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A retrospective review of the histopathological features seen in a series of 4241 endometrial biopsy samples collected from UK Thoroughbred mares over a 25 year period

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Summary

A retrospective review of the histopathological features and the groups in which they are most frequently seen in combination, seen in 4214 endometrial biopsy samples collected from UK Thoroughbred mares over a 25 year period revealed data which supports widely held views on their pathogenesis and significance. Endometrial hypoplasia is most commonly seen in young mares with delayed endometrial maturity. Mononuclear cell infiltrations develop in the endometrial stroma as a reflection of local immune responses to challenge by seminal proteins, micro-organisms, environmental debris and the products of pregnancy, during a brood mare's life. Endometrial hyperplasia most commonly persists during delayed post-partum or post-pregnancy failure of uterine involution, more commonly in younger mares. Glandular degenerative changes and stromal fibrosis develop as an inevitable consequence of ageing, eventually leading to endometrial atrophy, a reflection of gynaecological senility. These degenerative changes result in endometrial incompetence, progressively reducing mares' foaling potentials and increasing their potential for barren years, early pregnancy failures and abortions.

keywords: endometrial biopsy, histopathological features

Eine retrospektive Analyse histopathologischer Befunde bei der Untersuchung von Endometriumsbiopsieproben von 4241 Vollblutstuten im Vereinigten Königreich über einen Zeitraum von 25 Jahren

Dieser retrospektive Überblick über histopathologische Befunde und ihrer häufigsten Kombinationen anhand eines Untersuchungsgutes von 4214 Endomtriumsbiopsien von Vollblutstuten im Vereinigten Königreich aus den letzten 25 Jahren erlaubt weitgehende Aussagen hinsichtlich ihrer Pathogenese und Bedeutung. Endometriale Hypoplasien werden am häufigsten bei jungen Stuten mit verzögerter endometrialer Reife angetroffen. Mononukleäre Zellinfiltrate innerhalb des endometrialen Stromas sind Ausdruck einer lokalen Immunantwort auf die Herausforderungen während des Lebens einer Zuchtstute: Seminaleiweiße, Mikroorganismen, Zelldetritus und Nebenprodukte der Trächtigkeit. Eine endometriale Hyperplasie persistiert am häufigsten im Zuge einer verzögerten Involution nach Trächtigkeit oder Geburt und wird meistens bei jüngeren Stuten beobachtet. Degenerative Veränderungen der Drüsen und Stromafibrosen sind als unvermeidliche Konsequenzen des Alterns anzusehen. Sie führen unter Umständen zur endometrialen Atrophie, die Ausdruck des gynäkologischen Seniums ist. Diese degenerativen Veränderungen führen zu endometrial bedingten Fertilitätsstörungen mit fortschreitender Reduktion der Abfohlrate, einer Verlängerung der Güstzeit sowie Resorptionen und Aborten.

Schlüsselwörter: Endometriumsbiopsie, histopathologische Befunde

Introduction

Endometrial biopsy is a safe, practical and useful technique which provides an aid to the diagnosis of endometrial pathology in mares *(Ricketts, 1975a; Ricketts, 1975b; Kenney, 1977; Shideler et al., 1977; Kenney 1978a; Kenney 1978b; Ricketts, 1978; Doig et al., 1981; Slusher et al., 1985; Van Camp, 1988; Doig and Waelchli, 1993)*. In UK equine practice, biopsy samples are most commonly collected from mares who repeatedly fail to conceive during the breeding season or from mares during routine post-season barren mare examinations *(Rossdale and Ricketts, 1980)*. The histopathological information gained aids gynaecological treatment and subsequent management, leading to improved chances for conception, either during the immediate or the subsequent breeding season *(Ricketts, 1989a; Ricketts 1989b)*. The aim of this study was to review the histopathological features of 4241 endometrial biopsy samples collected over a 25 year period from UK Thoroughbred mares and their effect on subsequent fertility potential.

Materials and Methods

The records of 4241 endometrial biopsy samples collected from UK Thoroughbred mares and examined by the authors, between 1972 and 1997, were retrospectively reviewed and their histopathological features analysed with respect to the age, pre-and post-biopsy breeding history of the mares involved. Histopathological features were clas-

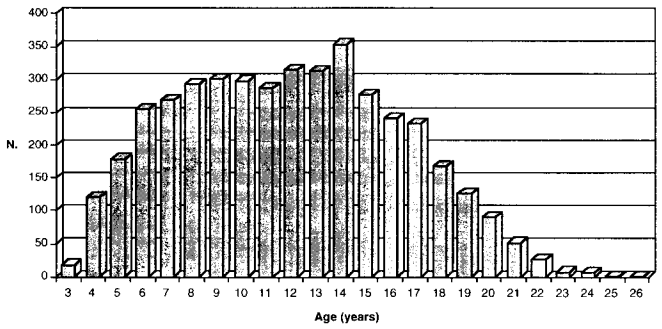

Fig. 1: 4241 Endometrial Biopsy Samples – age of mares at biopsy – frequency distribution histogram.

Lebensalter der untersuchten Stuten (n=4241): Häufigkeitsverteilung.

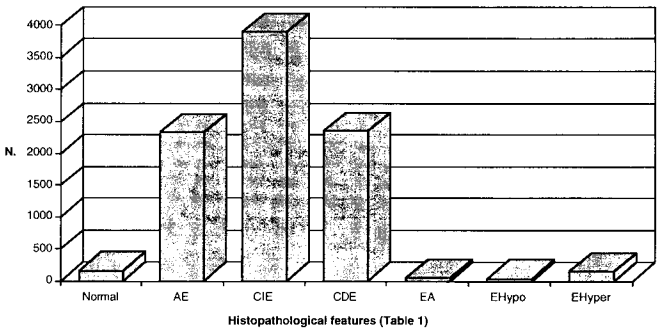

Fig. 2: Endometrial Biopsy Samples – histopathological features – frequency distribution histogram.

Häufigkeitsverteilung der histopathologischen Befunde bei der Untersuchung von 4241 Endometriumsbiopsien.

sified as described by *Ricketts (1975a; Ricketts 1975b)* (Table 1). The histopathological features were frequently seen in combinations, as grouped by *Ricketts (1978)* (Table 2). Where appropriate, mares were treated before preparation and presentation for mating again (Table 3). Not all mares were re-examined by endometrial biopsy following treatment *(Ricketts & Alonso, 1991a)* and therefore follow-up biopsies were not investigated in this survey. Ages, pre- and post-biopsy breeding histories of the mares biopsied were obtained from "The General Stud Book" and its supplements, "The Statistical Record - Return of Mares", published annually by Weatherbys Group Ltd., Wellingborough, Northamptonshire, UK.

Results

The ages, number of foals born and numbers of barren years, early pregnancy failures and abortions suffered by the mares at the time of biopsy collection are shown in the form of frequency histograms (Figs. 1, 2, 3 and 4). The youngest mare examined was 3 and the oldest 26 years old, but the majority (81 %) of the biopsy samples were collected from mares between the ages of 6 and 17 years (Fig. 1) , reflecting the age of the active UK Thoroughbred broodmare population. The largest number of foals born to a mare prior to biopsy was 19 (one 24 year-old mare), but the majority (95 %) of the mares examined had produced

Tab. 1: Endometrial Biopsy – Histopathological Features *(Ricketts 1975a; Ricketts 1975b).*

Endometriumsbiopsie – histopathologische Befunde *(Ricketts 1975a; Ricketts 1975b).*

Histopathological description	Features
Acute Endometritis (AE)	Polymorphonuclear leucocytes infiltrating the luminal epithelium and stroma, sometimes with eosinophils.
Chronic Infiltrative Endometritis (CIE)	Mononuclear cells, including histiocytes, and plasma calls, infiltrating the stroma.
Chronic Endometrial Degenerative Disease (CDE) (endometrosis)	Glandular degenerative changes (nests and/or cysts) with associated periglandular and/or diffuse stromal fibrosis.
Endometrial Atrophy (EA)	Glandular atrophy.
Endometrial Hypoplasia (EHypo)	Glandular hypoplasia.
Endometrial Hyperplasia (EHyper)	Glandular hyperplasia.

Tab. 2: Endometrial Biopsy Histopathological Feature Combination Groups *(Ricketts, 1978)*
(for key to abbreviations, see Table 1).

Kombinationen histopathologischer Befunde in Endometriumsbiopsieproben *(Ricketts, 1978)*
(Abkürzungen siehe Tabelle 1).

Histopathological group	Combination of features
1	No histopathological features
2	AE
3	CIE
4	AE + CIE
5	CDE
6	AE + CIE + CDE
7	CIE + CDE
8	EA
9	AE + EA
10	CIE + CDE + EA
11	EHypo
12	EHyper
13	AE + EHyper
14	CIE + CDE + EHyper
15	AE + CIE + CDE + EHyper

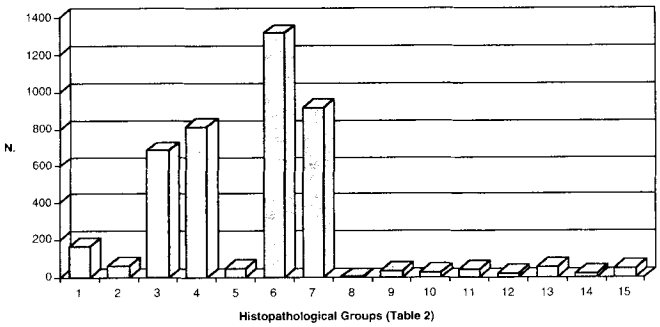

Fig. 3: Endometrial Biopsy Samples – histopathological feature combination groups – frequency distribution histogram.

Häufigkeitsverteilung von Kombinationen histopathologischer Befunde.

between one and 10 foals (Fig. 2). The largest number of barren years prior to biopsy was 11 (one 17 year-old mare), but the majority (96 %) of the mares examined had previously been barren between one and 5 years (Fig. 3). The largest number of early pregnancy failures and abortions suffered by mares prior to biopsy was 4 (one 7 year-old mare) and 5 (two mares, who were 17 and 22 years old) but the majority had suffered 1 or 2 early pregnancy failures or between one and 3 abortions (Figs. 4 & 5).

The histopathological features seen in the biopsy samples examined are shown in the form of a frequency histogram (Fig. 5). 166 (4 %) showed no significant histopathological features. 3903 (92 %) showed stromal mononuclear cell infiltrations (chronic infiltrative endometritis, CIE), 2356 (56 %) showed chronic glandular degenerative change and stromal fibrosis (chronic degenerative endometrial disease, CDE), 2327 (55 %) showed polymorphonuclear cell infiltration (acute endometritis, AE), 151 (4 %) showed glandular hyperplasia (endometrial hyperplasia, EHyper), 58 (1.4 %) showed glandular atrophy (endometrial atrophy, EA) and 45 (1.1 %) showed signs of luminal epithelial and glandular hypoplasia (endometrial hypoplasia, EHypo). 232 mares (5 %) of the biopsies examined showed signs of diffuse stromal fibrosis. These mares were on average 15.1 years old, had previously produced an average of 5.5 foals and had suffered 2.4 barren years, 0.8 early pregnancy failures and 1.5 abortions.

The histopathological groups classified in the biopsy samples are shown in Table 4 and in the form of a frequency histogram (Fig. 6). 1316 (31 %) of the biopsy samples examined had combinations of AE, CIE and CDE (Group 6), 918 (22 %) had CIE and CDE (Group 7), 784 (18 %) had AE and CIE (Group 4) and 683 (16 %) had CIE (Group 3). All the other groups were classified in less than 1.5 % of the biopsies examined (Table 4). Biopsies with EHypo (Group 11) and those with no significant histopathological features (Group 1) came from the youngest mares (mean ages 5.2 and 7.0 years, respectively) whereas biopsies with EA (Groups 8, 9 & 10) came from the oldest mares (mean ages 16.0, 17.6 and 18.5 years, respectively). Similar trends were seen with mean numbers of foals born, barren years, early pregnancy failures and abortions suffered (Table 4).

Tab. 3: Treatments used for Endometrial Biopsy Histopathological Features (where appropriate).

Mögliche Behandlungsmethoden bei Vorliegen verschiedener histopathologischer Befunde.

HP Feature	Treatment
AE	Intrauterine antibiotic irrigations (Ricketts & Mackintosh, 1987) +/– Caslick's vulvoplasty surgery (Ricketts & Curnow, 1988)
CIE	None
CDE	Uterine curettage (Ricketts, 1975) if considered excessive for age (Ricketts & Alonso, 1991b)
EA	None
EHypo	None
EHyper	Intravenous oxytocin infusion (Ricketts, 1978)

An analysis of each histopathological group in terms of percentage of foals born, barren years, early pregnancy failures and abortions suffered by mares following biopsy and treatment (where indicated, Table 3) (Table 5) show that foaling percentages were highest for those groups with un-

Tab. 4: Histopathological Feature Combination Groups – numbers and mean ages, foals born, barren years, early pregnancy failures and abortions suffered by mares prior to biopsy.

Vergleich anamnestischer Angaben (mittleres Lebensalter, Abfohlungen, Güstzeit [Jahre], Frühresorptionen [EPF] und Aborte) mit Gruppen, gebildet auf der Basis von histopathologischen Befundkombinationen (siehe Tabelle 2)

Histo-path. Groups	N.	Mean Age (years)	Mean Foals (N.)	Mean EPFs (N.)	Mean Aborts (N.)	Mean Barren (N.)
1	166	7.0	1.9	0.5	0.8	1.5
2	62	9.6	3.3	1.3	1.4	1.7
3	683	8.7	3.1	0.6	0.8	1.4
4	784	8.6	2.8	0.8	1.0	1.6
5	46	13.0	4.3	2.0	1.2	2.7
6	1316	14.4	5.4	0.7	1.2	2.5
7	918	14.3	5.5	0.6	1.0	2.4
8	2	16.0	6.5	0	1.0	2.0
9	33	17.6	7.7	0.6	0.9	3.3
10	26	18.5	6.6	0.8	1.8	3.0
11	39	5.2	0.3	0.3	0.6	1.1
12	21	10.0	4.2	1.0	1.3	2.1
13	52	10.4	4.0	1.0	1.3	2.3
14	17	11.3	3.4	0.8	1.4	1.7
15	50	12.3	4.2	0.8	1.5	2.5
All biopsies	4241	11.8	4.4	0.7	1.0	2.1

complicated EHyper (Group 12, 81 %), those with no significant histopathological features (Group 1, 75 %), those with EHypo (Group 11, 68 %) and those with uncomplicated CIE (Group 3, 66 %) and were lowest for those with EA complicated with CIE and CDE (Group 9, 17 %) and EA complicated with AE (Group 10, 21 %). The foaling percentage for the whole population studied was 54 %. The highest early pregnancy failure and abortion rates were seen in biopsies from mares with EA complicated with CIE and CDE (Group 9, 4 % and 13 % respectively). The early pregnancy failure and abortion rates for the whole population studied were 2 % and 5 %, respectively. For the biopsies with signs of diffuse stromal fibrosis, the mares subsequently showed a foaling percentage of 48 %. Their early pregnancy failure and abortion rates were both 3 %.

Discussion

This retrospective survey of the histopathological features seen in 4124 endometrial biopsy samples collected from UK Thoroughbred mares, over a 25 year period, provides data which supports widely held views *(Kenney, 1978; Ricketts, 1978; Ricketts, 1995)* on their pathogenesis and significance. Acute endometritis, most commonly associated with microbial infection, less commonly with non-infectious inflammation induced by intrauterine irritants, is seen in mares of all ages, and is followed by foaling, barren, early pregnancy failure and abortion rates similar to the examined population mean (except for cases complicating endometrial atrophy), suggesting that most respond to treatment. Endometrial hypoplasia is most commonly seen in young mares with delayed endometrial maturity, which usually resolves, without treatment, in time, to normality. Mononuclear cell infiltrations (chronic infiltrative endometritis) develop in the endometrial stroma as a reflection of local immune responses to challenge by seminal proteins, micro-organisms, environmental debris and the products of pregnancy, during a brood mare's life. Uncomplicated, they have no depressant effect on fertility potential and are not an indication for treatment. Endometrial hyperplasia most commonly persists during delayed post-partum or post-pregnancy failure uterine involution, more commonly in younger mares. Its resolution may be helped by intravenous oxytocin therapy *(Ricketts, 1978)*. Glandular degenerative changes and stromal fibrosis (chronic degenerative endometrial disease) develop as an inevitable consequence of ageing *(Ricketts and Alonso, 1991b)*, eventually leading to endometrial atrophy, a reflection of gynaecological senility. These degenerative changes result in endometrial incompetence, progressively reducing mares' foaling potentials and increasing their potential for barren years, early pregnancy failures and abortions. Whereas endometrial curettage may appear to have beneficial effects on the degree of CDE seen in endometrial biopsy samples collected before and after treatment in some younger mares *(Ricketts, 1975)* and return to normal cyclic regularity and pregnancy may reduce the degree of diffuse stromal fibrosis seen in some cases induced by hyperoestrogenism *(Ricketts, 1978)*, no currently recognised treatment can be relied upon to correct these endometrial degenerative changes and careful gynaecological management must be used to help the mare to compensate *(Kenney, Bergman, Cooper and Morse, 1975; Ricketts, 1989a; Ricketts 1989b)*.

Tab. 5: Histopathological Feature Combination Groups – percentages of foals born, barren years, early pregnancy failures and abortions suffered by mares when mates following biopsy and treatment (where indicated).

Vergleich von Kombinationen histopathologischer Befunde mit Parametern, die nach Biopsieentnahme und Therapie (falls indiziert) erhoben wurden: Abfohlrate, Güstzeit, Frühresorption (EPF) und Abort.

Histopath. Groups	Foals (%)	EPFs (%)	Abortions (%)	Barren (%)	NR*/ Died %
1	75	0	5	18	2
2	48	2	2	43	5
3	66	2	5	18	9
4	60	2	5	23	10
5	58	3	0	28	11
6	43	1	4	31	21
7	53	2	4	20	21
8	50	0	0	50	0
9	17	4	13	39	27
10	21	0	0	47	32
11	68	0	0	25	7
12	81	0	0	19	0
13	41	0	0	51	8
14	42	0	8	33	17
15	55	3	0	29	13
All biopsies	54	2	5	25	14

* NR = No returns (owners did not complete an official return to Weatherbys)

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