no recorded history of dystocia.

Discussion

Breed, either thoroughbred or non-thoroughbred, was not associated with biopsy grade. Arguably, this could reflect a limitation of this study by its strong bias towards thoroughbreds. A diversity of factors including the different breeding systems such as reliance on natural covering alone in thoroughbreds compared with other breeds utilizing assisted reproduction and differences in their breeding lifespans in addition to genetic factors might have played a role. An example might include the consignment for breeding of young thoroughbred mares following a racing career that generally ends at a relatively early age when compared with the competitive lifespan associated with sport horses. The latter in addition, typically commence their sporting career at a far later age than racehorses, typically only when already into equine "middle-age". At a comparative age, many thoroughbreds are multiparous and already display appreciable changes to their reproductive tracts associated with their breed and parity such as poor perineal conformation that have been associated with both endometrial degeneration and reduced reproductive performance (Hurtgen 2006).

In the current study, mare age was the only variable that was significantly associated with an individual's assigned biopsy grade. The increasing biopsy grade with age is consistent with various reports that biopsy grade, largely an index of endometrial fibrosis, was more common in older mares (Grüninger et al. 1998) and that pregnancy rate and pregnancy losses can be linked to age (Morris and Allen 2002). A recent literature review reported that age critically affected cyclicity, folliculogenesis, oocyte and embryo quality as well as presence of oviductal masses and uterine tract function (Derisoud et al. 2021). Increased parity and age was associated with a progressive decline in both endometrial quality and uterine clearance capacity (Derisoud et al. 2021). Australian thoroughbred mares at 20 years of age reportedly had < 70% covering success between the years 2000 - 2017 (Todd et al. 2020). This association may at least partially reflect the effects of age on the endometrial response to hemodynamic stressors and vascular injury (Grüninger et al. 1998). In turn, the association between chronic endometrial degeneration (endometrosis) is widely accepted as an important contributor to reduced reproductive performance in the mare (Todd et al. 2020; Tibary and Ruiz 2018). In the current study, a probable limitation resulted from the few mares < 7 years-old, arguably due to the bias associated with the selection of sub-fertile mares.

Barren status did not appear to be associated with biopsy grade. This might have been due to the small study population. Previously, number of years barren was associated with biopsy categories (Leishman, Miller, and Doig

1982) with apparent effects on pregnancy rate and pregnancy losses (Morris and Allen 2002). This current study's finding was also seemingly, albeit indirectly, at odds with one reporting a correlation between barren mares having fewer endometrial glands and increased peri-glandular fibrosis with observed subfertility (Leishman, Miller, and Doig 1982).

Furthermore, there was no association in this population of sub-fertile mares between the biopsy grade and the variables selected from their accompanying history. This might also reflect a limitation of a retrospective study that examined an incompletely reported data set pertaining to presence or absence of uterine fluid retention, acute endometritis, and bacterial culture. The sample size was small and a further complication is that these three are not independent variables.

Endometrial biopsy is considered to have a high sensitivity and specificity for diagnosis of endometritis (Diel de Amorim et al. 2016). Uterine fluid retention is both a precursor and sequela of infection (Causey 2006) and is a frequently observed clinical sign associated with an endometrial inflammatory response. This usually has an associated bacterial pathogenesis prompting the sampling for cytological, bacteriological or histological diagnosis of acute endometritis (Causey 2006). In contrast to results from a previous study (Woodward et al. 2012), this current study failed to support an association between biopsy grade and presence of intraluminal uterine fluid retention, acute endometritis or bacterial culture.

Biopsy results inform the reproductive management of the barren mare and could aid in the identification of susceptibility to uterine infection (Leishman, Miller, and Doig 1982; Woodward et al. 2012) and other structural and functional lesions encountered including endometrial fibrosis (Kenney 1978; Kenney and Doig 1986) that have been associated with poor reproductive performance. The current study did not support an association between a poorer biopsy grade in mares with an extended barren period; however, the limited sample size available in this study prevents definitive conclusions. The mares with recorded dystocia had a median biopsy grade of IIB and a range of grade IIA to IIB, supporting reports that dystocia is not necessarily associated with reduced reproductive performance (Byron et al. 2010; Carluccio et al. 2007). Mares bred two to three months post-fetotomy for resolution of dystocia had favorable short-term fertility (Carluccio et al. 2007) and similarly 59% of those that produced live foals post dystocia were bred in that same year of dystocia occurrence (Byron et al. 2010) . Following intervention via caesarean section, there was a favorable prognosis for subsequent birth of a live foal following surgical time of <90 min in mares <16 years-old (Abernathy-Young et al. 2012). The study did not anticipate finding an association between dystocia and biopsy grade, but believed it was noteworthy.

Conclusion

There was a significant association of increasing mare age with increasing biopsy grade. These results suggest that mare age is an important indication

for obtaining an endometrial biopsy during a breeding soundness examination. Current study results did not support that thoroughbred or non-thoroughbred breed, barren assignation, uterine fluid retention, acute bacterial endometritis and previous dystocia were associated with endometrial biopsy grade.

This study's limitations included a small, non-randomly selected sub-fertile mare population. The limited amount of younger mares available for this study, with resulted bias towards older mares, is likely reflected by the sub-fertile population selection and by the thoroughbred bias; with consideration of the age that these mares are first consigned for breeding (Allen et al. 2007; Bosh, Powell, Shelton, et al. 2009; Hanlon et al. 2012; Roach et al. 2021). The small study population size was particularly relevant to the variables of uterine fluid retention, acute endometritis, and positive bacterial culture. Furthermore, data were historical instead of prospectively collected and missing information further limited the sample size available for analysis. A further limitation included the inability to record the interval between dystocia and biopsy collection in those affected mares.

This study examined a combination of currently recommended indications for incorporating an endometrial biopsy in the reproductive examination of a mare. Results provided baseline information for future studies to investigate using a larger, more representative mare population in attempting to

establish the associated utility and limitations of endometrial biopsy in veterinary practice.

Authors' declaration of interest

No conflicts of interest have been declared.

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