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Distribution of degenerative changes in the equine endometrium as observed in a single versus two biopsies

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A R T I C L E I N F O

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A B S T R A C T

Equine endometrial degeneration is diagnosed by microscopy of an endometrial biopsy but it is uncertain if findings in a single biopsy represent the state of the entire endometrium. Previous studies have compared samples from multiple sites but conclusions are inconsistent. Further clarification is therefore needed. In this study, presence and characteristics of endometrial degeneration were compared in two full thickness specimens from the ventral base of both uterine horns, obtained post mortem from cyclic mares (n = 82). Hematoxylin and eosin-stained sections were blinded evaluated for periglandular fibrosis, glandular nests, dilated glands, glandular dilation, excessive lymphatic vessels, and lymphatic lacunae. Each section was also assigned to a modified Kenney and Doig categorization. Statistical analysis included multiple logistic regression models for fraction of mares with disagreement in the modified Kenney and Doig category and fraction of mares with disagreement for each parameter, multiple generalized linear regression models for fraction of parameters with disagreement, and equivalence tests for agreement of a cumulative score of degenerative parameters. Possible effect of age, mare type and estrous cycle stage were included in the analyses. For the Kenney and Doig categorization, 49% of the mares had good agreement, 46% had moderate agreement and 5% had poor agreement between the two biopsies. Disagreement in the Kenney and Doig categorization between the two biopsies increased with age (OR = 1.1, P = 0.009). Disagreement within each parameter ranged from 10% of mares (glandular nests), to 31% (periglandular fibroblasts) and 37% (lymphatic lacunae). Disagreement for changes in endometrial glands increased with age (OR = 1.15–1.16, P < 0.040). Several mares (33%) had disagreement in at least two parameters, and the number of parameters with disagreement increased with age (HR = 1.04, P = 0.010). When tested for equivalence, the cumulative scores for paired endometrial samples did not differ for mares younger than 18 years (P = 0.011). In conclusion, the degenerative changes were not uniformly distributed, revealed by a disagreement between paired endometrial samples for individual parameters and for the Kenney and Doig categorization, which increased with age. This suggests that conclusions based on a single biopsy, particularly in mares over 17 years of age, should be interpreted with caution, and evaluation of two biopsies should be considered.

1. Introduction

Histological interpretation of endometrial biopsies is an essential part of assessing fertility in mares [1,2]. A grading system that categorizes mares into four groups (I, IIA, IIB and III), based on findings of periglandular fibrosis, glandular nesting and dilation, inflammation and lymphatic lacunae, in association with reproductive history and findings upon a gynecological examination, was proposed by Kenney and Doig [1].

Endometrial degeneration is a chronic condition and one of the primary causes for subfertility in mares [1,3–5]. Periglandular fibrosis is an important feature of the degenerative process that alters the stromal structure, and is associated with cystic glandular dilation, fibrotic nesting of glands and formation of lymphatic lacunae [1,4,6,7]. The

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pathological alterations of the endometrial glands are associated with abnormal glandular function [4,8], and the degeneration is often associated with concurrent endometritis [4,9], as well as susceptibility to persistent breeding induced endometritis [10].

An endometrial biopsy obtained by alligator jaw biopsy forceps only represents 0.1–0.2% of the endometrial surface in a non-pregnant mare, and is regarded as the minimal size required for proper interpretation of endometrial health [1,11,12]. Despite the small size of a biopsy, it has been suggested that histopathological findings in a single biopsy are representative for the state of the entire endometrium [6,11–14]. This view has, however, been challenged by studies showing an uneven distribution and severity of endometrial degeneration [15–20]. While the conclusions of most studies on the representativeness of a single endometrial biopsy have been limited by a low number of observations, ranging from 5 to 40 mares [11–13,16,18,21], a few reports include 50 mares or more. Results from these studies are conflicting, with some suggesting that one sample is sufficient [14], while others show a discrepancy in severity of findings between different locations of the endometrium [15,17,19].

A further complicating factor for endometrial biopsy interpretation is the age of the mare. Several studies have demonstrated that the level of endometrial degeneration increases as the mare ages [5,22–26], with only one study investigating the correlation between the mare's age and representativeness of a single biopsy [19].

The aim of the present study was to investigate if the histopathological changes of paired left and right uterine horn samples differed in severity and if this let to discrepancies in the Kenney and Doig categorization, and to determine how differences were influenced by age, horse type and estrous cycle stage.

2. Materials and methods

2.1. Animals

Uteri from mares (n = 106) were collected post mortem at Danish abattoirs and the Copenhagen Zoo from September 2021 to March 2022. The mares were slaughtered for human or animal consumption according to European mandates (European Food Safety Authority, AHAW/04–027). Mares' age and breed were obtained from their passports, and time and date of slaughter was recorded on site. The internal genital tract was isolated within 15 min post-mortem, and placed in a chilled styrofoam box. Within a maximum of 8 h, the reproductive tracts were examined and processed at the University of Copenhagen.

The estrous cycle stage was determined by evaluation of the ovaries for presence of follicles and corpus luteum. A mare was regarded to be in follicular phase when one or more follicles with a diameter of >25 mm were present with an absence of a corpus luteum, and in luteal phase when a corpus luteum was present. Only uteri from mares that had follicles >25 mm or a corpus luteum, were non-pregnant, and free from endometrial or ovarian tumors and other internal genital tract gross pathology, except uterine cysts, were included (n = 82).

Transmural samples were excised surgically from the ventral aspect of the base of both uterine horns. The samples were fixed in 10% neutral buffered formalin for nine to ten days, after which the tissue was trimmed to a rectangular form with a length of approximately 20 mm. The samples were placed in cassettes, post-fixed in 10% formalin for an additional six days after which they were processed, paraffin embedded, sectioned at 1–2 μm and stained with hematoxylin and eosin (H&E).

2.2. Histology

The sections were evaluated under light microscopy (Olympus BX40 microscope), by one evaluator (NM), who was blinded to origin of the sample, and assessed for five specific degenerative parameters: 1) Layers of periglandular fibroblasts, 2) Glandular nests, 3) Dilated glands, 4) Degree of glandular dilation, 5) Lymphatic lacunae. Furthermore, the presence of polymorphonuclear leukocytes (PMNs) was noted in five random locations for inclusion in the modified Kenney and Doig categorization.

Initially the entire endometrial sample was evaluated at magnification 100. The number of glandular nests and dilated glands was counted per viewing field while moving across the endometrium and the mode (i.e. the number that appeared the most often) was noted. Level of glandular dilation was assessed (none/mild, moderate, pronounced), and presence of lymphatic lacunae were noted. The entire endometrium was then re-evaluated at magnification 400 for layers of periglandular fibroblasts (Table 1).

Each endometrial sample was assigned to category I, IIA, IIB or III based on the histopathologic parameters, according to the modified Kenney and Doig grading system [1]. The assignment of category did not include breeding history or gynecological examination data as these data were unavailable. For paired endometrial samples with a difference in the modified Kenney and Doig categorization it was noted if the difference was due to layers of periglandular fibroblasts, degenerative glandular changes, inflammation or lymphatic lacunae.

Each endometrial sample was scored for the five specific degenerative parameters individually, as per criteria in Table 1. Each endometrial sample was given a cumulative degenerative score, by transcribing the scores of each degenerative parameter (mild = 0, moderate = 1, severe = 2, absent = 0, present = 1), and summing up the scores from all five parameters (cumulative scores: 0–9).

2.3. Statistical analysis

Data were analyzed using the R statistical software version 4.2.1 (https://www.r-project.org). by use of packages "epitools" (https://CRAN.R-project.org/package=epitools), "LMMstar" (https://CRAN.R-project.org/package=LMMstar), and "publish" (https://CRAN.R-project.org/package=publish). Significance was defined as P-values <0.05, and values between 0.05 and 0.07 were considered a trend.

Agreement of modified Kenney and Doig category was considered good if the paired endometrial samples from a mare were assigned to the same category; moderate if the paired samples were assigned to adjacent categories (e.g., I and IIA); and poor if the paired samples varied with

Table 1
Parameters and scoring values used in analysis of endometrial biopsies.

Parameter	Magnification[a]	Scoring		
		None/Mild	Moderate	Severe
Layers of periglandular fibroblasts	x400	0–3	4–10	>10
Glandular nests per field	x100	0–2	3–4	>4
Dilated glands per field	x100	0–3	4–6	>6
Degree of glandular dilation	x100	None/Mild	Moderate	Pronounced
Parameter		Absent		Present
Lymphatic lacunae	x100	–		+

[a] The magnification is calculated by multiplying the ocular magnification (10) with the lens magnification used (either 10 or 40).

two or three categories. For statistical analysis, moderate and poor agreements were considered disagreement and grouped together. Two multiple logistic regression models were applied to analyze possible association between disagreement of modified Kenney and Doig category and risk factors. Risk factors included in the first model were age (years, continuous), type (horse, pony) and estrous cycle stage (estrus, diestrus). Risk factors included in the second model were age (categorical), type (horse, pony) and estrous cycle stage (estrus, diestrus). Age categories were designated as young (1–10 years; n = 31); middle-aged (11–17 years; n = 25) and old (18–29 years; n = 24). The model covariates each individual risk factor to all other risk factors included.

Comparison between paired endometrial samples for any specific parameter was defined as agreement if the score was the same in paired endometrial samples (0), and disagreement if the score was different between paired endometrial samples (1).

Agreement within each of the six degenerative parameters was analyzed descriptively and with 95% confidence intervals (binomial test). Two multiple logistic regression models were applied for each parameter to analyze possible risk factors association with number of mares with disagreement between the paired endometrial samples. Risk factors included in the first and second model were the same as described for the previous analysis of agreement of modified Kenney and Doig category. Backwards stepwise regression was performed and based on Akaike Information Criteria, risk factors were eliminated from both models, which were reanalyzed with the remaining risk factor.

Descriptive analysis and two multiple generalized linear regression models for count data (i.e., number of parameters with disagreement) were applied to analyze possible risk factors association with number of degenerative parameters with disagreement between the paired endometrial samples. Risk factors included in the first and second model were the same as described for the previous analysis of agreement of modified Kenney and Doig category.

The agreement between the cumulative scores in the paired endometrial samples was analyzed by an equivalence test, using the absolute difference in cumulative scores, with the non-inferiority margin set at 1.

3. Results

3.1. Sample population

Eighty-two mares fulfilled the inclusion criteria. Their age ranged from 1 to 29 years (mean = 13.4 years, with lower quadrant at 8.0 years and upper quadrant at 18.3 years (two mares with unknown age were excluded from analyses including "age")). For the young mares the median age was 7 years (mean = 6.6 years, with lower quadrant at 4.5 years and upper quadrant at 9 years), for the middle-aged mares the median age was 15 years (mean = 14.7 years, with lower quadrant at 14.0 years and upper quadrant at 16.0 years), for the old mares the median age was 21 years (mean = 21.0 years, with the lower quadrant at 19 years and the upper quadrant at 22 years). The mare population had an equal distribution of mares in follicular phase (n = 41) and luteal

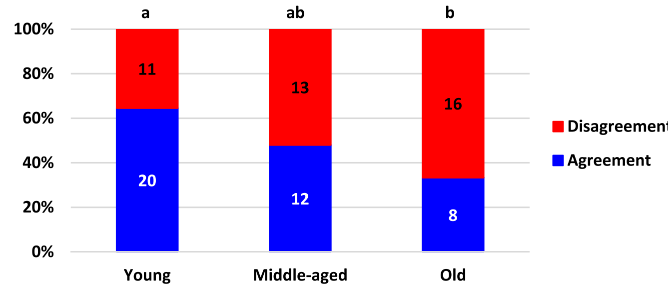

Fig. 1. Distribution of mares with agreement and disagreement in the modified Kenney and Doig categorization within the three age groups.
Categorical age groups are defined as young: 1–10 years, middle-aged: 11–17 years and old: 18–29 years. [a,b] groups with different subscript have statistically different proportions of mares with agreement vs. disagreement, $P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Multiple logistic regression model with risk factors associated with disagreement of modified Kenney and Doig categorization between the paired endometrial samples.

Risk factor	Level	Odds Ratio	95% CI	P-value
Mare age (years)	Continuous	1.11	1.03–1.19	0.009
Type	Horse	Reference		
	Pony	0.86	0.21–3.57	0.835
Estrous cycle stage	Estrus	Reference		
	Diestrus	0.65	0.25–1.69	0.376
Mare age (categorical)	Young	Reference		
	Middle-aged	2.00	0.67–5.94	0.214
	Old	3.93	1.25–12.38	0.019
	Middle-aged	Reference		
	Old	1.97	0.60–6.43	0.263

Categorical age groups are defined as young: 1–10 years, middle-aged: 11–17 years and old: 18–29 years.

Table 2
Grouping of 82 mares based on agreement in modified Kenney and Doig categorization between the paired endometrial samples.

Group	Modified Kenney and Doig category	Number (%)	Dissimilarities in (Number):			
			PGF	DGC	IFLM	LL
Good agreement	I	7 (8.5)				
	IIA	21 (25.6)				
	IIB	10 (12.2)				
	III	2 (2.4)				
Subtotal		40 (48.8)				
Moderate agreement	I/IIA	7 (8.5)				
	IIA/IIB	23 (28.0)				
	IIB/III	8 (9.8)				
Subtotal		38 (46.3)	22[a]	11	4	1
Poor agreement	I/IIB	3 (3.7)				
	IIA/III	1 (1.2)				
	I/III	0 (0.0)				
Subtotal		4 (4.9)	3[b]	1	0	0

Disagreement was due to differences in 'Layers of periglandular fibroblasts' (PGF), degenerative glandular changes (DGC), inflammation (IFLM) or 'Lymphatic lacunae' (LL).
[a] Of which three mares also had a difference in degenerative glandular changes, and six in lymphatic lacunae.
[b] Of which all three mares also had a difference in degenerative glandular changes and two in lymphatic lacunae.

Table 4
Number of mares with disagreement within each parameter, effect of age as continuous factor, and difference between young and old mares.

Parameter	Disagree-ment	Binomial test	Multiple log. regression Risk factor age (continuous)	Multiple log. regression Risk factor age (young vs. old)
	Number of mares (%)	95% CI in %	OR, (95% CI), P-value	OR, (95% CI), P-value
Layers of periglandular fibroblasts	25 (30.5)	20.8–41.6	1.07, (0.99; 1.16), 0.083	2.06, (0.63; 6.69), 0.231
Glandular nests per field	8 (9.8)	4.3–18.3	1.16, (1.01; 1.32), 0.037	10.0, (1.11; 89.91), 0.040[a]
Dilated glands per field	9 (11.0)	5.1–19.8	1.16, (1.02; 1.32), 0.027	3.82, (0.67; 21.72), 0.131
Degree of glandular dilation	21 (25.6)	16.6–36.4	1.15, (1.05; 1.27), 0.003	6.67, (1.58; 28.26), 0.010
Lymphatic lacunae	30 (36.6)	26.2–48.0	0.95, (0.88; 1.02), 0.177	0.61, (0.20; 1.83), 0.376

OR = Odds ratio, y = year(s) old. Categorical age groups are defined as young: 1–10 years, middle-aged: 11–17 years and old: 18–29 years.
[a] Tendency for old mares to have a higher risk of disagreement than middle-aged mares (P = 0.064).

phase (n = 41). Their type consisted of 72 horses (Standardbreds (n = 36), Warmbloods (n = 19) and other breeds (n = 17)) and 10 ponies (Icelandic horses (n = 7) and other pony breeds (n = 3)).

3.2. Disagreement in Kenney and Doig category

Paired endometrial samples from approximately half of the mares (48.8%) showed good agreement, 46.3% showed moderate agreement and 4.9% showed poor agreement. The primary cause for disagreement was dissimilarities in 'Layers of periglandular fibroblasts' (Table 2).

The distribution of mares with agreement and disagreement of the modified Kenney and Doig categorization within the categorical age groups is shown in Fig. 1. Approximately two thirds of the young mares had agreement, while two thirds of the old mares had disagreement, between the paired samples.

Analysis of risk factors associated with disagreement in modified Kenney and Doig categories between the paired endometrial samples is shown in Table 3. Mare age was the only risk factor that had a significant association with disagreement. The chance for disagreement of modified Kenney and Doig categories between paired endometrial samples increased with 1.11 for each year of mare age. A significant difference was observed between young and old mares, but not between young and middle-aged, or middle-aged and old mares (Table 3).

3.3. Disagreement within each degenerative parameter

Parameters for which most mares had disagreement between the paired endometrial samples were 'Lymphatic lacunae' (36.6%) and 'Layers of periglandular fibroblasts' (30.5%). Parameters for which the lowest number of mares had disagreement were 'Glandular nests per field' (9.8%) and 'Dilated glands per field' (11.0%) (Table 4).

Binomial tests demonstrated a range of expected percentages of mares with disagreement (Table 4). While less than 20% of the mares can be expected to have disagreement between the paired endometrial samples for 'Glandular nests per field' and 'Dilated glands per field', up to 41.6% of the mares can be expected to have disagreement for 'Layers of periglandular fibroblasts'. Table 4 shows the data from the statistical models after backwards stepwise regression was performed, and the models were reanalyzed with the remaining risk factor "age" (continuous and categorical, respectively). Complete data set of initial models can be viewed in supplementary 1.

For each additional year of age, the risk of disagreement between the paired endometrial samples for the parameters 'Glandular nests per field', 'Dilated glands per field' and 'Degree of glandular dilation' increased significantly. The model prediction of disagreement as effect of age for these parameters is visualized in Fig. 2. The risk increased minimally up until 15–20 years of age, for the parameters 'Glandular nests per field' and 'Dilated glands per field', as visualized in Fig. 2. For the parameter 'Degree of glandular dilation' a faster increase in risk was observed at an earlier age than for 'Glandular nests per field' and 'Dilated glands per field' (Fig. 2). There was no significant effect of age as a continuous factor on other parameters.

Old mares had a significantly higher risk of disagreement between the paired endometrial samples in 'Glandular nests per field' and 'Degree of glandular dilation', compared to young mares (Table 4). Compared to middle-aged mares, old mares had a trend of a higher risk of disagreement for 'Glandular nests per field' (P = 0.064). There was no significant difference between these two age groups for any other parameter (all P > 0.210), and no significant difference between young mares and middle-aged mares (all P > 0.087).

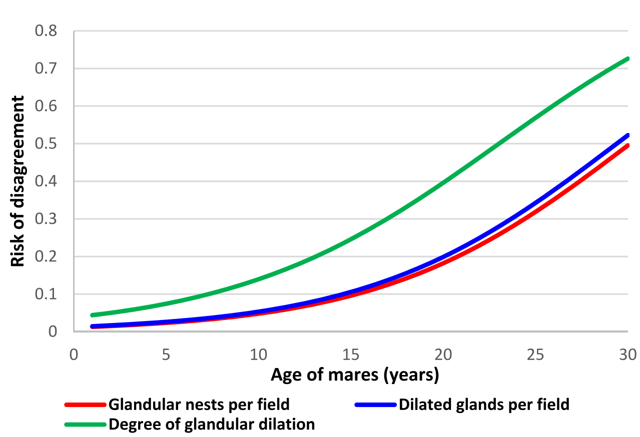

Fig. 2. Model prediction of the risk of disagreement for parameters with significant effect of age. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4. Total number of degenerative parameters with disagreement

Full agreement between paired endometrial samples for all five parameters was found in 28/82 mares (34%). Disagreement in only one parameter was found in 27/82 mares (33%), disagreement in two to four

Table 5
Multiple generalized linear regression model for count data with risk factors associated with number of parameters with disagreement between the paired endometrial samples.

Risk factor	Level	Hazard Ratio	95% CI	P-value
Mare age (years)	Continuous	1.04	1.01–1.08	0.010
Type	Horse	Reference		
	Pony	1.34	0.73–2.49	0.347
Estrous cycle stage	Estrus	Reference		
	Diestrus	1.30	0.84–2.03	0.243
Mare age (categorical)	Young	Reference		
	Middle-aged	1.06	0.61–1.85	0.844
	Old	1.75	1.06–2.87	0.028
	Middle-aged	Reference		
	Old	1.65	0.98–2.78	0.060

Count data represents the number of parameters with disagreement.
Categorical age groups are defined as young: 1–10 years, middle-aged: 11–17 years and old: 18–29 years.

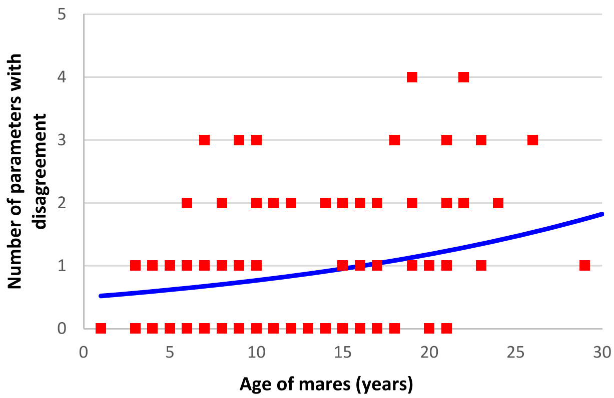

Fig. 3. Model prediction of the number of parameters with disagreement in relation to age.
Red squares depicting mares included in this study. Blue line depicting the prediction curve from the statistical model (Table 5). The figure shows the expected number of parameters with disagreement for each year of age. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

parameters was found in 33% of the mares (two parameters: 21%, three parameters: 10%, four parameters: 2%). No paired specimens differed in five parameters.

Risk factors associated with the number of disagreeing parameters between the paired endometrial samples are presented in Table 5. Age was the only risk factor with significant association. Old mares had significantly more parameters with disagreement, compared to young mares and a trend of more parameters with disagreement than middle-aged mares. The model showed that each additional year of age resulted in an increased risk of disagreement between the paired endometrial samples by 0.04 parameter. A prediction curve was created from the statistical model, depicting the most likely number of parameters, for which a mare will have disagreements between paired endometrial samples at a certain age (Fig. 3).

3.5. Difference in cumulative score

The cumulative scores of the paired endometrial samples were equivalent for young mares and middle-aged mares, but not for old mares (Table 6).

4. Discussion

Our results showed that 48.8% of mares had good agreement in scoring of paired endometrial samples when using the modified Kenney and Doig categorization; a finding that is supported by the findings of Sikora and co-workers, who reported that 51% of mares had good agreement [17]. Other studies have assessed the scoring agreement in three endometrial biopsies per mare and found a lower level of agreement e.g., 29% [15] and 35% [16]. This may be contributed to a statistically increased risk of disagreement when more biopsies are examined unless lesions are diffuse. The level of agreement is probably also affected by other factors such as severity of lesions and study inclusion criteria. Waelchli and Winder [14] found 73.8% agreement when evaluating five endometrial biopsies per mare. However, they did not include mares with grossly visible endometrial cysts. Presence of endometrial cysts is indicative of endometrial degeneration [6,15,19] and by excluding such cases the study population may potentially have comprised of a higher number of mares with normal endometrium or mild endometrial degeneration. This assumption is supported by 52.7% of the included mares having all five samples assigned to category I [14], whereas only 8.5% of mares in our study had both paired samples assigned to this category. The effect of age may partly explain the discrepancy between the prevalence of mares with poor agreement between samples in our study and those analyzed in other studies [14–17]. However, as the studies were not conducted in a similar manner, several other variables exist that may explain the discrepancy in findings. Discrepancies in findings may hypothetically be explained by sample sites being from the dorsal surface of the uterus [17], versus the ventral surface in the present study, and evaluation by one pathologist in the present study [14,16,17], versus two pathologists in other studies [15], for which a high inter-rater variability may have affected the results [27]. Also, the size of the samples varied from punch biopsies [17] to 2 cm excised samples in the present study, [14]. Lefranc and Allen observed a significant difference in endometrial gland surface density between Welsh Pony mares and Thoroughbred mares [28], probing the question if the difference may cause one breed to be more prone to disagreement in severity of degenerative glandular changes. We found no difference in the prevalence of disagreement between horses and ponies, or between mares in estrus and diestrus, and consider these risk factors less likely to explain the discrepancy between studies. However, we cannot exclude that the statistical power was too small ($n = 10$ ponies) or that the groups collectively termed ponies or horses were too diverse, to observe a difference in prevalence of disagreement.

The disagreement in the modified Kenney and Doig categorization between paired endometrial samples was mainly due to a variation in the number of periglandular fibroblast layers. Periglandular fibrosis constitutes an important part of endometrial degeneration. It has been implicated as contributing to embryonic death by altering the endometrial function and causing glandular changes [1,4,6,7,29]. Consequently, periglandular fibrosis is highly weighted in the Kenney and Doig categorization [1]. Adhering to the guidelines by Kenney and Doig for this parameter, 30.5% of mares in the present study had the paired samples assigned to different, although adjacent scores. Even though the observed difference was only one score unit, it would be enough to change the modified Kenney and Doig category and the subsequent fertility prognosis. For all three parameters involving glands, the risk of disagreement increased with age. That, along with a low number of mares with disagreement indicates a homogenous distribution of glandular degenerative changes in young and middle-aged mares. This may reflect glandular changes being caused by periglandular fibrosis, and therefore occurring later in life, resulting in disagreement when the mares age. For 'Lymphatic lacunae', 36.6% of the mares had disagreement between paired endometrial samples, while only one mare had disagreement in the modified Kenney and Doig category attributed to this parameter. These findings suggest that the parameter was not responsible for categorization. The disagreement between paired

Table 6
Difference in cumulative score between paired endometrial samples relative to mare age, and agreement between paired endometrial samples.

Difference in cumulative score	0	1	2	3	4	5	Agreement, mean, (95% CI), P-value
Mare age (categorical)							
Young	14	14	2	1	0	0	0.68, (0; 0.90), 0.011
Middle-aged	15	5	5	0	0	0	0.60, (0; 0.88), 0.011
Old	8	10	5	1	0	0	0.96, (0; 1.26), 0.407

Categorical age groups are defined as young: 1–10 years, middle-aged: 11–17 years and old: 18–29 years.

endometrial samples for 'Layers of periglandular fibroblasts' and 'Lymphatic lacunae' were not affected by any risk factors, suggesting that these parameters are unequally distributed in all mares.

As we have no data from gynecological examinations and no knowledge of the mares' reproductive history, we are unaware if the mare population included represents a standard broodmare population. However, the mean age of the mare population was 13.4 years, which is comparable to the broodmare population of Thoroughbreds reported by Weatherbys (2022) in Ireland (mean = 12.4 years, 67% of mares between 7 and 15 years) and Great Britain (mean = 11.4 years, 67% of mares between 6 and 13 years) [30]. Therefore the results of the present study appears to be applicable for broodmares. Geriatric horses are representing a significant part of the equine population [31], and mares of 21– >25years are represented among active broodmares listed in Weatherbys (2022) for Ireland (n = 761) and Great Britain (n = 210) [30]. Age is considered the most important factor for the onset of endometrial degeneration [4,7,22,25], and several studies have demonstrated a positive correlation between age of the mare and severity of endometrial degeneration [5,24,25]. The indication for obtaining biopsies for diagnosis of endometrial degeneration therefor increases with age. However, as the results of our study show, the risk of disagreement of degeneration between uterine sites also increases with age. Two studies investigating the endometrial conditions of mares >20 years categorized 2% of the mares as I and 13–15% of the mares as IIA [32,33]. In that context, our findings indicate that a categorization of I or IIA may not be accurate in mares older than 17 years. The categorization should be confirmed with a second biopsy obtained from a different part of the endometrium.

Twenty-seven mares (33%) had disagreement in at least two of the five specified degenerative parameters, with an increase in disagreement for each additional year of age. This is in contrast to a study by Hanada and co-workers, where the prevalence of disagreement decreased from 7 to 12 years to older ages [19]. However, that study did not differentiate between parameters or severity of endometrial degeneration. It is possible that endometrial degeneration was present at all sites, but it may not have been the same parameters present and not of a similar severity. Our results showed that even when endometrial degeneration was present in both uterine horns, the severity sometimes differed. In contrast to Ricketts and Alonso, who found that mares up to nine years of age had no signs of endometrial degeneration [22], the present study found disagreement in one parameter, and thus endometrial degeneration was present in at least one uterine horn, in mares as young as three years old. This is in line with the results from Rua and co-workers, and Schilling categorizing some mares younger than 5 or 10 years (respectively) as IIB (7.4% or 17%, respectively) and III (3% or 3.6%, respectively) [25,33].

5. Conclusion

Comparison of degenerative changes in the endometrium of the ventral surface of the uterine horns revealed a disagreement in severity in a modified Kenney and Doig categorization, for individual degenerative parameters and for the total number of parameters with disagreement. Age had a negative impact on the agreement between the findings. Nevertheless, type and estrous cycle stage had no impact. For both clinical and prognostic purposes, it is important to consider that degenerative changes are unequally distributed between paired endometrial samples. Furthermore, results from this study, suggest that diagnosis of endometrial degeneration based on a single biopsy, particularly in mares over 17 years of age, should be interpreted with caution, and evaluation of two biopsies is recommended.

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Declaration of competing interest

None.

CRediT authorship contribution statement

Natacha D. Muderspach: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Project administration. **Mats H.T. Troedsson:** Methodology, Writing – review & editing, Supervision. **Graça Ferreira-Dias:** Methodology, Writing – review & editing, Supervision. **Jorgen S. Agerholm:** Methodology, Resources, Writing – review & editing, Supervision. **Mette Christoffersen:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2023.09.018.

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