

# Endometrial biopsies of old mares – What to expect?!

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**Summary:** Based on the ageing of the equine population, the number of old mares presented for breeding will also increase. However, aged mares frequently exhibit fertility problems. The objective of this study was a detailed histomorphological characterisation of endometrial biopsies of old mares to identify causes for their reduced fertility. For this purpose 819 endometrial biopsies of 814 old mares (minimum age: 20 years) submitted to the Institute of Pathology (Faculty of Veterinary Medicine, University of Leipzig) between 1993 and 2014 and the related anamnestic data were evaluated retrospectively. The study comprised a detailed histomorphological characterisation of endometrial alterations by haemalaun and eosin (H.E.) stain, the categorisation of the cases according to Kenney and Doig (1986) without or in consideration of the duration of barrenness and, for some randomly selected samples ( $n = 157$ ), an assessment by Picro-Sirius Red stain for further investigation of degenerative angiopathies. Most of the cases were assigned to categories IIb and III (83%) without consideration of the duration of barrenness or category III (68%) in consideration of the duration of barrenness. The categorisation of the cases was based on the occurrence of endometrosis and, to a lesser extent, endometritis. In contrast, an endometrial atrophy or lymphatic lacunae were not observed in any sample. Nearly all biopsies (97%) showed endometrosis which was mostly moderate. Endometritis was diagnosed in 49% of all samples. The inflammation was predominantly mild and non-suppurative (subclinical). Histopathological findings which are not considered in the categorisation system of Kenney and Doig (1986) were frequently detected. This comprised degenerative lesions of blood vessels (angiosclerosis; 89% of all samples; mostly moderate to severe), periglandular accentuated mononuclear inflammatory cell infiltrates (PAMC; 58% of all samples; mostly mild), perivasculitis (43% of all samples; mostly mild) and 16% of all samples showed signs of endometrial maldifferentiation. The endometrium of old mares predominantly exhibited a mixture of endometrial alterations: mostly marked degenerative lesions (endometrosis and angiosclerosis), predominantly mild inflammatory processes (endometritis, PAMC and perivasculitis) and, to a lesser extent, functional disturbances. These findings are only partially considered in the evaluation scheme of Kenney and Doig (1986). However, histopathological findings which were left out of the categorisation scheme so far (e.g. angiosclerosis and other functional disorders than endometrial atrophy) also directly or at least indirectly influence the fertility of a mare. Therefore, these findings as well as the age of the mare have also to be evaluated for a precise prediction of the reproductive potential of the old mare. In any case the endometrial biopsy can be helpful for the owner or the veterinarian to decide whether breeding an old mare is an acceptable risk, especially in mares whose reproduction potential is completely unknown (aged maiden mares and mares with the last foaling a long time ago).

**Keywords:** reproduction, old mares, endometrial biopsy, subfertility, categorisation, age

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## Introduction

Geriatric horses represent a significant share of the equine population and their number seems to increase further (Mellor et al. 1999, Brosnahan and Paradis 2003, Cole et al. 2005, USDA 2007, McGowan et al. 2010a, 2010b, Ireland et al. 2011, USDA 2016). In contrast to women (Gindoff and Jewelewicz 1986, te Velde and Pearson 2002, Videan et al. 2008), mares are able to reproduce despite advanced age (McCue 1991, Horohov et al. 2002, Traub-Dargatz et al. 2006, Burns 2016). Therefore, the number of old mares presented for breeding and reproductive management by veterinarians will also increase (Burns 2016). However, with increasing age reproductive potential declines in mares (Gordon and Sartin 1978, McCue 1991, Ginther 1992, Vanderwall et al. 1993, Kriesten 1995, Heilkenbrinker et al. 1997, Schoon et al. 1997b, Schoon and Schoon 2003, Carnevale 2006, Scoggin 2015). The causes for this association between increasing age and decline in fertility are numerous and multifactorial (Scoggin 2015) but uterine pathology has been implicated as a major cause of age-associated subfertility

(Carnevale 2006). Besides the age of the mare, the number of foalings also has an effect on the occurrence of endometrial alterations and both factors do not cause the same lesions (Bracher et al. 1997a). Fertility problems are common in old mares and therefore breeding an aged mare is often challenging (King 1999, Pycock 2003, Smith Thomas 2007). As a consequence, these animals should be evaluated carefully before breeding (Carnevale 2006). In this context the uterine biopsy can be useful to predict the ability of the mare to carry a foal to term and therefore can help the owner to determine whether breeding the mare is an acceptable risk (Carnevale 2006). The categorisation system of Kenney and Doig (1986) is accepted internationally for predicting the expected foaling rate of a mare based on histopathological findings and the duration of barrenness. However, certain factors affecting the reproductive potential (the age of the mare, endometrial alterations such as angiosclerosis) are not considered (Schoon et al. 1997b, 1997c, Schoon and Schoon 2003).

The aim of this study was a detailed histomorphological characterisation of endometrial biopsies of old mares and categorisa-

tion according to the system of Kenney and Doig (1986). Furthermore, correlations among the histopathological findings and the anamnestic data should be demonstrated. Finally, the causes for sub-/infertility in old mares and the usefulness of the endometrial biopsy in this context should be pointed out.

## Material and Methods

819 endometrial biopsies and related anamnestic data of 814 old mares (minimum age: 20 years) were analysed retrospectively. All samples were submitted to the Institute of Pathology (Faculty of Veterinary Medicine, University of Leipzig) between 1993 and 2014 for routine examination. The fixed tissue specimens were embedded in paraplast using an automatic processor (Hypercenter XP, Shandon, Frankfurt, Germany), sectioned at 3–4 µm thickness and stained with haemalaun and eosin (H.E.). All samples were characterised histomorphologically in detail with regard to inflammatory processes (endometritis, periglandular accentuated mononuclear inflammatory cell infiltrates (PAMC), ((peri-)vasculitis), degenerative lesions (endometrosis, angiosclerosis, lymphatic lacunae) and functional disturbances (irregular endometrial differentiation (ID), unequal endometrial differentiation (UD), endometrial atrophy, endometrial inactivity during the breeding season). Additionally, 157 samples with angiosclerosis were selected randomly for further investigation of degenerative angiopathies by Picro-Sirius Red stain. Finally, endometrial biopsies were graded according to the categorisation system of Kenney and Doig (1986) in/without consideration of the duration of barrenness of the mare. The statistical evaluation of the data was performed using the software "IBM SPSS Statistics 25®". The Chi-Quadrat-Test according to Pearson and the Fisher's exact-test were applied to determine a significant coherence. A p-value ≤ 0.05 was consistent with a statistically significant result.

## Results

### General characteristics of the examined geriatric mares

The average age of the animals was 21.4 years, the oldest mare was 32 years old. However, 93% of all mares were younger than 25 years. For 429 mares (53%) details about the reproductive status (number of foalings) were available. 8% of the horses had never given birth to a foal and 12% of the horses only had foaled one time, respectively. The other mares (80%) had produced at least 2 foals in their life time with a maximum of 20 offspring. The duration of barrenness was known for 622 mares (76%). Only 6% were not barren at the time of sample collection. The vast majority of the individuals (94%) had been unsuccessfully bred in the past whereby 59% of the mares had been barren for at least 2 years.

### Categorisation according to Kenney and Doig (1986)

The results of categorisation without consideration (blue bars) and in consideration (red bars) of the duration of barrenness of the mare is provided in Fig. 1.

A categorisation exclusively based on the histopathological findings without consideration of the time of barrenness was achievable for 816 samples. In 3 of the 819 samples the occurrence of endometritis and/or endometrosis (criteria of the categorisation system) could not definitely be assessed due to widespread artefacts. As shown in Fig. 1, approx. a similar percentage of biopsies (40% and 43%, respectively) was classified both to category IIb and III. Category IIa was less frequently diagnosed (15%) and only 2% of the samples were assigned to category I.

The duration of barrenness of the mare was known in 625 cases. Therefore, in these cases, the categorisation was performed in consideration of the length of barrenness. More than 2/3 of the samples (68%) were assigned to category III. Category IIb was diagnosed in 24% of all cases while categories IIa and I were rarely diagnosed (6% and 2%, respectively).

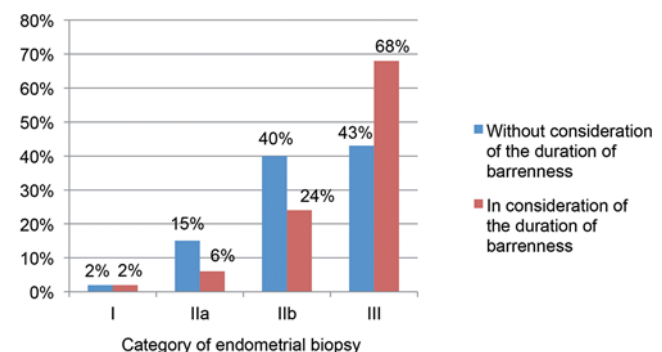
### Histopathological findings

The biopsy specimens showed a variety of histopathological findings (inflammatory processes, degenerative lesions and functional disturbances; see Fig. 2) which could only partially be considered in the categorisation system of Kenney and Doig (1986).

Histopathological findings considered in the categorisation system

#### (Partially) reversible findings

**Endometritis:** An endometritis was diagnosed in 49% of the biopsies. The occurrence of endometritis could not definitely be assessed in 3 samples due to widespread artefacts and every second endometrium did not show an endometritis. The inflammation was mostly (76%) mild. A moderate and severe endometritis was diagnosed in 21% and 3% of the cases, respectively. According to the cellular composition (cellular character) most of the biopsies showed a non-suppurative (subclinical) endometritis (73%). A mixed-cellular and suppurative inflammation was seen in 19% and 7%, respectively. In contrast an eosinophilic and granulomatous endometritis was



**Fig. 1** Categorisation of the endometrial biopsies of old mares according to Kenney and Doig (1986) without consideration (blue bars) and in consideration (red bars) of the duration of barrenness. *Kategorisierung der Endometriumbiopate alter Stuten gemäß Kenney und Doig (1986) ohne (blaue Balken) und mit (rote Balken) Berücksichtigung der Günstzeit*

rarely observed (3% and 1%, respectively). In some cases different forms of endometritis were detected in the same biopsy.

**Irreversible findings**

**Endometriosis:** Endometriosis was detected in almost all biopsies (97%). Only 3% of all biopsies showed no signs of endometriosis. Due to widespread artefacts the occurrence of endometriosis could not definitely be assessed in 3 samples. Moderate lesions dominated (60%), a mild (27%) or severe (12%) endometriosis was less frequently observed. In 70% of the cases at least one endometrotic focus in the sample showed signs of epithelial degeneration (destructive endometriosis). The endometriosis was mostly inactive (77%), mixed (22%) and especially active forms (1%) were less frequently detected. A stromal fibrosis was seen in only 5 samples.

**Lymphatic lacunae:** Lymphatic lacunae were not observed in any sample.

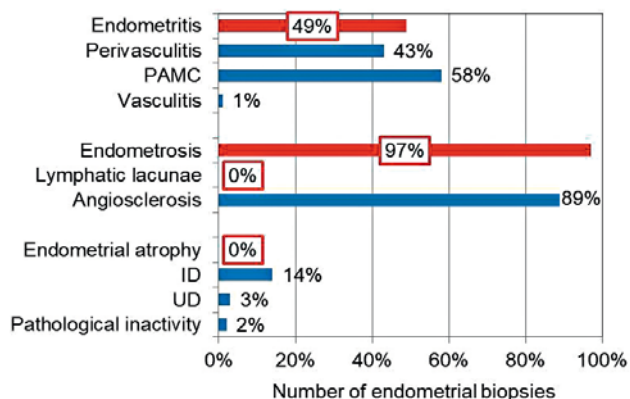
**Reversibility debatable**

**Endometrial atrophy:** No sample showed an endometrial atrophy.

Histopathological findings which are not considered in the categorisation system

**Perivasculitis:** A perivasculitis was diagnosed in 43% of all samples. The inflammation was predominantly mild (67%) and non-suppurative (94%). A moderate or severe perivasculitis was less frequently observed (21% and 12%, respectively). In most cases (73%) more than one vessel was affected.

**PAMC:** In 58% of the samples periglandular accentuated mononuclear inflammatory cell infiltrates (PAMC) were detected which were mostly mild (53%) or moderate (35%).

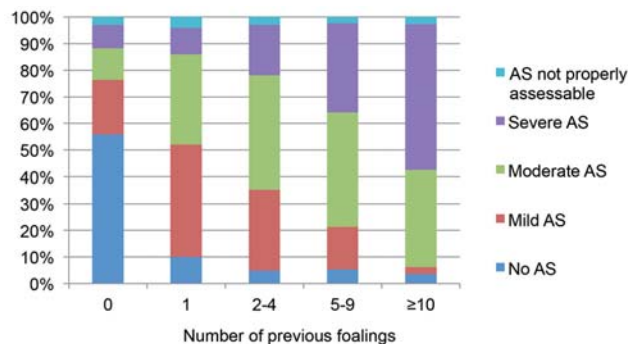


**Fig. 2** Incidence of histopathological findings in the examined endometrial biopsies: histopathological alterations in red colour are considered in the categorisation system of Kenney and Doig (1986), whereas the alterations in blue colour are left out. PAMC: periglandular accentuated mononuclear inflammatory cell infiltrates; ID: irregular endometrial differentiation; UD: unequal endometrial differentiation | *Inzidenz der histopathologischen Befunde in den untersuchten Endometriumbiopsaten: histopathologische Alterationen in roter Farbe werden im Kategorisierungssystem von Kenney und Doig (1986) berücksichtigt, während die Alterationen in blauer Farbe nicht berücksichtigt werden. PAMC: periglandulär akzentuierte mononukleäre Entzündungszellinfiltrate; ID: irreguläre glanduläre Differenzierung; UD: ungleichmäßige glanduläre Differenzierung*

**Vasculitis:** In 9 samples (1%) a vasculitis was diagnosed.

**Angiosclerosis:** Angiosclerosis was detected in 89% of all samples by routine examination (H.E.). Only in 66 specimens (8%) no degenerative lesions of blood vessels were seen. In 3% of the samples this finding could not definitely be assessed. The lesions were generally classified as moderate (45%) or severe (37%), only 18% exhibited mild angiosclerosis. In the subsequent assessment of the 66 biopsies without angiosclerosis (in H.E. stained slides) by Picro-Sirius Red stain degenerative lesions of blood vessels were detected in nearly all biopsies.

**Functional disturbances:** 16% of all biopsies showed at least one type of endometrial maldifferentiation. An irregular endometrial differentiation was observed in 14% of all samples and represented the most frequent manifestation of maldifferentiation. In contrast, unequal endometrial differentiation and endometrial inactivity during the breeding season were detected only in 3% and 2% of all cases, respectively. During the breeding season (01/05–31/08, definition according to Killisch et al. 2014) 17% of all samples showed an endometrial maldifferentiation (ID: 11%; UD: 4%; endometrial inactivity: 2%).



**Fig. 3** Association between parity of the mare and incidence and degree of angiosclerosis (AS) | *Zusammenhang zwischen Parität der Stute und der Inzidenz von Angiosklerosen (AS) und deren gradueller Ausprägung*

**Table 1** Categorisation of endometrial biopsies without consideration of the duration of barrenness: influencing factor age of the mare (own results and references) | *Kategorisierung der Endometriumbiopsate ohne Berücksichtigung der Günstzeit: Einflussfaktor Stutenalter (eigene Ergebnisse und Literaturangaben)*

Age of mares in years	Number of endometrial biopsies				Source
	I	IIa	IIb	III	
25–32	4%	4%	41%	52%	Own results
20–24	2%	15%	40%	43%	
>20	2%	13%	37%	48%	Schilling (2017)
1–5	43%	36%	17%	3%	
17–24	7%	29%	50%	14%	Brinsko et al. (1994)
2–7	32%	63%	5%	0%	
≥12	31%	40%	26%	3%	Waelchli (1990)
≤11	70%	21%	4%	5%	

### Influencing factor: age

Older mares ( $\geq 25$  years) were more frequently assigned to the prognostic unfavourable category III and less often to category IIa than mares in their early twenties (see Table 1, differences not statistically significant,  $p = 0.08$ ).

A statistically significant correlation between the age of the mares and the incidence and degree ( $p = 0.199$ ) or quality ( $p = 0.26$ ) of endometrosis was not detected. However, the following tendencies were observed: Older mares ( $\geq 25$  years) showed more frequently moderate to severe endometrosis and destructive forms of endometrosis than younger animals (see Table 2).

Functional disturbances were more often detected in the older age group (24%) than in younger animals (16%) although these differences were not statistically significant ( $p = 0.113$ ). The incidence of perivasculitis did not differ between the two age groups ( $p = 0.968$ ). There was no statistically significant association between the age of the mares and the incidence and degree of angiosclerosis ( $p = 0.439$ ), endometritis ( $p = 0.490$ ) and PAMC ( $p = 0.747$ ).

### Influencing factor: parity

There was no statistically significant correlation between parity of the mare and the categorisation of her endometrial biopsy without consideration of the duration of barrenness ( $p = 0.407$ ; nulli- and uniparous mares pooled).

Only a few biopsies did not show endometrosis independent of the number of foalings. Furthermore, there was no statistically significant association between parity and degree of endometrosis ( $p = 0.684$ ). A statistically significant association between the number of previous foalings and incidence and degree of perivasculitis ( $p = 0.568$ ), PAMC ( $p = 0.446$ ) and endometritis ( $p = 0.759$ , moderate and severe endometritis pooled) was not observed in this study. There was a statistically significant correlation between the occurrence of the functional disturbances and parity of a mare ( $p = 0.036$ ). 35% of the nulliparous mares showed an endometrial maldifferentiation while the incidence in the other foaling groups varied between 12–16%. Moreover, a statistically significant association was

detected between the parity of the mare and the incidence and degree of angiosclerosis ( $p = 0.001$ ; see Fig. 3). More than half of all nulliparous mares showed no degenerative lesions of blood vessels. However, severe angiosclerosis was also seen in these mares. Despite these quantitative distinctions there were also qualitative differences in angiosclerosis detected by Picro-Sirius Red stain. The vessels of nulliparous mares had only very mild lesions within the intima and the media while the adventitia was partially marked altered. In contrast, even uniparous animals had generally marked alterations within the intima and the media. Nulliparous mares predominantly showed a fibrosis/fibroelastosis and it seemed that the amount of elastic fibers increased with the number of foalings. In mares with at least 10 offspring the lesions of the vascular wall predominantly consisted of elastic fibers.

### Special anamnestic characteristics of the examined geriatric mares

The comprehensiveness of anamnestic data was quite variable in the individual case. Many mares (39%) showed pathological findings of the reproductive system in the clinical examination. These primarily included endometrial cysts (20%), intrauterine fluid accumulations (12%), and anatomical abnormalities (e.g. changes in the vulvar conformation, 14%). 7% of all mares had exhibited aberrations of the cyclic activity, especially a missing cycle/oestrus and an irregular, prolonged or shortened cycle. However, these mares did not show functional disturbances more often than animals with a regular oestrus cycle. 13% of all mares had at least one embryonic/fetal loss in the past. Some animals (6%) had given birth to a foal in the year of the examination. 14 animals were retired sport mares (partially maiden mares).

## Discussion

The aim of the current study was the analysis and histomorphological characterisation of endometrial biopsies of old mares. Besides the age of the mare, the number of foalings also has an effect on the occurrence of endometrial alterations (Bracher et al. 1997a). A distinct differentiation between these two factors is almost impossible because a mare will always age during pregnancy.

The categorisation system of Kenney and Doig (1986) is an internationally accepted evaluation scheme to predict the ability of a mare to carry a foal to term (Schoon et al. 1992, Schoon and Schoon 2003, Aurich et al. 2011). There is a wide age range in each category (Doig et al. 1981, Ricketts and Alonso 1991). However, the age of a mare has a significant effect on the category to which she is assigned. As shown in Table 1, advanced age is associated with classification in prognostic unfavourable categories (Doig et al. 1981, Waelchli 1990, Ricketts and Alonso 1991, Brinsko et al. 1994, Kriesten 1995, Heilkenbrinker et al. 1997, Aurich et al. 2011, Schilling 2017). In the present study, the vast majority of examined endometria also belonged to categories IIb and III without consideration of the duration of barrenness or category III in consideration of the duration of barrenness. These results are comparable to the conclusions of Schilling (2017), whereas in the study of Brinsko et al. (1994) aged mares were mostly assigned to categories IIa and IIb (see

**Table 2** Characterisation of endometrosis: influencing factor age of the mare | Charakterisierung der Endometrose: Einflussfaktor Stutenalter

Characterisation of endometrosis		Number of endometrial biopsies	
		Age of the mares 20–24 years	Age of the mares $\geq 25$ years
Quantity	No	3%	4%
	Mild	27%	15%
	Moderate	58%	65%
	Severe	12%	17%
Quality	Nondestructive	31%	23%
	Destructive	69%	77%

Table 1). These differences are likely explained by the different classification of the age groups. Furthermore, in this study, mares of the older age group were more frequently classified as category III mare than animals in their early twenties.

Old mares showed a variety of histopathological findings. However, some of these alterations are not considered in the categorisation system of Kenney and Doig (1986). The categorisation of old mares is particularly based on the occurrence of endometrosis which is a degenerative, irreversible and clinical silent disease (Bracher et al. 1997a, Schoon et al. 1997b, Aurich and Palm 2009). Endometrosis can only be detected by histopathological examination (Schoon et al. 1995, 1997b). Nearly all samples showed endometrosis which was mostly moderate. The incidence and the degree of endometrosis in this study were higher than in the investigations of Schoon et al. (1997b) and Schilling (2017) (see Table 3). These results confirm the assumption of endometrosis as an age-related disease (Doig et al. 1981, Ricketts and Alonso 1991, Brinsko et al. 1994, Schoon et al. 1994, Flores et al. 1995, Bracher et al. 1997b, Schoon et al. 1997b, Hoffmann 2006, Lehmann 2010, Aurich et al. 2011) and moreover, the degree of endometrosis increases with ageing (Schoon et al. 1995, Heilkenbrinker et al. 1997, Schilling 2017). The occurrence of endometrosis in infertile (“nulliparous”) female mules (Huth et al. 2008, Jäger et al. 2008) is further indicative of an age-related pathogenesis which is independent of parity. The marked individual disparities in quantity of endometrosis in this and former studies (Doig et al. 1981, Ricketts and Alonso 1991) and the low incidence of severe forms in this study despite advanced age of the animals indicate that other factors than age, e.g. genetics (Schilling 2017), may play an important role in the pathogenesis of endometrosis. As shown, a correlation to the number of previous foalings does not exist (Schoon et al. 1994, 1995, Bracher et al. 1997b, Schoon et al. 1997b, Hoffmann 2006, Schilling 2017). Destructive forms of endometrosis which were frequently detected in this study are associated with a poor breeding prognosis (Schoon et al. 1997b, Lehmann et al. 2011).

Endometritis being part of the categorisation schema is a (partially) reversible lesion (Brunckhorst and Schoon 1990, Schoon et al. 1992, 1994, 1997b). The incidence of endometritis in old mares is higher than in the investigations of Schoon et al. (1997b) and Schilling (2017) (see Table 3). Endometritis occurs more often in older mares than in younger animals (Carnevale and Ginther 1992, Schilling 2017). The increased susceptibility of older mares to uterine infections may be caused by failure of natural uterine defensive mechanisms and/or inadequate physical clearance (McCue 1991). According to Schilling (2017) the parity of a mare has also an effect on the occurrence of endometritis and maiden mares are least often affected. Such a correlation could not be established in this study. This is likely to have been caused by the special fertility problems of old maiden mares. These mares are often susceptible to persistent breeding induced endometritis as a result of an abnormal tight cervix and consequential intrauterine fluid accumulations (Pycock 2003, Carnevale 2006).

Lymphatic lacunae and endometrial atrophy (part of the categorisation system) seem to play only a minor role in old mares (see Table 3). In contrast, histopathological findings which are not considered in the categorisation system and have a direct or at least an indirect impact on fertility prognosis are frequently diagnosed in old mares.

Most biopsies exhibited moderate to severe angiosclerosis. Incidence and degree of angiosclerosis in old mares is higher than in the studies of Schoon et al. (1997b) and Schilling (2017) (see Table 3). Degenerative angiopathies seem to have no direct but an indirect negative effect on fertility of the mare (Schoon et al. 1997a, 1999a, Schoon and Schoon 2003). The alterations are closely related to endometrosis and result in a decreased endometrial perfusion and drainage (Schoon et al. 1997a, 1999a, Schoon and Schoon 2003). Blaich et al. (1999) demonstrated by Doppler sonography that a marked fibrosis in the A. uterina is associated with an enhanced vascular resistance. Furthermore, endometrial angiopathies have

**Table 3** Incidence and degree of different histopathological lesions: own results and references | Inzidenz und Ausprägungsgrad der unterschiedlichen histopathologischen Befunde: eigene Ergebnisse und Literaturangaben

Histopathological lesion	Incidence of the histopathological lesion (predominant degree)		
	Own results	Schoon et al. (1997b) n=2500	Schilling (2017) n=11698
Endometrosis	97% (++)	60% (+)	76% (+)
Endometritis	49% (+)	35%	38 or 46% (without or in consideration of inflammatory cell infiltrates; +)
Lymphatic lacunae	0%	3%	–
Angiosclerosis	89% (++)/+++)	80%	59% (+/++)
Functional disturbances	Breeding season: 17% – ID: 11% – UD: 4% – endometrial inactivity: 2% – endometrial atrophy: 0%	Whole year: ca. 13–14% – endometrial atrophy: ca. 3–4%	Breeding season: 18% – UD and/or ID: 16% – endometrial inactivity: 1% – endometrial atrophy: 1%
Perivasculitis	43% (+)	40%	

+ : mild; ++ : moderate; +++ : severe; n: number of endometrial biopsies; ID: irregular endometrial differentiation; UD: unequal endometrial differentiation | + : geringgradig; ++ : mittelgradig; +++ : hochgradig; n: Anzahl untersuchter Endometriumbiopsate; ID: irreguläre glanduläre Differenzierung; UD: ungleichmäßige glanduläre Differenzierung

a negative effect on fetal placental development (Kersten 2000, Schoon and Schoon 2003). Mares suffering from angiosclerosis have a lower density and branching of the chorionic villi (Kersten 2000). At least two factors, age and parity, have to be considered as causes of angiosclerosis (Schoon et al. 1997a, 1999a, Schoon and Schoon 2003, Schilling 2017) with parity having the greater influence (Kriesten 1995, Schoon et al. 1997a, 1997b, 1999a, Wrede 1999, Schilling 2017). Accordingly, even in old mares a correlation between incidence and degree of angiosclerosis and the number of previous foalings was observed. Old nulliparous mares often showed, as already described by Kriesten (1995), Schoon et al. (1997b) and Grüninger et al. (1998), an angiosclerosis especially affecting the adventitia. This can be interpreted as a predominantly age-related finding (Grüninger et al. 1998). Infertile (“nulliparous”) female mules also exhibit angiosclerosis relating to aging (Jäger 2009). In multiparous animals the alterations affect all layers of the vascular wall and resemble the pregnancy sclerosis in other species (Schoon et al. 1997b, Grüninger et al. 1998, Schoon and Schoon 2003). Older multiparous mares with marked angiosclerosis diagnosed in endometrial biopsies can be identified as risk patients for fatal ruptures of extrauterine vessels during late pregnancy or birth (Ludwig et al. 2001). Some uni-/oligoparous mares in this study showed marked angiosclerosis affecting all layers of the vascular wall and therefore these mares could be risk patients as well. With regard to the age-related declining regeneration capacity of blood vessels (Kriesten 1995, Schoon et al. 1997a) the age of the mare at the time of the last pregnancy could play an important role in this context.

Functional disturbances may lead to permanent or temporary infertility depending on the individual cause (Schoon et al. 1999b, Schoon and Schoon 2003, Ellenberger et al. 2005). Their prognostic significance during winter anoestrus (Aupperle et al. 2003) and transition periods (Killisch et al. 2017) is questionable whereas they are an important finding with impact on the fertility of the mare in the breeding season. There is no association between the occurrence of functional disturbances and the age of the mare (Schoon et al. 1999b, 2000, Schilling 2017). The incidence of functional disturbances during the breeding season in this study is consistent with the incidence of these lesions in routine examinations (Schilling 2017) (see Table 3). Therefore, these alterations seem to be no special problem of old mares except of nulliparous ones. In accordance with Schilling (2017) nulliparous mares frequently showed functional disorders. Possible causes are hormonal treatments (Klug et al. 1997), recent retirement from sport (Kilgenstein et al. 2015) or long non-reproductive periods (Schilling 2017). The negative effect of long non-reproductive periods on fertility is described for nulliparous elephants and rhinoceroses. The prolonged exposure to endogenous sex steroids during these periods induces an asymmetric reproductive ageing with development of genital pathologies and erratic/absent luteal activity (Hermes et al. 2004, 2006).

Perivasculitis which mostly involved more than one vessel in one biopsy and PAMC were also common findings in endometrial biopsies of old mares. In case of multifocal perivasculitis a significant negative effect on fertility is detectable, even in biopsies without any other lesions (Kriesten 1995, Schoon

and Schoon 2003). In contrast, the significance of PAMC is still unknown (Klose 2015, Klose and Schoon 2016).

The endometrium of old mares predominantly exhibits a mixture of alterations: mostly marked degenerative lesions, predominantly mild inflammatory processes and to lesser extent functional disturbances. Many of these findings are not considered in the categorisation system of Kenney and Doig (1986) but have to be regarded as important factors contributing to reduced fertility of old mares. Histopathological findings which were left out of the categorisation scheme so far (e.g. angiosclerosis), the quality and reversibility of lesions and as well as the age of a mare should be also considered for a precise prediction of a mare’s reproductive potential (Schoon et al. 1997b, Schoon and Schoon 2003). Anamnestic data, especially the duration of barrenness, are furthermore important for prediction of the reproductive potential of an old mare. Many old mares had been barren for years and therefore have a poorer fertility prognosis according to the categorisation system of Kenney and Doig (1986). It is recommended to investigate endometrial biopsies of old mares before breeding, especially of animals whose reproduction potential is completely unknown (aged maiden mares and mares with the last foaling a long time ago), for different reasons: marked individual differences in degree of endometrosis and in occurrence of endometrial maldifferentiation, to diagnose and to treat reversible lesions for optimizing the breeding prognosis as well as to help the owner to determine whether breeding the mare is consistent with an acceptable risk.

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