

Histopathological features of endometritis eosinophilica in mares

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Summary. Equine endometritis eosinophilica (EE) is rarely described and its diagnostic criteria are not defined. The aim of this study was to characterize histological features of EE. A data base (1995-2013) was searched for biopsies with increased eosinophils. This study included all biopsies with this diagnosis and representative biopsies without this record. The definition of equine EE was based on criteria for EE in women and the results of the determination of physiological numbers of eosinophils within the equine endometrium. EE was diagnosed in 55 mares. Biopsies of 10 mares contained eosinophils exceeding the physiological range, but no EE; the diagnosis of eosinophilic infiltrates (EI) was applied. Those of the remaining mares (n=126) displayed eosinophils within the physiological range (EWPR). An irregular glandular differentiation during the breeding season was detected in 25% of mares with EE, 33% of mares with EI and 24% of the mares with EWPR. Most mares with EE (93%), EI (90%) and EWPR (72%) showed endometrosis; it was high grade in 11% with EE and 7% with EWPR. Endometritis was diagnosed within 56% of mares with EE, 40% of mares with EI and 37% of those with EWPR. In mares with EE suppurative endometritis dominated (58%) and in those with EWPR non-suppurative endometritis (58%). This study indicates EE as a primary fertility reducing disease. Results suggest an association between eosinophilic infiltration and the presence of neutrophils. Further, they provide the basis

for future studies into the pathogenesis, prognosis and therapy of EE.

Key words: Histology, Endometrium. Endometritis eosinophilica, Equine, Mare

Introduction

Eosinophils are physiological components of several tissues, i.e. the gastrointestinal tract, lungs, skin and the reproductive system (McEwen, 1992; Rothenberg and Hogan, 2006) including the cervix (Wehrend et al., 2005) and endometrium of mares (Brunckhorst et al., 1991; Blüthgen, 2002). They participate in the regulation of tissue homeostasis, contribute to inflammatory reactions and tissue remodeling and are involved in innate and adaptive immunity (Rothenberg and Hogan, 2006).

Eosinophils directly interact with mast cells in a reciprocal way. Mast cells release chemotactic factors that attract eosinophils, whereas eosinophils can regulate mast cell functions by releasing their granules (Schulz, 1997; Rothenberg and Hogan, 2006).

Inflammatory conditions with the presence of eosinophils can be subdivided into those with a known aetiology, i.e. reactive eosinophilic inflammation (McEwen, 1992; Rothenberg and Hogan, 2006) and those with an unknown cause that are sometimes referred to as primary or idiopathic eosinophilic inflammatory conditions (Archer et al., 2006; Mäkinen et al., 2008). In regard to the former, eosinophils play a role in hypersensitivity reactions, parasitic diseases as well as infection with certain virus, fungi and/or bacteria

(McEwen, 1992; Rothenberg and Hogan, 2006). The latter are recognized in different species and can affect one or several tissues. Examples in human beings are eosinophilic oesophagitis (Chang and Anderson, 2008), eosinophilic gastrointestinal disorders (Rothenberg, 2004) as well as eosinophilic endometritis (Desai and Shinagare, 2010) and in horses eosinophilic enteritis (Archer et al., 2006; Mäkinen et al., 2008) and multisystemic eosinophilic epitheliotropic disease (Bosseler et al., 2013).

Endometrial disorders are an important cause of reduced fertility in mares (Kenney and Doig, 1986; Bracher et al., 1997; Schoon et al., 1997) and thus can have a major financial impact on the horse breeding industry. These disorders include periglandular fibrosis (endometrosis) and endometritis (Kenney and Doig, 1986; Schoon et al., 1992, 1997) as well as glandular maldifferentiation (Schoon et al., 2000). Endometritis can be classified into exudative (suppurative), or non-suppurative inflammation (Schoon et al., 1997). Exudative endometritis includes acute and subacute forms (Schoon et al., 1997; Schöniger et al., 2013). By routine gynaecological examination only suppurative endometritis can be recognized, whereas the remaining endometrial diseases are subclinical and require the histopathological examination of an endometrial biopsy (Schoon et al., 1997).

Occasionally, increased numbers of eosinophils can be observed within the endometrium of mares with fungal infections (Hurtgen and Cummings, 1982), pneumouterus or at least conditions predisposing to the entry of air within the uterine lumen (Slusher et al., 1984) or hysteroscopy with air inflation (Schiemann et al., 2001). The terminology "endometritis eosinophilica" was applied as a diagnosis for accumulation of eosinophils within the equine endometrium (Schoon et al., 1997). This lesion was considered predominantly as a primary entity, although the histopathological features of this disease condition have not been described in detail (Schoon et al., 1997). In women, increased numbers of eosinophils within the endo- and/or myometrium have been observed after surgical intervention such as biopsy collection or curettage (Bjersing and Borglin, 1962; Mikó et al., 1988; Desai and Shinagare, 2010).

In regard to the equine endometrium concise diagnostic criteria for endometritis eosinophilica are not defined so far. Their establishment is important to obtain a uniform disease diagnosis by different investigators. This in turn will allow a direct comparison of future investigations into the pathogenesis of endometritis eosinophilica, its impact on fertility and/or response to treatment.

Therefore, the aims of the present study were as follows: The first goal was to obtain reference values for the numbers of eosinophils within the equine endometrium over the course of the endometrial cycle and the second to establish diagnostic criteria for equine endometritis eosinophilica. The third goal was to further

examine the existence of possible predisposing conditions for this disease or sequelae to its occurrence.

Materials and methods

Animals and tissue samples

This retrospective study was performed on equine endometrial biopsies that were submitted for histopathological evaluation and prognosis of fertility between 1995 and 2013 to the Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig. The data base of the institute was searched for cases with the diagnosis of increased numbers of eosinophils within the equine endometrium. This study includes all evaluable cases with this diagnosis and for comparison a representative number of biopsies without this record. In total, biopsies of 191 mares were examined. These were collected during different stages of the endometrial cycle. For all selected cases (n=191), the seasonal time at biopsy collection (breeding season, transitional period or winter anoestrus) was recorded. The age was known for 143 mares and the length of barrenness for 110 mares. The presence/absence of lesions of the genital tract was reported in 60 mares. Information about the treatment of a genital tract disease or covering/insemination within 30 days prior to the biopsy procedure was provided for 43 mares.

To investigate numbers of eosinophils during the course of the endometrial cycle, this study also included endometrial biopsies of 10 adult mares that were collected repeatedly during the same endometrial cycle. Biopsies were retrieved during the breeding season at days 0, 5, 10, 13, 16, 19 and 21 of the endometrial cycle, whereas day 0 was defined as ovulation day.

Histology

Endometrial biopsies had been fixed in 10% buffered formalin (Overlack GmbH, Leipzig, Germany) and embedded in paraplast (Firma Engelbrecht, Medizin- und Labortechnik GmbH, Edermünde, Germany). They had been processed routinely for histological examination. From the biopsies of the retrospective investigation (n=191) haematoxylin-eosin (HE) and picosirius red stained sections were available. All biopsies diagnosed with increased eosinophilic infiltrates were also examined with a Periodic acid-Schiff reaction (PAS reaction) for the presence of intralésional fungal hyphae.

The endometrial biopsies of the 10 mares that had been repeatedly collected during the course of the same endometrial cycle were stained with HE.

Histopathological evaluation

Determination of numbers of eosinophils

Within each endometrial biopsy, eosinophil count

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was recorded in ten 40x high power fields (HPFs) by the use of an Olympus CH-2 microscope within the stratum compactum and the stratum spongiosum. In tissue sections with an uneven distribution of eosinophils 5 HPFs were selected from areas with the highest numbers of eosinophils and 5 HPFs from the remaining areas. In the remaining biopsies 10 randomly selected HPFs were examined. Subsequently, for each biopsy, the mean number of eosinophils per HPF was calculated separately for the stratum compactum and the stratum spongiosum.

Due to the lack of reference values, the physiological range of eosinophils during the course of the endometrial cycle was determined using the biopsies of the 10 mares that were collected repeatedly during the course of the same endometrial cycle. The highest mean values per HPF were 11 eosinophils within the stratum compactum of one mare and 5 eosinophils within the stratum spongiosum of another mare.

These values were applied to biopsies of the retrospective evaluation and eosinophil counts within the stratum compactum and stratum spongiosum were defined as those within the physiological range (EWPR) and those exceeding the physiological range (EEPR). EEPR was characterized by a mean value of >11 eosinophils per HPF within the stratum compactum and >5 eosinophils per HPF within the stratum spongiosum. Biopsies with EEPR were further subdivided into those with endometritis eosinophilica (EE) and those with eosinophilic infiltrates (EI). Since reference values for eosinophilic disease conditions in horses were missing, EE was defined according to definitions reported in the human literature (Chang and Anderson, 2008; Desai and Shinagare, 2010). The criteria for EE and EI are listed in Tables 1 and 2, respectively. EE or EI restricted to the stratum compactum were defined as “superficial”, EE or EI present solely within the stratum spongiosum as “deep” and EE or EI within both layers as “superficial and deep”.

Determination of mast cells numbers

Mast cells were enumerated within biopsies of the retrospective evaluation using the picosirius red stained sections. Mast cells were always evenly distributed within the stratum compactum and the stratum spongiosum. Therefore, in each biopsy their numbers in 10 randomly selected 40x HPFs were counted within the stratum compactum and the stratum spongiosum. For all biopsies of one group, i.e. those with EE, EI and EWPR, the total mean of mast cells per HPF was determined.

Further evaluation of endometrial tissue sections

Biopsies of the retrospective study were also evaluated for the functional morphology of endometrial glands (Kenney and Doig, 1986; Schoon et al., 1992) as well as the presence of endometritis (Kenney and Doig, 1986; Schoon et al., 1992, 1997), periglandular fibrosis

(endometriosis: Schoon et al., 1997) and glandular maldifferentiation (Schoon et al., 1997, 2000).

Endometriosis was further subclassified into destructive, non-destructive or combined destructive/non-destructive as well as active, inactive or combined active/inactive (Hoffmann et al., 2009). A destructive, non-destructive, active or inactive endometriosis was diagnosed if >75% of the glands within the examined biopsy showed the evaluated change, respectively (Hoffmann et al., 2009). In a combined destructive/non-destructive or active/inactive endometriosis a similar number of glands within the evaluated biopsy were affected by each of the two alterations, respectively (Hoffmann et al., 2009).

Endometritis was further classified in acute suppurative, subacute suppurative and non-suppurative as described by Schöniger et al. (2013). A superficial endometritis was defined when inflammation was restricted to the stratum compactum, whereas in deep endometritis only the stratum spongiosum was affected.

Results

Eosinophilic infiltration within endometrial biopsies collected repeatedly during the course of the same endometrial cycle

The age of the 10 mares ranged from 4 to 20 years.

Table 1. Applied criteria for the diagnosis of equine endometritis eosinophilica.

Distribution pattern of endometritis eosinophilica	Definition of endometritis eosinophilica
Focal	One HPF with ≥ 40 eosinophils
Multifocal	Two or more HPFs with ≥ 20 eosinophils
Diffuse	Mean value ≥ 15 eosinophils per HPF ¹
Multifocal to confluent	Two or more HPFs with ≥ 40 eosinophils and mean value ≥ 15 eosinophils per HPF ¹

HPF, high power field, i.e. 40x magnification of a CH-2 Olympus microscope. ¹For evaluation of the mean value per HPF, ten HPFs were enumerated.

Table 2. Applied criteria for the diagnosis of eosinophilic infiltrates.

Location of eosinophilic infiltrates	Definition of eosinophilic infiltrates
Stratum compactum	Mean value >11 eosinophils per HPF No EE within the stratum spongiosum
Stratum spongiosum	Mean value >5 eosinophils per HPF No EE within the stratum compactum

HPF, high power field, i.e. 40x magnification of a CH-2 Olympus microscope. ¹For evaluation of the mean value per HPF, ten HPFs were enumerated.

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The average age was 9.7 years with a standard deviation (SD) of 5.5. For each examined time point, the eosinophil counts varied more or less between the individual mares. Slightly more eosinophils were detected within the stratum compactum than within the stratum spongiosum. The highest individual mean values were 11 eosinophils per HPF within the stratum compactum of one mare and 5 eosinophils per HPF within the stratum spongiosum of another mare (Fig. 1).

In regard to the total mean, within the stratum compactum it was highest during the mid interoestrus (day 13: 2.9 eosinophils per HPF) and lowest during the postoestrus (day 5: 0.2 eosinophils per HPF). Within the stratum spongiosum the highest total mean values of 0.9 eosinophils per HPF were observed during mid and late interoestrus (days 13 and 16) and the lowest count during the postoestrus (day 5: 0 eosinophils per HPF) (Table 3, Fig. 1).

Retrospective evaluation of endometrial biopsies collected once during the stage of the endometrial cycle

Endometritis eosinophila

This disease condition was diagnosed in 55 mares (Figs. 2, 3).

Available anamnestic data: Mares with an EE had an average age of 12.5 years (SD ± 4.3); the age was known for 37 of 55 mares. The mean age of mares within each of the three types of EE (detailed below) was similar, i.e. 12-13 years.

Barrenness at the time of biopsy collection was reported for 34 mares. This information was known for 37 out of 55 mares with EE. The length of barrenness was 2 years in 13 mares (24%).

Per clinical history 2 mares showed an insufficient

Table 3. Total mean eosinophil counts within endometrial biopsies of ten mares during the endometrial cycle.

Endometrium	Endometrial cycle						
	Day 0	Day 5	Day 10	Day 13	Day 16	Day 19	Day 21
Stratum compactum	0.4 SD: 0.6	0.2 SD: 0.3	0.6 SD: 0.8	2.9 SD: 3.6	1.1 SD: 1.4	0.4 SD: 0.5	0.9 SD: 1.3
Stratum spongiosum	0.3 SD: 0.4	0.0	0.4 SD: 0.8	0.9 SD: 1.6	0.9 SD: 0.9	0.4 SD: 0.4	0.7 SD: 1.2

Data reported as total mean eosinophil numbers per 40x high power field; SD, standard deviation. Day 0 defined as ovulation day.

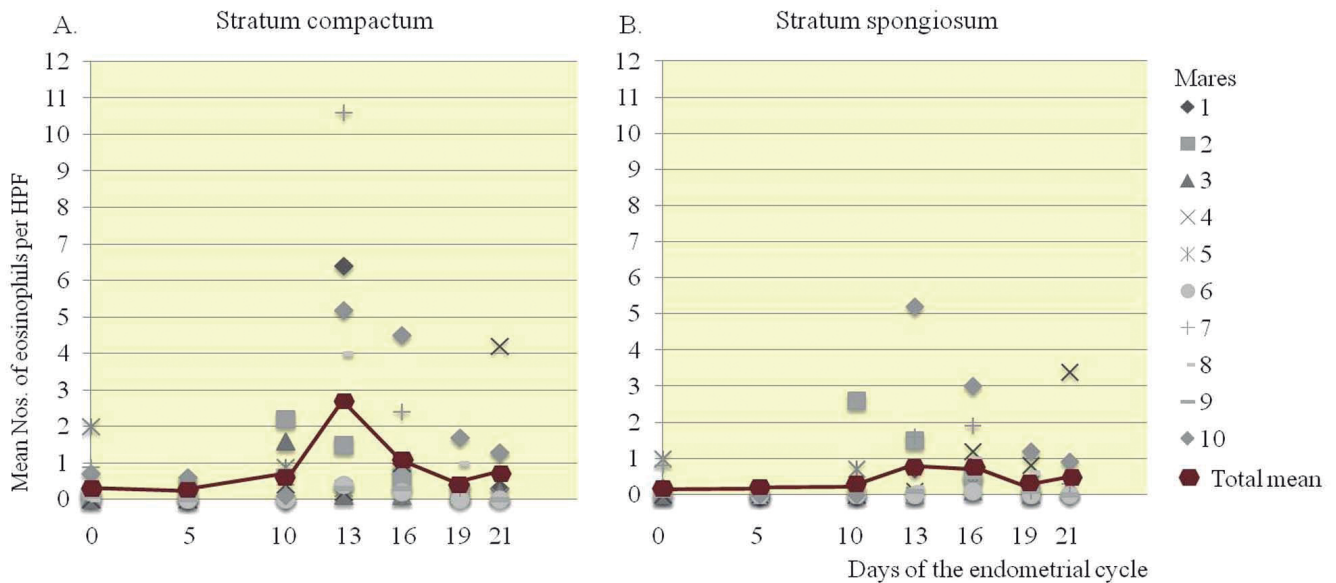


Fig. 1. Eosinophil counts within endometrial biopsies of ten mares during the endometrial cycle. From each mare, endometrial biopsies are collected at different days of the endometrial cycle. In regard to each endometrial biopsy, eosinophils are counted within ten 40x high power fields (HPFs; Olympus CH-microscope), separately for the stratum compactum and stratum spongiosum, and the mean eosinophil count per 40x HPF is calculated. Higher mean numbers of eosinophils per 40x HPF are present within the stratum compactum than within the stratum spongiosum. The highest mean numbers of eosinophils per HPF are detected during the mid interoestrus (day 13; mares 7 and 10). The total mean numbers of eosinophils per HPF are indicated by the red squares. The highest values are observed within the stratum compactum at day 13 and within the stratum spongiosum at days 13 and 16. Nos., numbers.

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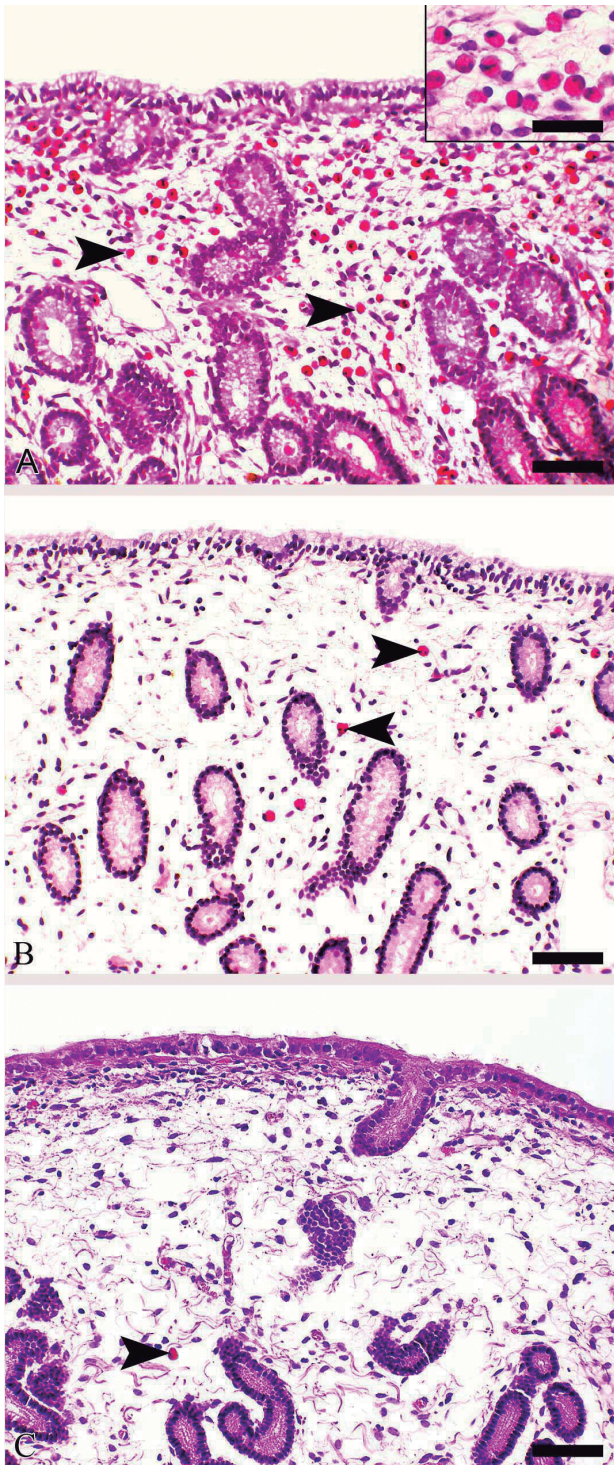


Fig. 2. A. Endometrium of a mare with a diffuse endometritis eosinophilica. The stratum compactum and the stratum spongiosum are diffusely infiltrated by numerous eosinophils (arrowheads). Inset: Eosinophils are depicted at a higher magnification. B. Endometrium of a mare with eosinophilic infiltrates. The stratum compactum and the stratum spongiosum contain some eosinophils (arrowheads). C. Endometrium of a mare with eosinophils within the physiological range. Rare eosinophils are observed within the stratum compactum and the stratum spongiosum (arrowhead). Scale bars, A-C, 50 μ m; Inset, 20 μ m.

vulvar labial closure, 3 mares had a bacterial infection and 14 mares had alterations that were not further specified (Table 4: data reported for 24 mares, 9 of these without lesions). Within 30 days prior to the biopsy collection, 6 mares had received intrauterine treatment and 4 mares were bred (Table 5: data known for 14 mares, 6 of these had not received an intrauterine treatment and were also not bred).

Most of the examined biopsies (58%, n=32) were collected during the physiological breeding season, and the remaining during the spring or fall transition periods (33%, n=18) and the winter anoestrus (9%, n=5).

Histopathological features of EE: EE within the stratum compactum and/or stratum spongiosum was diagnosed in 55 mares, respectively. The following forms of EE were recognized: superficial EE (n=31 mares), superficial and deep EE (n=20 mares) and deep EE (n=4 mares).

The distribution of the EE within the stratum compactum was diffuse (n=24), multifocal to confluent (n=24) and focal (n=3). The distribution of EE within

Table 4. Available data about genital diseases of mares with EE, EI and EWPR.

Genital disease	EE (24 mares) ¹	EI (4 mares)	EWPR (32 mares) ¹
Total reported data	28 (100%)	4 (100%)	37 (100%)
No disease	9 (32%)	1 (25%)	14 (38%)
Bacterial infection	3 (11%)	1 (25%)	2 (5%)
Insufficient labial closure	2 (7%)	None	6 (16%)
Pneumovagina/-uterus	None	None	1 (3%)
Not specified	14 (50%)	2 (50%)	14 (38%)

¹Three mares had 2 or 3 lesions, respectively. EE, endometritis eosinophilica; EI, endometrial eosinophilic infiltrates; EWPR, endometrial eosinophil numbers within the physiological range.

Table 5. Available data about manipulations within the genital tract of mares with EE, EI and EWPR.

Manipulations	EE (14 mares) ¹	EI (3 mares) ²	EWPR (26 mares) ²
Total findings	16 (100%)	4 (100%)	27 (100%)
None	6 (37%)	None	15 (57%)
Intrauterine treatment			
NaCl	1 (6%)	None	4 (15%)
Iodine solution	1 (6%)	None	2 (7%)
Antibiotics	2 (13%)	None	2 (7%)
Not specified	2 (13%)	1 (25%)	None
Insemination/Covering	4 (25%)	2 (50%)	2 (7%)
Not specified ¹	None	1 (25%)	2 (7%)

¹Two mares received two types of treatment. ²One mare received two types of treatment. EE, endometritis eosinophilica; EI, endometrial eosinophilic infiltrates; EWPR, endometrial eosinophil numbers within the physiological range.

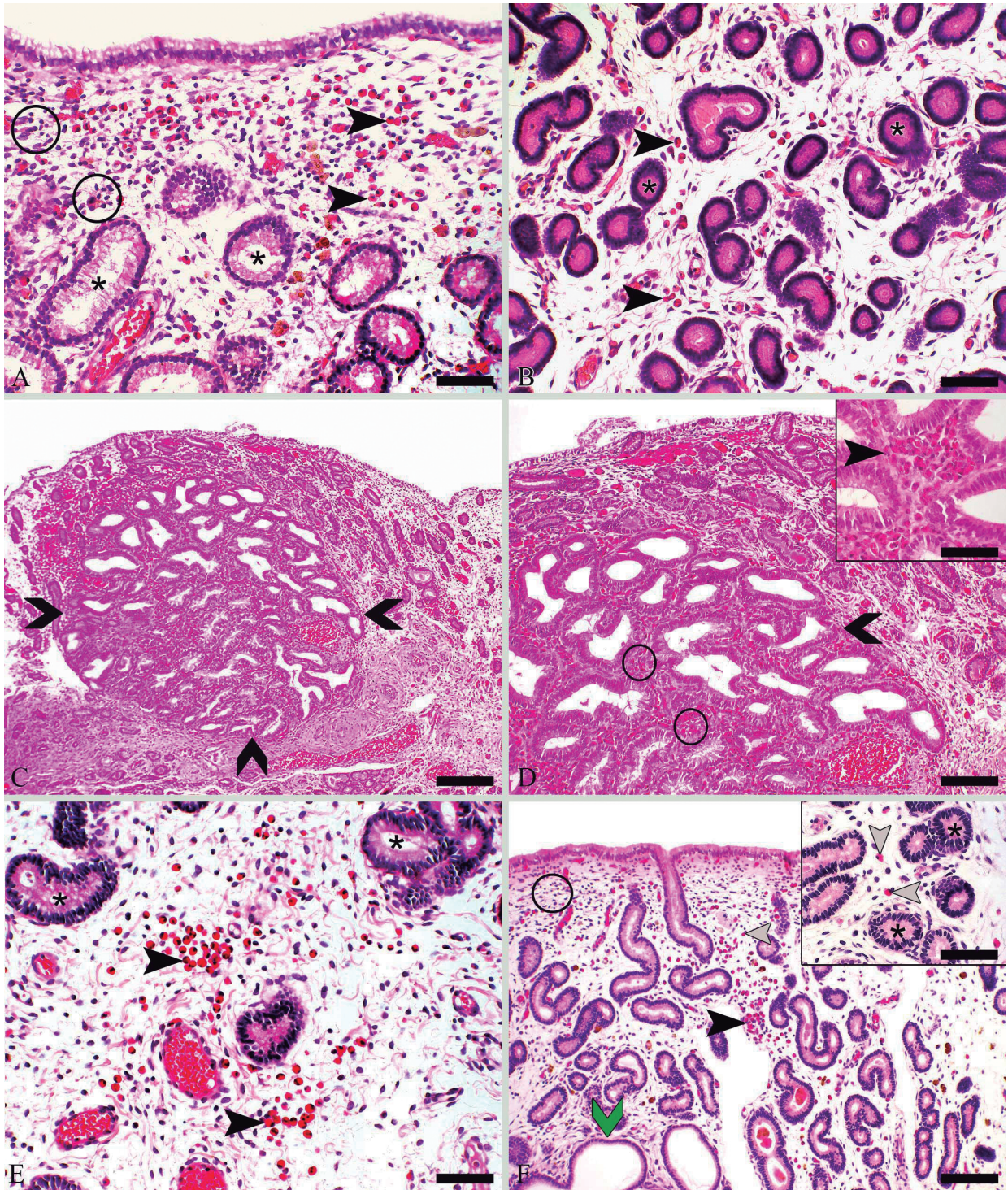


Fig. 3. **A.** Endometrium of a mare with a diffuse endometritis eosinophilica within the stratum compactum and the stratum spongiosum; infiltrating eosinophils are labeled by arrowheads. There is a concurrent subacute suppurative endometritis (circles). Endometrial glands show a secretory morphology (asterisks). **B.** Endometrium of a mare with a diffuse endometritis eosinophilica. Depicted is the stratum spongiosum showing a diffuse infiltration with high numbers of eosinophils (arrowheads). There is only a mild activity of the uterine glands (asterisks). **C and D.** Equine endometrium with a focal endometritis eosinophilica associated with nested endometrotic glands. **C.** Nested endometrotic glands are indicated by arrowheads. **D.** The stroma within the endometrotic nest (arrowhead), but not the adjacent stratum spongiosum, is infiltrated by numerous often aggregated eosinophils (circles). Inset: The presence of numerous eosinophils within the stroma between the uterine glands of the endometrotic nest is depicted at a higher magnification. **E.** Multifocal endometritis eosinophilica within the endometrium of a mare. The multifocal aggregates of eosinophils are labeled by arrowheads. Endometrial glands (asterisks) show an irregular maldifferentiation. **F.** Endometrium of a mare with a diffuse to confluent endometritis eosinophilica. In addition to aggregates of eosinophils (black arrowhead), the stroma also shows a diffuse infiltration with eosinophils (grey arrowhead). There is a concurrent subacute suppurative endometritis (circle) and irregular glandular maldifferentiation. An endometrotic gland is indicated by the green arrowhead. Inset: Endometrial glands with an irregular differentiation (asterisks) are depicted in detail. Eosinophils are labeled by grey arrowheads. Scale bars: A, B, Insets in D, F, 20 μ m; C, D, E, 50 μ m; F, 200 μ m.

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the stratum spongiosum was diffuse (n=9), multifocal to confluent (n=7), multifocal (n=6) and focal (n=2). In one mare with a focal deep EE, the eosinophilic infiltrates were located within nested endometrotic glands (Fig. 3).

In regard to mares with a superficial and deep EE, the distribution of the eosinophils within both layers differed within 12 cases. In these the stratum compactum showed always a diffuse or multifocal to confluent distribution, whereas in the stratum spongiosum all four distribution patterns were detected.

Of the mares with EE restricted to the stratum compactum (n=31), the stratum spongiosum showed EEPR in 15 mares and EWPR in 16 mares. Of the mares with EE restricted to the stratum spongiosum (n=4), the stratum compactum contained EEPR in 1 mare and EWPR in 3 mares.

The PAS reaction was negative for the detection of intralésional fungal hyphae within all biopsies.

Mast cell count: These could be evaluated within 53 biopsies. In general, in all forms of EE the stratum spongiosum contained a higher number of mast cells than the stratum compactum (Table 6).

Additional microscopic findings within examined biopsies: The functional morphology of endometrial glands was proliferative (n=14), secretory (n=9), transitional between the former two stages (n=13) and inactive (n=3). In 16 biopsies an irregular glandular maldifferentiation was diagnosed. Of these biopsies, two were collected during the winter anoestrus, 6 during the transitional periods and 8 during the breeding season. The latter represented 25% of all biopsies collected within the breeding season.

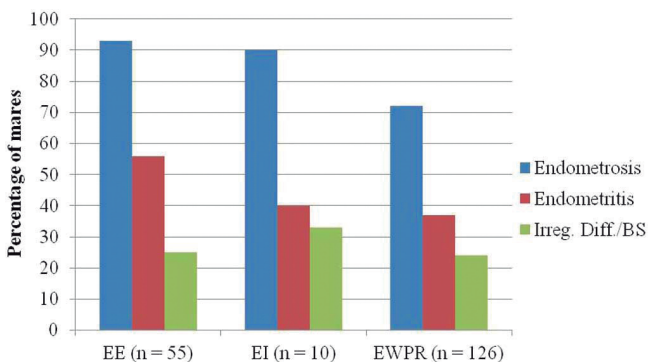


Fig. 4. Concurrent diseases within endometrial biopsies of mares with endometritis eosinophila (EE), endometrial eosinophilic infiltrates (EI) and endometrial eosinophils within the physiological range (EWPR). Endometrosis and endometritis are diagnosed within 93% and 56% of mares with EE (n=55), 90% and 40% of those with EI (n=10) and 72% and 37% of mares with EWPR (n=126), respectively. Biopsies collected during the breeding season (EE: n=32; EI: n=6; EWPR: n=58) show an irregular glandular maldifferentiation within 25%, 33% and 24% of mares with EE, EI and EWPR, respectively.

Mares with EE most commonly had the presence of additional endometrial diseases, i.e. 93% (n=51) displayed endometrosis, 56% (n=31) endometritis. In regard to the 32 mares with a biopsy collection during the breeding season, 25% (n=8) showed an irregular glandular maldifferentiation (Figs. 3, 4).

Endometrosis was mostly mild (46%, n=25) or moderate (36%, n=20) and it was marked in 11% of the mares (n=6). Mixed active and inactive endometrosis (40%, n=22) was more frequently than active (26%, n=14) or inactive (27%, n=15) forms. Non-destructive (47%, n=26) and combined destructive/non-destructive forms (31%, n=17) occurred more frequently than destructive endometrosis (15%, n=8) (Fig. 5).

In regard to endometritis, mild forms dominated (33%, n=18); moderate or marked endometritis was diagnosed in 16% (n=9) or 7% (n=4) of the mares, respectively. It was superficial in 20 mares and superficial and deep in 11 mares. Non-suppurative (24%, n=13) and subacute suppurative inflammatory lesions (29%, n=16) dominated, whereas acute suppurative endometritis was rarely observed (3%, n=2) (Fig. 6).

Notably, in mares with a superficial EE (n=31), the additional type of endometritis was superficial (32%, n=10) or superficial and deep (16%, n=5). In mares with a superficial and deep EE (n=20), the other form of endometritis was also either superficial (45%, n=9) or superficial and deep (30%, n=6). One mare with a deep EE (n=4) had a superficial endometritis. Two mares with a superficial EE displayed an acute suppurative endometritis, while all other mares with EE had either a non-suppurative or subacute suppurative inflammation.

Biopsies with eosinophilic infiltrates

This finding was observed within biopsies of 10 mares (Fig. 2).

Available anamnestic data: The average age of the 7 mares with a reported age was 12 years \pm 4.6 (Mean \pm S.D.).

The time of barrenness was known for 7 mares; none of these showed barrenness of more than 1 year.

Based on mares clinical history, 1 mare showed a

Table 6. Mast cell numbers within biopsies of mares with endometritis eosinophila.

Endometritis eosinophila	Total mean mast cell numbers		
	Superficial EE (n=30) ¹	Superficial and deep EE (n=19) ²	Deep EE (n=4)
Stratum compactum	1.3 (SD: 1.5)	1.6 (SD: 1.9)	1.8 (SD: 2.6)
Stratum spongiosum	2.2 (SD: 2.4)	2.4 (SD: 2.6)	3.2 (SD: 3.8)

EE, endometritis eosinophila; SD, standard deviation; n, numbers of mares. ¹Data available for 30 of 31 mares. ²Data available for 19 of 20 mares. Data reported as total mean per 40x high power field.

bacterial infection and 2 mares had genital lesions that were not further specified (Table 4: information provided for 4 mares; one of these had no genital tract alteration). Within 30 days prior to the biopsy procedure 1 mare had received intrauterine treatment and two mares were bred. For 1 mare the type of performed procedure was not further specified (Table 5: data known for 4 mares).

The examined biopsies were most commonly collected during the breeding season (n=6) and less frequently during the transitional periods (n=2) and the winter anoestrus (n=2 mares).

Histopathological features of EI: This condition (n=10) was superficial in 4 mares, superficial and deep in 1 mare and deep in 5 mares. The mare with superficial and deep EI showed a concurrent mild endometrosis and had received uterine treatment for a bacterial infection as well as being bred within 30 days prior to the biopsy collection.

The PAS reaction was negative for the detection of intralesional fungal hyphae within all biopsies (n=10).

Mast cell count: The stratum spongiosum contained a higher number of mast cells than the stratum compactum (Table 7).

Additional microscopic findings within examined biopsies: Endometrial glands showed the following functional morphologies: proliferative (n=1), secretory (n=1), transition between the former two stages (n=4) or an irregular maldifferentiation (n=4). Two of the latter were collected during the breeding season and one each during the fall transition and the winter anoestrus. Thus, an irregular glandular differentiation was observed within 33% of all biopsies collected during breeding season (Fig. 4).

Endometrosis was diagnosed in 9 mares; it was mild in 3 mares, moderate in 4 mares and marked in 2 mares. The non-destructive form occurred in 4 mares, whereas in the remaining mares it was combined non-destructive/destructive. Most commonly, the combined active/inactive type was observed (5 mares); active and inactive endometrosis was detected in 3 mares and 1 mare, respectively (Figs. 4, 5).

Table 7. Mast cell numbers within biopsies of mares with endometrial eosinophilic infiltrates.

Endometrial eosinophilic infiltrates	Total mean mast cell numbers		
	Superficial EI (n=4)	Superficial and deep EI (n=1)	Deep EI (n=4) ¹
Stratum compactum	2.6 (SD: 2.3)	0.6	0.5 (SD: 0.4)
Stratum spongiosum	6.6 (SD: 4.5)	5.1	1.3 (SD: 1.1)

EI, endometrial eosinophilic infiltrates; SD, standard deviation; n, numbers of mares. ¹Data available for 4 of 5 mares. Data reported as total mean per 40x high power field.

Table 8. Mast cell numbers within biopsies of mares with endometrial eosinophil numbers within the physiological range.

EWPR	Total mean mast cell numbers/EWPR (n=126/125) ¹
Stratum compactum	1.0 (SD: 1.7)
Stratum spongiosum	1.7 (SD: 2.4) ¹

EWPR, endometrial eosinophil numbers within the physiological range; SD, standard deviation; n, numbers of mares. ¹Data available for 125 of 126 mares. Data reported as total mean per 40x high power field.

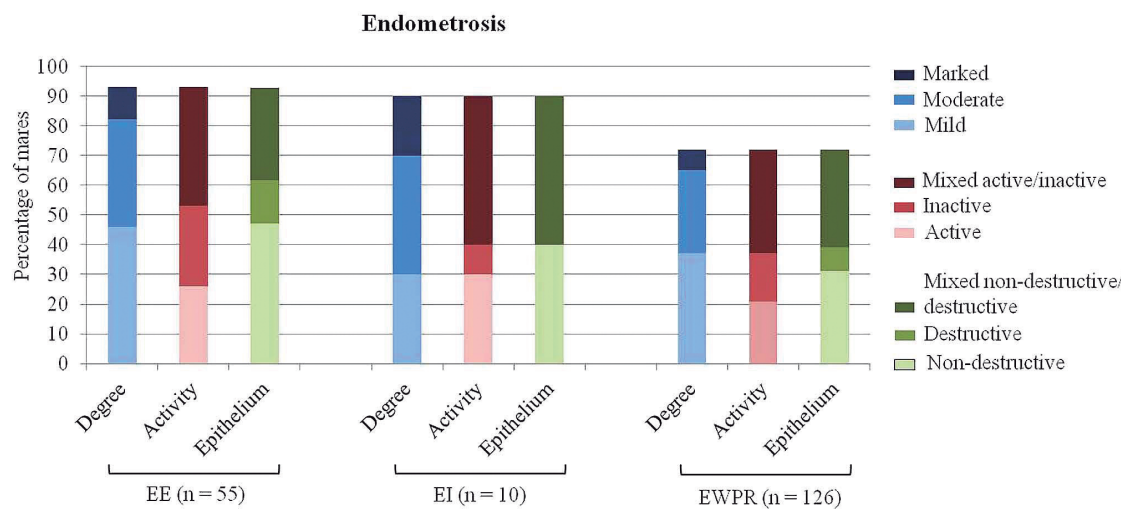


Fig. 5. Degree and forms of endometrosis observed within endometrial biopsies of mares with endometritis eosinophilica (EE), endometrial eosinophilic infiltrates (EI) and endometrial eosinophils within the physiological range (EWPR). A higher percentage of endometrosis is observed within mares with EE (93%) and EI (90%) than in those with EWPR (72%). Within all three groups of mares, mixed

active/inactive endometrosis is more frequently observed (EE: 40%; EI: 50%; EWPR: 35%) than active (EE: 26%; EI: 30%; EWPR: 21%) and inactive forms (EE: 27%; EI: 10%; EWPR: 16%). Non-destructive endometrosis dominates in mares with EE (47%) and EI (40%), whereas mares with EWPR show similar frequencies of non-destructive (31%) and mixed non-destructive/destructive endometrosis (33%).

Endometritis eosinophilica in mares

Four mares showed endometritis, it was mild in 3 mares, moderate in 1 mare, non-suppurative in 3 mares and subacute suppurative in 1 mare. In 2 mares it was superficial; in the remaining 2 mares the inflammation involved the stratum compactum and stratum spongiosum (Figs. 4, 6).

Biopsies containing eosinophil numbers that are within the physiological range

No elevated numbers of eosinophils were observed in biopsies of 126 mares (Fig. 2).

Available anamnestic data: This group of mares had an average age of 10 years (SD \pm 4.7) (age known for 99 mares).

Information about the time of barrenness was known for 66 mares; in regard to barren mares (n=61) the duration of barrenness was 2 years for 28 mares (22 %).

Per clinical history, insufficient labial closure was diagnosed in 6 mares, pneumovagina or pneumouterus in 1 mare, bacterial infection in 2 mares and 14 mares had not further specified alterations (table 4: information was available for 32 mares, 14 of these had no alteration). Within 30 days prior to the biopsy procedure, an intrauterine treatment had been performed in 8 mares and 2 had been bred. Two other mares had received a not further specified procedure (table 5: data provided for 26 mares).

The reported time of the biopsy collection was the breeding season in 58 mares (46%), the spring or fall transitional periods in 42 mares (33%) and the winter anoestrus in 26 mares (21%).

Mast cell count: The stratum compactum contained lower mast cell numbers than the stratum spongiosum (Table 8).

Microscopic findings within examined biopsies: The functional morphology of uterine glands was proliferative (n=31), secretory (n=36), transitional between the former two stages (n=24) and inactive

(n=9). An irregular glandular differentiation was diagnosed in biopsies of 26 mares, of these 14 were collected during the breeding season, 5 during the transitional periods and 7 during the winter anoestrus. This is consistent with the presence of an irregular glandular differentiation within 24% of all biopsies collected during breeding season (Fig. 4).

Biopsy samples of 91 mares (72%) showed endometrosis. The degree was mostly mild (37%, 47 mares) and moderate (28%, 35 mares); marked endometrosis was diagnosed in 7% of the mares (n=9). Non-destructive (31%, 39 mares) and combined non-destructive/destructive forms (33%, 42 mares) were more frequently detected than the destructive type of endometrosis. It was more commonly combined active/inactive (35%, 44 mares) than active (21%, 27 mares) or inactive (16%, 20 mares) (Fig. 5).

Endometritis was present in 46 mares (37%). It was mild in 37 mares (29%) and moderate in 8 mares (6%) and either superficial (20%, 25 mares) or superficial and deep (17%, 21 mares). Non-suppurative inflammation was observed in 27 mares (21%) and subacute suppurative endometritis in 19 mares (15%) (Fig. 6).

Discussion

The present study confirms endometritis eosinophilica as a rare disease of mares, defines the diagnostic criteria of this disease and shows the presence of different subtypes of equine endometritis eosinophilica.

Proposed diagnostic criteria of endometritis eosinophilica in mares

By the evaluation of biopsies collected repeatedly during the same endometrial cycle, the physiological range of numbers of eosinophils was determined. Based on these data, it was possible to define a group of mares showing EWPR and compare this group to those with EI and EE.

To the authors' knowledge, only one publication is

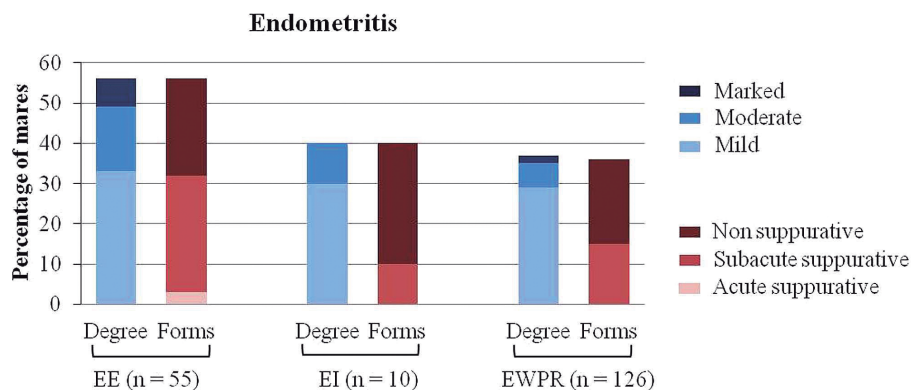


Fig. 6. Degree and forms of endometritis observed within endometrial biopsies of mares with endometritis eosinophilica (EE), endometrial eosinophilic infiltrates (EI) and endometrial eosinophils within the physiological range (EWPR). Mares with EE show more commonly (56%) concurrent endometritis than those with EI and EWPR. In mares from each group (EE, EI, EWPR) mild endometritis is most frequently observed. Moderate and marked endometritis is most commonly detected in mares with EE. Suppurative forms (acute and subacute) dominate in mares with EE, whereas the prevailing form in mares with EI and EWPR is non-suppurative endometritis. Acute suppurative inflammation is exclusively seen in mares with EE.

available that provides reference numbers for eosinophilic infiltrates of EE, in a woman (Desai and Shinagare, 2010) by using the diagnostic criteria of human eosinophilic oesophagitis (Chang and Anderson, 2008). In addition to a multifocal and diffuse distribution of eosinophilic infiltrates (Chang and Anderson, 2008; Desai and Shinagare, 2010), in mares also focal and multifocal to confluent types of EE were observed. In this study, the proposed diagnostic criteria for equine EE were elected under consideration of the physiological range of eosinophils within the equine endometrium, using as reference numbers those provided in human medicine (Chang and Anderson, 2008; Desai and Shinagare, 2010) and the distribution patterns of eosinophilic infiltrates within endometrial biopsies in affected mares.

Physiological numbers of eosinophils within the endometrial stroma

Under physiological conditions Kenney and Doig (1986) did not observe eosinophils within the endometrial stroma. This observation, however, could not be confirmed by this study and the results of other investigations (Brunckhorst et al., 1991; Gilbert, 1992; Schiemann et al., 2001; Blüthgen, 2002; Palm et al., 2008). Therefore, the presence of a few eosinophils within the endometrial stroma of the mare has to be considered a normal component of the local immune system. In this study as well as in previous investigations numbers of eosinophils varied not only between individual mares, but also during the different stages of the endometrial cycle (Brunckhorst et al., 1991; Blüthgen, 2002).

The expression of oestrogen receptors on endometrial eosinophils in rats has been shown (Lyttle et al., 1984, 1989; Katayama et al., 1998), whereas no such information is available for eosinophils within the endometrium of mares. Further, several studies in rats revealed an oestrogen dependent eosinophilic infiltration within the uterus (Tchernitchin et al., 1976; Katayama et al., 1998). Moreover, numbers of eosinophils within the cervix and uterus decreased under the influence of progesterone (Luque et al., 1996; Ramos et al., 2000; Blüthgen, 2002). In this study, a hormonal influence has to be considered as well, in particular since progesterone and oestrogen levels are highly variable between individual mares (Brunckhorst et al., 1991; Blüthgen, 2002; Hoffmann, 2006).

Injury or mechanical irritation is supposed to stimulate tissue infiltration with eosinophils as well (Bjersing and Borglin, 1962; Mikó et al., 1988; Desai and Shinagare, 2010; Hopster-Iversen et al., 2011). In the present study, however, this appears to be an unlikely explanation for the increased numbers of eosinophils within some biopsies due to the absence of histological findings consistent with tissue injury or a healing response. Further, endometrial biopsies of mares with intrauterine devices also did not contain elevated

numbers of eosinophils (Klein et al., 2016).

Endometritis eosinophilica as an idiopathic disease condition or a bystander reaction?

Taking into consideration the literature data as well as the results of this study, EE most likely represents a separate endometrial disease, as has been suggested already by Schoon et al. (1997). Increased numbers of eosinophils within the endometrium have been detected only in some, but not all mares with bacterial (Oddsdóttir et al., 2008) or mycotic infections (Hurtgen and Cummings, 1982), genital alterations that could predispose to pneumouterus (Slusher et al., 1984) as well as after hysteroscopy (Slusher et al., 1984; Schiemann et al., 2001), the application of certain insemination protocols (Palm et al., 2008) and hormonal treatment (Klug et al., 1997). In the endometrium of women increased numbers of eosinophils were also associated with tissue injury (Bjersing and Borglin, 1962; Mikó et al., 1988; Desai and Shinagare, 2010).

In regard to mares of this study with EE and a provided clinical history, 32% showed no genital lesions and 37% had not been treated or bred within 30 days prior to the biopsy collection. Further, in the present investigation no mycotic infection was detected within biopsies with EE and EI. An association between increased numbers of endometrial eosinophils and the presence of fungi/yeast was excluded by other studies as well (Schiemann et al., 2001; Blüthgen, 2002).

Unfortunately, most previous studies either did not include a quantitative evaluation of tissue eosinophils, or a direct comparison of obtained eosinophilic counts with those of this study was not possible due to methodical differences. Palm et al. (2008), however, analyzed the numbers of subepithelial eosinophils within four 40x HPFs. The eosinophilic counts obtained by Palm et al. (2008) were markedly below those defined in this study as threshold level for the diagnosis of superficial EE.

Pathogenesis of equine endometritis eosinophilica

The pathogenesis of equine EE is uncertain so far. Because of the lack of detection of an initiating primary cause, it has to be considered as an idiopathic condition. This, however, does not exclude an immune mediated pathogenesis, for example a hypersensitivity reaction, as has been proposed for idiopathic eosinophilic oesophagitis in human beings (Straumann et al., 2001) and for idiopathic eosinophilic enteritis in horses (Mäkinen et al., 2008). In patients with idiopathic eosinophilic oesophagitis, exposure to certain food allergens may trigger eosinophil tissue infiltration (Mulder and Justinich, 2011), and a genetic signature was identified that leads to an overexpression of the eotaxin-3 gene (Blanchard et al., 2006).

The finding that equine EE is mostly either superficial or superficial and deep suggests the extension of a superficial EE into the deeper layers of the

endometrium. This hypothesis would be further supported by the detection of milder forms of EEPR also within the stratum spongiosum of about 50% of mares with a “superficial” EE. The latter indicates that EI could also represent a precursor stage of EE. Interestingly, one focal deep EE was located within a fibrotic gland. Under the assumption that equine EE may be triggered by the exposure to certain allergens, these would probably be present within the lumina of the uterus and/or endometrial glands.

In hypersensitivity reactions and some eosinophilic disorders, for example idiopathic eosinophilic oesophagitis, there is a concurrent increase in mast cell numbers (Straumann et al., 2001). Thus, in the present study, the numbers of mast cells were examined as well. To identify tissue mast cells in the formalin fixed and paraplast embedded biopsies, picosirius red was used (Rodenbusch, 2011). This method stains the mast cytoplasm turquoise (Rodenbusch, 2011). It was elected in this study, since it allows the detection of all mast cells, regardless of the presence/absence of mast cell granules (Rodenbusch, 2011).

The detection of more mast cells within the stratum spongiosum than the stratum compactum (EE, EI and EWPR) confirms the results of Schulz (1997). The higher mean numbers of mast cells within biopsies from mares with EE and EI compared to those present in biopsies with EWPR indicates an involvement of tissue mast cells in the pathogenesis of equine EE as well. It has been shown that the equine endometrium contains only tryptase positive mast cells, but no chymase positive mast cells (Schulz, 1997; Welle et al., 1997) and that mast cell tryptase recruits eosinophils (He et al., 1997). In regard to the density of mast cells, no significant differences exist within endometrial tissue samples with secretory and proliferative endometrial glands (Schulz, 1997).

Further studies are necessary to find out more details about the pathogenesis of equine EE and possible associated genetic alterations that may predispose mares to this disease.

Association of equine endometritis eosinophilica with other endometrial diseases

Compared to mares with EE and EWPR, the results obtained from the investigation of biopsies with EI have to be interpreted with caution due to the small case numbers (10 mares).

Whereas an irregular glandular differentiation may be detected during the transitional periods, its presence during the breeding season always represents a pathological finding (Schoon et al., 2000). An irregular glandular differentiation was observed with similar frequencies in biopsies with EE and EWPR collected during the breeding season. The most common cause of an irregular differentiation of uterine glands is a hormonal imbalance (Klug et al., 1997; Ellenberger et al., 2002).

The mares of the present study, from which biopsies were collected repeatedly during the course of the same endometrial cycle, had the highest numbers of endometrial eosinophils in the mid and late interoestrus. At this stage of the endometrial cycle low serum progesterone levels and low or high serum oestrogen concentrations exist (Brunckhorst et al., 1991; Hoffmann, 2006). Further investigations are necessary to test, whether imbalances of oestrogen and/or progesterone predispose mares to develop EE.

Within each of the three groups (EE, EI and EWPR) of this study, the vast majority of mares showed endometrosis and a moderate number of mares had endometritis.

The less frequent occurrence of endometrosis including its moderate and marked forms within the EWPR group of mares may be explained by their slightly younger age compared to mares with EE and EI (Schoon et al., 1997). Eosinophilic inflammation is often associated with fibrosis and tissue remodeling (Mäkinen et al., 2008; Aceves, 2014). Thus, it cannot be ruled out that the eosinophilic infiltrates have contributed to the endometrosis within the EE and EI groups of mares.

The higher percentage of suppurative endometritis (acute and subacute) within the group of mares with EE may indicate an increased susceptibility of these mares to bacterial infection. A direct association between EE and increased endometrial numbers of neutrophils, however, has also to be considered, since mast cell tryptase not only simulates the tissue influx of eosinophils, but also attracts neutrophils (He et al., 1997).

Equine endometritis eosinophilica as separate factor of subfertility

Within the categorization scheme of Kenney and Doig (1986) modified by Schoon et al. (1997), endometritis is one factor to be considered for prognosis of a mare's fertility. Thus, the presence of eosinophils (solely or in conjunction with other inflammatory cells) will determine or at least influence the degree of inflammation and thus represents a fertility reducing factor.

Conclusions

This study confirms the existence of EE as a separate endometrial disease in mares, establishes the diagnostic criteria of this disease, provides consensus recommendations for the diagnosis of equine EE and shows that biopsies with EE have to be differentiated from those with EI. The use of identical diagnostic criteria is a necessary prerequisite to allow the direct comparison of different investigations. The present examination raises the awareness of equine theriogenologists for the existence of this fertility reducing disease of mares and provides the basis for further investigations into the pathogenesis, prognosis and treatment of this disease.

Acknowledgements. The authors thank the histology laboratory, Institute of Pathology, Faculty of Veterinary Medicine, University Leipzig, for excellent technical support.

References

- Aceves S.S. (2014). Remodeling and fibrosis in chronic eosinophil inflammation. *Dig. Dis.* 32, 15-21.
- Archer D.C., Barrie E.G., Kelly D.F., French N.P. and Proudman C.J. (2006). Obstruction of equine small intestine associated with focal idiopathic eosinophilic enteritis: an emerging disease? *Vet. J.* 171, 504-512.
- Bjersing L. and Borglin N.E. (1962). Eosinophilia in the myometrium of the human uterus. *Acta Pathol. Microbiol. Scand.* 54, 353-364.
- Blüthgen U. (2002). Untersuchungen zu Vorkommen, Nachweis und Bedeutung von eosinophilen Granulozyten im Endometrium der Stute unter besonderer Berücksichtigung des Vorkommens von Sproßpilzen. [Dissertation med. vet.]. Leipzig, Germany: Faculty of Veterinary Medicine, Leipzig-University.
- Bosseler L., Verryken K., Bauwens C., de Vries C., Deprez P., Ducatelle R. and Vandenabeele S. (2013). Equine multisystemic eosinophilic epitheliotropic disease: a case report and review of literature. *N Z Vet J.* 61, 177-182.
- Bracher V., Gerstenberg C. and Allen W.R. (1997). Der Einfluss von Endometrose (degenerativer Endometriumserkrankungen) auf Fruchtbarkeit, Plazentation und fötale Entwicklung beim Pferd. *Pferdeheilkunde* 13, 465-473.
- Brunckhorst D., Schoon H.-A., Bader H. and Sieme H. (1991). Morphologische, enzym- und immunhistochemische Charakteristika des endometrialen Zyklus bei der Stute. *Fertilität* 7, 44-51.
- Blanchard C., Wang N., Stringer K.F., Mishra A., Fulkerson P.C., Abonia J.P., Jameson S.C., Kirby C., Konikoff M.R., Collins M.H., Cohen M.B., Akers R., Hogan S.P., Assa'ad A.H., Putnam P.E., Aronow B.J. and Rothenberg M.E. (2006). Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J. Clin. Invest.* 116, 536-547.
- Chang F. and Anderson S. (2008). Clinical and pathological features of eosinophilic oesophagitis: a review. *Pathology* 40, 3-8.
- Desai S.R. and Shinagare S.A. (2010). Severe eosinophilic endometritis following diagnostic curettage. *Online J. Health Allied Sci.* 9, 1-2.
- Ellenberger C., Aupperle H., Bartmann C.-P., Hoppen H.-O., Schoon D. and Schoon H.-A. (2002). Endometrial maldifferentiation caused by ovarian disorders in the mare - morphological and immunohistochemical studies. *Theriogenology* 58, 499-502.
- Gilbert R.O. (1992). Cyclic changes in equine endometrial histology. *Proceedings of the 12th Int. Conf. Anim. Reprod. The Hague.* pp 1867-1869.
- He S., Peng Q. and Walls A.F. (1997). Potent induction of a neutrophil and eosinophil-rich infiltrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. *J. Immunol.* 159, 6216-6225.
- Hoffmann C. (2006). Morphologisch-funktionelle Untersuchungen zur Pathogenese der equinen Endometrose unter besonderer Berücksichtigung endometrialer Proteine und Kohlenhydrate. [Dissertation med. vet.]. Leipzig, Germany: Faculty of Veterinary Medicine, Leipzig-University.
- Hoffmann C., Ellenberger C., Rodrigo Costa M., Aupperle H., Dhein S., Stief B., Schoon H.-A. (2009). The equine endometrosis: new insights into the pathogenesis. *Anim. Reprod. Sci.* 111, 261-278.
- Hopster-Iversen C., Hopster K., Staszky C., Rohn K., Freeman D. and Rötting A.K. (2011). Influence of mechanical manipulations on the local inflammatory reaction in the equine colon. *Equine Vet. J. Suppl.* 1-7.
- Hurtgen J.P. and Cummings M.R. (1982). Diagnosis and treatment of fungal endometritis in mares. *Proc. Ann. Meeting Soc. Theriogen.* 82, 18-22.
- Katayama M.L., Federico M.H., Brentani R.R. and Brentani M.M. (1998). Eosinophil accumulation in rat uterus following estradiol administration is modulated by laminin and its integrin receptors. *Cell Adhes. Commun.* 5, 409-424.
- Kenney R.M. and Doig P.A. (1986). Equine endometrial biopsy. In: *Current Therapy in Theriogenology*. 2nd ed. Morrow D.A. (ed.) WB Saunders. Philadelphia. USA pp 723-729.
- Klein V., Müller K., Schoon H.-A., Reilas T., Rivera del Alamo M.M. and Katila T. (2016). Effects of intrauterine devices in mares: A histomorphological and immunohistochemical evaluation of the endometrium. *Reprod. Domest. Anim.* 51, 98-104.
- Klug E., Bartmann C.P., Schöning A., Schoon D. and Schoon H.-A. (1997). Auswirkung einer Progesteron-Langzeit-Applikation auf Sexualzyklus und Endometrium des Pferdes – Zwei Seiten einer therapeutischen Medaille. *Pferdeheilkunde* 13, 490-498.
- Luque E.H., Ramos J.G., Rodriguez H.A. and Muñoz de Toro M.M. (1996). Dissociation in the control of cervical eosinophilic infiltration and collagenolysis at the end of pregnancy or after pseudopregnancy in ovariectomized steroid-treated rats. *Biol. Reprod.* 55, 1206-1212.
- Lyttle C.R., Medlock K.L. and Sheehan D.M. (1984). Eosinophils as the source of uterine nuclear type II estrogen binding sites. *J. Biol. Chem.* 259, 2697-2700.
- Lyttle C.R., Teuscher C., Medlock K.L. and Sheehan D.M. (1989). Estradiol-stimulated increases in uterine eosinophils and nuclear type II estrogen-binding sites are prevented by pertussis toxin. *Endocrinology* 125, 2773-2779.
- Mäkinen P.E., Archer D.C., Baptiste K.E., Malbon A., Proudman C.J. and Kipar A. (2008). Characterisation of the inflammatory reaction in equine idiopathic focal eosinophilic enteritis and diffuse eosinophilic enteritis. *Equine Vet. J.* 40, 386-392.
- McEwen B.J. (1992). Eosinophils: a review. *Vet. Res. Commun.* 16, 11-44.
- Mikó T.L., Lampe L.G., Thomazy V.A., Molnár P., and Endes P. (1988). Eosinophilic endomyometritis associated with diagnostic curettage. *Int. J. Gynecol. Pathol.* 7, 162-172.
- Mulder D.J. and Justinich C.J. (2011). Understanding eosinophilic esophagitis: the cellular and molecular mechanisms of an emerging disease. *Mucosal Immunol.* 4, 139-147.
- Oddsóttir C., Smith S. and Watson E.D. (2008). Leukocyte distribution in the equine endometrium as a response to acute induced endometritis. *Pferdeheilkunde* 24, 66-70.
- Palm F., Walter I., Budik S., Kolodziejek J., Nowotny N. and Aurich C. (2008). Influence of different semen extenders and seminal plasma on PMN migration and on expression of IL-1beta, IL-6, TNF-alpha and COX-2 mRNA in the equine endometrium. *Theriogenology* 70, 843-851.
- Ramos J.G., Varayoud J., Kass L., Rodriguez H., Muñoz de Toro M., Montes G.S. and Luque E.H. (2000). Estrogen and progesterone modulation of eosinophilic infiltration of the rat uterine cervix.

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- Steroids 65, 409-414.
- Rodenbusch S. (2011) Makroskopische und histopathologische Untersuchungen am Genittrakt sub- und infertiler weiblicher Rinder im klinischen Kontext unter besonderer Berücksichtigung der Endometriumbiopsie. [Dissertation med. vet.]. Leipzig, Germany: Faculty of Veterinary Medicine, Leipzig-University.
- Rothenberg M.E. (2004). Eosinophilic gastrointestinal disorders (EGID). *J. Allergy Clin. Immunol.* 113, 11-28.
- Rothenberg M.E. and Hogan S.P. (2006). The eosinophil. *Annu. Rev. Immunol.* 24, 147-174.
- Schiemann V., Bartmann C.P., Kirpal G., v. Reisz A., Schoon H.-A. and Klug E. (2001). Diagnostic hysteroscopy in the mare – uterine contamination and endometrial reaction. *Pferdeheilkunde* 17, 557-564.
- Schöniger S., Gräfe H. and Schoon H.-A. (2013). Beta-defensin is a component of the endometrial immune defence in the mare. *Pferdeheilkunde* 29, 335-346.
- Schoon H.-A.; Schoon D. and Klug E. (1992). Uterusbiopsien als Hilfsmittel für Diagnose und Prognose von Fertilitätsstörungen der Stute. *Pferdeheilkunde* 8, 355-362.
- Schoon H.-A., Schoon D. and Klug E. (1997). Die Endometriumbiopsie bei der Stute im klinisch-gynäkologischen Kontext. *Pferdeheilkunde* 13, 453-464.
- Schoon H.-A., Wiegandt I., Schoon D., Aupperle H. and Bartmann C.-P. (2000). Functional disturbances in the endometrium of barren mares: a histological and immunohistological study. *J. Reprod. Fertil. Suppl.* 56, 381-391.
- Schulz S. (1997). Untersuchungen zu Vorkommen, Nachweis und Bedeutung von Mastzellen im Endometrium der Stute. [Dissertation med. vet.]. Leipzig, Germany: Faculty of Veterinary Medicine, Leipzig-University.
- Slusher S.H., Freeman K.P. and Roszel J.F. (1984). Eosinophils in equine uterine cytology and histology specimens. *J. Am. Vet. Med. Assoc.* 184, 665-670.
- Straumann A., Bauer M., Fischer B., Blaser K. and Simon H.U. (2001). Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. *J. Allergy Clin. Immunol.* 108, 954-961.
- Tchernitchin X., Tchernitchin A. and Galand P. (1976). Dynamics of eosinophils in the uterus after oestrogen administration. *Differentiation* 5, 151-154.
- Wehrend A., Huchzermeyer S. and Bostedt H. (2005). Distribution of eosinophils and mast cells in the cervical tissue of non-gravid mares during dioestrus. *Reprod. Dom. Anim.* 40, 562-563.
- Welle M.M., Audigé L. and Belz J.P. (1997). The equine endometrial mast cell during the puerperal period: evaluation of mast cell numbers and types in comparison to other inflammatory changes. *Vet. Pathol.* 34, 23-30.

Accepted January 20, 2017