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Histology of the Post Partum Equine Uterus as Determined by Endometrial Biopsies

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Katila, T: Histology of the post partum equine uterus as determined by endometrial biopsies. Acta vet. scand. 1988, 29, 173-180. Altogether 156 equine endometrial biopsies were carried out during the post partum (p.p.) period: immediately after parturition until 15 days after foaling. The histological changes are described.

The rapid restoration of the equine endometrium is remarkable. The destruction of the luminal epithelium is minor and regeneration takes place rapidly. Remnants of the placenta, the microcotyledons and desquamated cells, have disappeared by 5 p.p. The transformation of glands from a pronounced secretory pattern to normal appearance also takes only 5 days. The occurrence of neutrophils and lymphocytes is normally connected with the restoration of the endometrium and should not always be interpreted as a sign of infection.

mare; uterine biopsy; post partum period.

Introduction

Andrews & McKenzie (1941) were the first to study the uterine histology in the post partum (p.p.) mare. Since that time, however, the histology of the post partum equine uterus has received little attention (*Ricketts* 1978, *Gygax et al.* 1979, *Bailey & Bristol,* 1983, *Sexton & Bristol* 1985).

The events during involution are of particular interest in the mare, because it is able to conceive again only 1-2 weeks after parturition, although fertility for the first p.p. oestrus has been reported to be lower than for subsequent oestrous periods (*Caslick* 1937, *Chieffi et al.* 1962, *Lieux* 1980, *Badi et al.* 1981, *Fiolka et al.* 1985). The uterus undergoes remarkable changes after parturition. It decreases tremendously in size, the microcaruncles rapidly disappear and the dilated glands return to their initial size (*Gygax et al.* 1979). Bacterial infections, which originate at the time of parturition, have to be eliminated. Leukocytes invade the uterus and their numbers then gradually decrease (*Andrews & McKenzie* 1941, *Ricketts* 1978). The pathogenesis of the changes that are found in uterine biopsies of problem mares is still obscure (*Kenney* 1978). It is not known to what extent these changes arise during parturition and the involutionary period. This paper gives a detailed description of the morphological changes of the endometrium after foaling.

Material and methods

The histological changes during 58 post partum periods of brood mares owned by the Finnish State Horse Breeding Institute were studied. The mares were mainly of the Finnhorse breed. The average age was

12.7 ± 4.5 years (5-23) and the number of foalings 4.1 ± 2.7 (1-13). Uterine biopsy specimens were taken on the 2nd, 5th, 10th and 15th days p.p. Since most of the mares were bred during the foal heat, only a few biopsies were carried out on the 10th and 15th days p.p. (Table 1). A further 6 research mares were used to obtain specimens immediately p.p. (0 h-24 h) and on days that were missing from the previous experiment (3rd, 4th and 6th-8th days).

The biopsy specimen was taken with an alligator type equine biopsy punch using the accepted technique (*Kenney* 1978). The specimen was fixed in Bouin's solution for 24 h and then transferred to 70% etanol, embedded in paraffin, sectioned in 4-6 µm thicknesses and stained with haematoxylin-eosin in the conventional way.

The principles and terminology presented by *Kenney* (1978) were followed in the interpretation and description of the biopsy specimens. Luminal contents, luminal epithelium, microcaruncles, glands and stroma of the stratum compactum and stratum spongiosum were individually evaluated in each sample. The amount of cellular infiltration was classified as slight (+), moderate (++) or heavy (+++) depending on the distribution, frequency, size and density of the infiltration. The numbers of neutrophilic and eosinophilic leukocytes and lymphocytes were evaluated separately. The term siderophage has been used for a macrophage which has phagocytized erythrocytes, whose remnants have been converted to haemosiderin.

Table 1. Number of uterine biopsy specimens obtained at different times following parturition.

Hours (h) or days (d) p.p.	Number of specimens
0 h	1
1 h	2
2 h	2
4 h	2
6 h	2
12 h	2
1 d	1
2 d	58
3 d	2
4 d	2
5 d	57
6 d	2
7 d	2
10 d	14
15 d	5
Total	156

Results

Luminal contents

Most biopsy specimens had luminal contents: blood, secretory material, and cells. Detached epithelial cells or strips of epithelium were commonly seen. Neutrophils occurred as individual cells, cell aggregations, or as a layer above the epithelium. When lymphocytes, eosinophils or siderophages were present, they occurred as individual cells. Various kinds of cell debris were also noted. Abundant luminal contents were found in 42 (27%) biopsy specimens. In these samples neutrophils were also usually present in the lumen. If neutrophils occurred in the lumen, they were also found in the endometrium. A large number of neutrophils were present in 33 (21%) tissue specimens.

Surface epithelium

Most of the epithelium was desquamated in 25 (16%) samples and large areas were free from epithelium in 25 other specimens. In the specimens taken less than 24 h p.p. the epithelium in the centre of the microcaruncles had often disappeared (Fig. 1). If the epithelium above microcaruncles was intact, it tended to be lower than between microcarunles in the biopsy specimens taken <2 days p.p. (Fig. 2). The height of the epithe-

lium varied between 10 and 50 µm, being lower early p.p. and higher >5 days p.p.
If leukocytes were present in large numbers in the lamina propria or lumen, simultaneous transepithelial migration was often seen. The most common migrating cell type was the neutrophil, but intraepithelial lympocytes were also frequent. Cytoplasmic vacuoles were often seen in the supranuclear area of epithelial cells 2 days p.p. They had the following kind of contents: 1) no contents, 2) droplets, 3) degenerated karyorrhectic cells and fragments of nuclei and 4) erythrocytes.

Microcaruncles
Microcaruncles were large (height 500-800 µm) and covered most of the sample <24 h p.p. Two days p.p. they had already diminished somewhat in size. Gradually the structure of the microcaruncle disappeared and only cell-dense stromal areas were left. Occasionally, some collapsed lumina were still present (Fig. 3).
Cryptal cells were intact 0-1 h p.p. The first degenerative changes were cytoplasmic vacuoles at 2-6 h p.p. Degenerative changes were more distinctive at 12-24 h with pyknosis, karyorrhexis, karyolysis and intracytoplasmic vacuoles. Hyperaemia of septal capillaries was noted in some microcaruncles (Fig. 4).
The cryptal lumina were large 0-4 h p.p. but later collapsed. In these early specimens the lumina were either empty or contained eosinophilic material. Some specimens (>6 h) contained cell debris, neutrophils and other cells in their cryptal lumina. Biopsy specimens taken 1-12 h p.p. had low numbers of neutrophils under the luminal epithelium and in the capillaries between the cryptal septa. The localization of neutrophils depended on the degree of the infiltration. The slightest infiltration consisted of neutrophils located in the capillaries and subepithelially. In heavier infiltrations neutrophils were also seen inside the cryptal lumina and extending from subepithelial tissues between the microcaruncles to the deeper stromal layers. In more extensive infiltrations they were present outside the microcaruncles in the str. spongiosum. Lymphocytes occurred rarely and in low numbers in specimens taken <3 days. Later they were almost always present. In the specimens taken 10 and 15 days p.p. the former areas of microcaruncles were detectable by the occurrence of sideophages and lymphocytes. Subepithelial lymphocytes were also seen in all these samples.

Glands
The glands were noticeably dilated 2 days p.p. and earlier. The glandular epithelium appeared pleomorphic. The cytoplasm stained pale and contained vacuoles. The luminal surface was uneven. Secretory granules were seen in the apical areas of the cytoplasm. Cellular debris was common in the glandular lumen (Fig. 5). There were areas in which glandular cells were apparently necrotic. The architecture of these glands was disappearing and their cytoplasm was basophilic.
Some specimens contained fibrotic gland nests with cystically distended glands (Fig. 6). They were located in the deeper layers of the str. spongiosum. These glands had an epithelium which varied from hypertrophic to atrophic (Fig. 6 & 7).
Five days p.p. the glands in the upper layers of the str. spongiosum had regenerated, whereas glands situated deeper sometimes still had the same heterogeneous and vacuolated appearance as described above. The regenerating glandular epithelium not only stained homogeneously and darker than previously but the cytoplasm had no vacuoles and the cytoplasmic luminal border was even

(Fig. 8). Mitotic figures were frequently seen. Siderophages were present in large numbers around the glands and in the glandular epithelium. By 10 days p.p. the majority of glands had returned to normal while the others were still slightly dilated. By 15 days p.p. almost all glands had returned to the pregravid size, but mitotic figures were still common.

Cellular infiltration of lamina propria

Neutrophils were common in the str. compactum under the luminal epithelium, but lymphocytes occurred more often in the str. spongiosum (Table 2). Neutrophils occurred with the same frequency on days 2 and 5, whereas lymphocytes, eosinophils and siderophages were more common 5 days p.p. The difference in lymphocyte infiltration on days 2 and 5 was statistically very highly significant, both in str. compactum and in str. spongiosum (x^2, $p<0.001$).

Periglandular aggregations of siderophages and lymphocytes occurred in all specimens taken on day 10, at which time lymphocytic infiltrations were often also seen perivascularly, but subepithelial neutrophils were detected in only 3 specimens.

All samples obtained 15 days p.p. had varying numbers of lymphocytes and siderophages. Lymphocytes were present in the str. compactum and periglandularly. Siderophages occurred at the sites of former microcaruncles and around the glands.

Discussion

The maximum surface area of the uterine biopsy site is 2 cm^2 or, on average, about 0.2% of the whole endometrial surface area (*Kenney* 1975). In spite of this, a single biopsy specimen is normally representative of the entire endometrium (*Kenney* 1975). The mechanical trauma caused by the biopsy is minor. There is usually 1-2 ml of haemorrhage

Figure 1. Section through a microcaruncle 2 h post partum. The luminal epithelium is largely intact but desquamated in the centre (a). Vacuolization of the septal epithelial calls can be seen (b). Cryptal lumina are large and a small number of neutrophils are present between the septa (c). HE × 160.

Figure 2. Section through a microcaruncle immediately after parturition. The luminal epithelium is intact and cubiodal (a). Cryptal lumina are large (b) and septal cells still unchanged (c). HE × 160.

Figure 3. Section through a microcaruncle 4 d post partum, the structure of which is disappearing but some cryptal lumina are still visible. Much karyorrhectic debris. HE × 160.

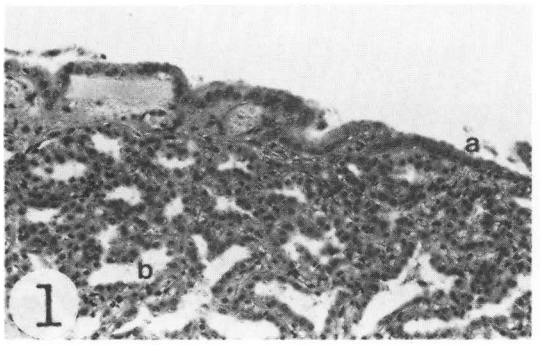
Figure 4. Section through a microcaruncle 1 d after foaling. Septal capillaries are hyperaemic (a). Vacuolization of cytoplasm, pyknosis and karyorrhexis are evident in septal epithelial cells. HE × 160.

Figure 5. Section through endometrial glands immediately post partum. The glands are dilated and contain cell debris (a). The glandular epithelium is tall columnar, while the cytoplasm is pale and vacuolated giving a heterogenous appearance (b). HE × 250.

Figure 6. Section through glands 3 d post partum with a fibrotic gland nest, in which the glands are cystically dilated and have a cuboidal darkly basophilic epithelium (a). Nonfibrosed glands have a paler staining, tall columnar and vacuolated epithelium. HE × 62.5.

Figure 7. Section through endometrium 3 d post partum. The gland in the upper part of the field is fibrotic, dilated and has a low columnar epithelium and papilliform infoldnings (a). The glands at the lower right have a tall secretory type epithelium (b). HE × 250.

Figure 8. Section through glands 7 d post partum. The glands have returned to their pregravid size and appearance. Numerous mitotic stages are seen in the epithelium (a). Mononuclear cells occur periglandularly (b). HE × 160.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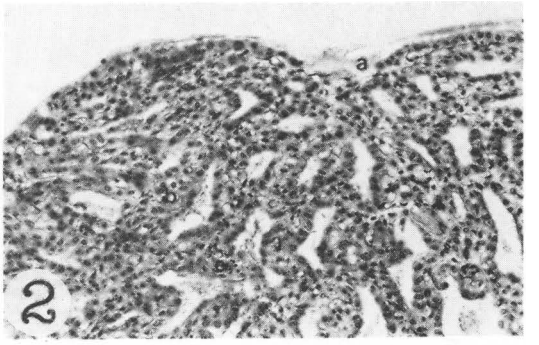
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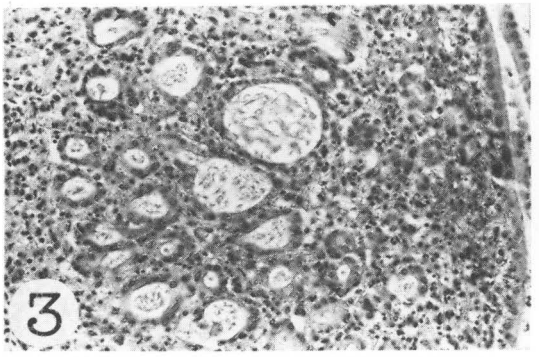
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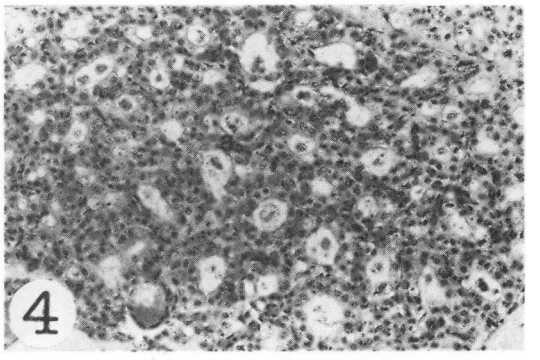
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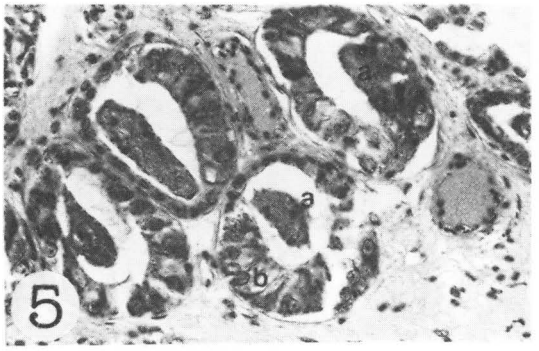
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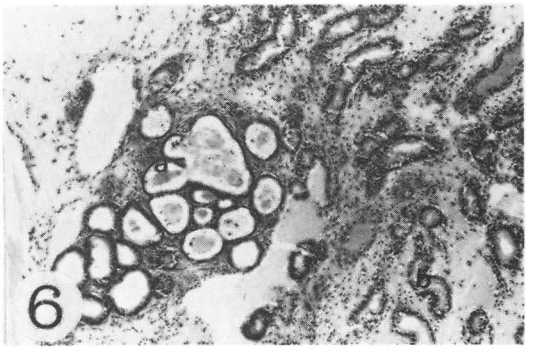
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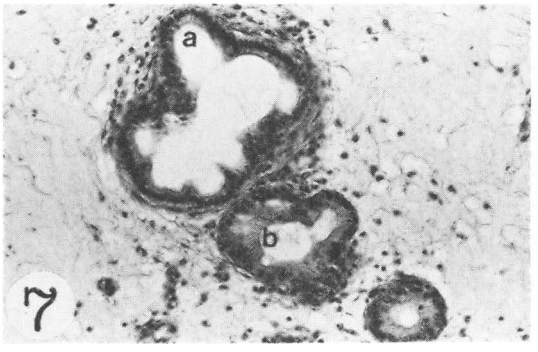
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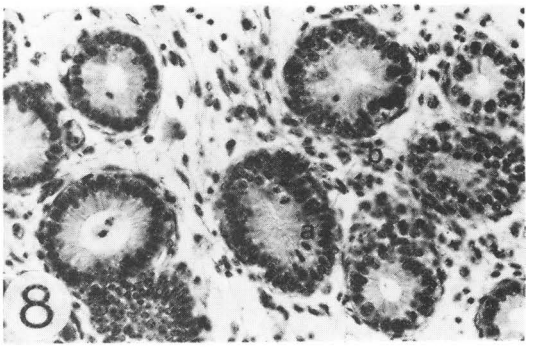
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Table 2. Occurrence of cellular infiltrations in lamina propria of post partum uterine biopsy specimens.

	Stratum compactum[a]		Stratum spongiosum			Stratum compactum[a]		Stratum spongiosum	
	n	%	n	%		n	%	n	%
2 days p.p. (n=58)					*5 days p.p. (n=57)*				
N-	12	20.7	35	60.3	N-	10	17.5	28	49.1
N+	24	41.4	9	15.5	N+	26	45.6	18	31.6
N++	16	27.6	10	17.2	N++	17	29.8	10	17.5
N+++	6	10.3	4	6.9	N+++	4	7.0	1	1.8
N present, total	46	79.3	23	39.7	N present, total	47	82.5	29	50.9
L-	52	89.7	20	34.5	L-	21	36.8	2	3.5
L+	5	8.6	34	58.6	L+	22	38.6	37	64.9
L++	1	1.7	4	6.9	L++	13	22.8	37	64.9
L+++	0	0.0	0	0.0	L+++	1	1.8	7	12.3
L present, total	6	10.3	38	65.5	L present, total	36	63.2	55	96.5
E-	56	96.6	54	93.1	E-	48	84.2	42	73.7
E+	2	3.4	3	5.2	E+	8	14.0	10	17.5
E++	0	0.0	1	1.7	E++	1	1.8	5	8.8
E present, total	2	3.4	4	6.9	E present, total	9	15.8	15	26.3
No infiltration	11	18.9	12	20.7	S-	0	0.0	30	52.6
					S+	0	0.0	15	26.3
					S++	0	0.0	5	8.8
					S+++	0	0.0	7	12.3
					S present, total	0	0.0	27	47.4
					No infiltration	2	3.5	2	3.5

[a]str. compactum does not contain microcotyledonary areas

N = neutrophils; L = lymphocytes; E = eosinophils; S = siderophages

- = no infiltration; + = slight infiltration; ++ = moderate infiltration; +++ = pronounced infiltration.

at the biopsy site (*Kenney* 1977). Manipulation of the equine uterus, however, easily causes neutrophilia (*Bennett et al.* 1980). Thus, biopsies may have increased the number of neutrophils and siderophages. Hyperaemia, oedema and haemorrhage are often related to surgical procurement of the specimen (*Kenney* 1978). Also, processing a biopsy specimen can cause some artefacts, such as detached luminal epithelium, distortion of the sample and intussusception of glands, particularly around the margins (*Kenney* 1978).

Surface epithelium

In the present study loss of epithelium was observed. Apparently loss can be caused by endometritis (*Vandeplassche et al.* 1983). However, there are conflicting reports about the loss and restoration of the surface epithelium in normal post partum periods. Thus, *Andrews & McKenzie* (1941) and *Ricketts* (1978) found that the luminal epithelium passed through a period of degeneration and subsequent restoration. Areas of ulceration were still visible at the beginning of

first p.p. oestrus (*Andrews & McKenzie* 1941, *Ricketts* 1978) and only 12 d p.p. the luminal epithelium was complete (*Ricketts* 1978). *Gygax et al.* (1979) observed that the luminal epithelium was intact again within 7 days p.p. *Blanchard et al.* (1985) administered endotoxin into the mare uterus 1-4 days p.p., which caused no clinical symptoms and the authors concluded that the intact uterine epithelium protected mares from endotoxin. Although histologic sections showed damage to the luminal epithelium 1-2 days p.p., scanning electron microscopy did not substantiate this finding (*Bailey & Bristol* 1983). These authors assumed that the absence of the epithelium was an artefact.

In the very early biopsy specimens (0-24 h) the epithelium above the centre of microcotyledons was frequently missing and secretory material was seen instead. The intact epithelium above the microcaruncles was low cuboidal until 2 d p.p. If epithelial loss truly occurs, according to the present study, it is only in small areas above the microcaruncles and is restored rapidly. The low epithelium above the microcaruncles may be epithelium that has already regenerated. It may also be epithelium which originates during the time of pregnancy, since the uterine epithelium is thinned during gestation to shorten the length of the diffusion pathway between foetal and maternal bloodstreams (*Samuel et al.* 1976). The increase in the height of the epithelium >2 days p.p. is probably due to the oestrogenic effect from the growing preovulatory follicle, as seen in the oestral cycling mare.

Microcaruncles

The involutionary changes observed in microcaruncles correspond closely with earlier studies (*Gygax et al.* 1979, *Bailey & Bristol* 1983). Some microcaruncles appeared hyperaemic as described by *Gygax et al.* (1979).

Glands

The cystic distention of glands from the duct to the basal portion was described in the present study, confirming the earlier findings by *Kenney* (1979), *Gygax et al.* (1979) and *Bailey & Bristol* (1983). Vacuolated glandular epithelium was a distinctive finding 2 days p.p. and earlier. *Ricketts* (1978) and *Bailey & Bristol* (1983) also noticed that epithelial cells had a vacuolated hypertrophic appearance. This may indicate either secretory activity or degenerative change. The fact that the glands had this appearance as early as 0 h p.p., suggests high secretory activity during pregnancy rather than degeneration. However, degeneration may start even before parturition as a result of hormonal changes.

An apocrine type of secretion has been observed in the bovine and human glandular epithelium (*Gordon* 1975, *Marinov & Lovell* 1968). The most probable source for the degenerated cells so commonly found in the lumina of the glands is epithelial cells shed from the glands. Whether this represents the apocrine type of secretion in horses is uncertain. Desquamation could also be caused by artificial disruption during tissue preparation, because the apical borders of the cell are fragile (*Gordon* 1975).

The fibrotic gland nests in the deeper layers of the str. spongiosum did not have the same appearance as the other glands. The epithelium was low, sometimes atrophic, the cytoplasm had no vacuoles, and the nuclei stained evenly and homogenously. The glandular epithelium resembled that of a cycling mare. These gland nests probably do not contribute to the secretion during pregnancy but remain nonfunctional. This may be one explanation for the decreased ability of mares with this kind of pathological change to carry a foal to full term (*Kenney* 1978).

Cellular infiltrations

The occurrence of neutrophils and lymphocytes may be considered a normal post partum phenomenon. Neutrophils occurred commonly (79%) in the str. compactum 2 d p.p. Almost all mares have bacteria in uterine samples immediately p.p. (*Farrelly & Mullaney* 1964, *Gygax et al.* 1979). The localization of neutrophils subepithelially in the str. compactum suggests that they are there to phagocytize bacteria that have entered the uterus at the time of parturition. Neutrophils may also be present because of aseptic inflammation as they play a role in the removal of microcaruncles.

One of the most common findings in endometrial biopsy specimens of older and problem mares is lymphocytic infiltrations (*Kenney* 1978). Some of these, especially those occurring periglandularly, may have arisen duuring the post partum period. Thus, they may represent a normal ageing phenomenon of the uterus that has gone through several pregnancies. It has been stated that inflammatory lesions are of less consequence to fertility than are fibrotic lesions (*Gordon & Sartin* 1978, *Leishman et al.* 1982, *Asbury* 1982).

Siderophages are known to be able to persist for a long time p.p. (*Kenney* 1978). These are macrophages that clear up cell debris, in this case haemorrhaged red cells, which explains why they were present in large numbers at the sites of former microcaruncles. They were never seen between the microcaruncles in the str. compactum. In the str. spongiosum they were frequently encountered around the glands and quite often also in the grandular epithelium. Perhaps they are needed to clear up degenerated glandular epithelium. *Gordon & Sartin* (1978) have also noted occurrence of haemosiderin in the glandular epithelium during the first p.p. oestrus. It is possible the the glandular epithelium itself has phagocytic activity.

Eosinophils were more common in str. spongiosum than in the str.compactum and slightly more frequent 5 days p.p. than 2 days p.p. *Kenney* (1978) reported that they occurred more frequently in inflammed uteri during oestrus. He assumed that they represent an immunologic reaction. *Slusher et al.* (1984) found no correlation with the stage of the oestrous cycle or cyclic activity, but they found that eosinophils were always accompanied by neutrophils. Most mares that had eosinophils exhibited a poor perineal conformation, which was thought to result in pneumouterus. Deliberate introduction of air into the uterus caused infiltration of eosinophils in uterine specimens (*Slusher et al.* 1984). Eosinophils of rats and humans are known to have oestrogen receptors and to increase during oestrus and oestrogen treatment (*Beeson & Bass* 1977). It has been postulated that they are carriers of oestrogen into the uterus (*Beeson & Bass* 1977). This could explain their increase in day 5 biopsy specimens since most mares have a developing follicle at that time.

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Sammanfattning

Puerperal histologi av livmodern hos häst med hjälp av endometriumbiopsi.

Sammanlagt 156 biopsier utfördes hos post partum (p.p.) ston: omedelbart efter fölning t.o.m. 15 dygn senare. De histologiska förändringarna beskrivs. Regenerationen av stoets endometrium p.p. är anmärkningsvärt snabb. Det luminala epiteliets destruktiva förändringar äro minimala och regenerationen snabb. Placentarester, mikrocotyledoner och deskvamerade celler försvinner inom 5 dygn p.p. Transformationen av körtlar från ett stadium av kraftig till normal sekretion tar ej heller mera än 5 dygn. Förekomsten av neutrofiler och lymfocyter är normal i samband med endometriets regeneration och behöver inte nödvändigtvis anses som ett tecken på infektion.

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