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# Histomorphometry of epithelial structures of the mare's endometrium

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## Abstract

The aim of this study was to analyze through histomorphometry, epithelial structures of the mare's endometrium classified according to Kenney and Doig (1986). Histomorphometric analysis of the luminal and glandular epithelial cell heights, glandular lumen diameter, glandular density, percentage of glands with apparent lumen and with intraluminal secretion, and a descriptive analysis of the endometrium was made in 65 endometrial biopsies. Samples were collected during estrus (n=30) and diestrus (n=35), fixed in Bouin's solution, embedded in paraffin HxE and classified according to Kenney and Doig (1986) categories. Groups of IIa category obtained the greatest medium cell heights for luminal and glandular epithelium, and groups of III category obtained the smallest values, during estrus and diestrus. The medium heights of luminal epithelium, glandular epithelium and glandular lumen diameter of samples collected during estrus were greater than those collected during diestrus. The gland density was greater during diestrus than estrus. This work showed that histomorphometry could improve the consistency in biopsy evaluation by furnishing objective data.

Keywords: Morphometry, mare, endometrium, epithelium.

## Introduction

The endometrium has a special role in fertility as it has been known for a number of years. The conceptus depends essentially on the several uterine secretions which furnishes a system for metabolic exchange of nutrients and waste products between the embryonic vesicle and the endometrium (Ginther 1992).

There are many aids to evaluate the uterus and endometrium, nevertheless changes that markedly affect fertility can be unavailable to be diagnosticated (Kenney 1978; Doig et al. 1981; Kenney and Doig, 1986). Many of these changes can be revealed only by endometrial biopsy. The technique of endometrial biopsy in the mare is simple, safe, and painless (Ricketts, 1975). According to the pathologic changes, the endometrium can be assigned to a prognostic category, of which the most widely accepted are Kenney's (1978) and Kenney and Doig's (1986) systems.

However the application of these methods to classify endometrial biopsies is dependent on a subjective evaluation. Histomorphometry may yield a more objective study of the endometrial morphology and more preciseness in diagnosis of mare's endometrial pathologies. Morphometry means the act of measuring anatomical structures, which may have a significant role in histology and histopathology (Mandarin-de-Lacerda, 1994).

A morphometric investigation of the epithelial structures could be useful since the uterine epithelium responds quickly and dramatically to the hormonal environment and to the presence of pathogenic organisms (Samuel et al. 1979)

The purpose of this study was the histomorphometric analysis of epithelial structures of the mare's endometrium, and to compare, through statistical analysis, the quantitative results obtained from endometrial samples categorized according to Kenney and Doig's (1986) system.

## Materials and methods

### *Materials*

Sixty-five archive endometrial biopsies from 65 mares (Mangalarga Marchador and Campolina), 4 to 25 years old, were used in this study.

Those endometrial samples were collected according to previously described technique (Kenney 1978), during estrus and diestrus, and categorized according to Kenney and Doig (1986) categories (Table 1).

### *Methods*

Histomorphometric analysis of the endometrial samples was staining by HxE and conducted to evaluate the height of the

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**Table 1:** Classification of endometrial biopsies according to Kenney and Doig (1986)

| | Diestrus | Estrus |
|---|---|---|
| Categories | n | n |
| I | 10 | 5 |
| IIa | 10 | 10 |
| IIb | 10 | 10 |
| III | 5 | 5 |
| Total | 35 | 30 |

luminal and glandular epithelium; glandular lumina diameter, glandular density, and percentage of glands with apparent lumen and with intraluminal secretion. A semiautomatic image analyser system composed of a light camera and a digitater table (Zeiss Kontron, Germany), with an ocular reticule (Figure 1) was used.


**Figure 1**: Ocular reticule associated with a semiautomatic image analyser system composed of a light camera and a digitater table (Zeiss Knotron, Germany)

Histomorphometric analysis of luminal and glandular epithelial cell heights was made by measuring the distance from the cell apical margin to the basement membrane, until one hundred cells was reached. A magnification of 400x was used.

Histomorphometric analysis of glandular lumen diameter was made through the smallest diameter of glandular lumen, until reaching one hundred glands. A magnification of 400x was used.

Glandular density was determined by counting the number of glands, its ducts or branches within the stratum compactum and spongiosum within a randomly determined area. A magnification of 100x was used. Percentage of glands with apparent lumen and glands with intraluminal secretion was made simultaneously to the counting of glands in gland density, using a manual cell counter.

A descriptive analysis of the endometrium was also made, to evaluate frequence of pseudostratification, citoplasmic vacuoles and mitosis in luminal and glandular epithelium, vascular changes (congestion, hemorrage, and edema).

Data obtained from histomorphometry were subjected to an analysis of variance (ANOVA) for unbalanced samples, and descriptive statistics, using the "Statistical Analysis System" (SAS) package version 6.01 for microcomputer.

## Results

Endometrial samples collected during estrus showed greater medium heights of luminal (Table 2) and glandular (Table 3) epithelial cells than those collected during diestrus. These differences haven't been significant, though, except between IIaD and IIaE groups, relating to luminal epithelium. IIa category samples showed the greatest medium height of glandular (Figure 2) and luminal (Figure 3) epithelium in estrus as well as in diestrus while III category had the smallest (Figure 4). These differences have been statistically significant, except for luminal epithelium of estrus samples.

**Table 2**: Descriptive statistical analysis and analysis of variance of the histomorphometrical measurements of luminal epithelial height in endometrial biopsies from mares in diestrus (D) and estrus (E) classified according to Kenney and Doig

| Group | n | X(µm) | SD | Group | n | X(µm) | SD |
|---|---|---|---|---|---|---|---|
| ID | 10 | 15,18 | 4,77 | IE | 5 | 19,74 | 3,86 |
| IIaD | 10 | 17,25$^{ab}$ | 3,17 | IIaE | 10 | 20,46$^{a}$ | 2,23 |
| IIbD | 9 | 14,43 | 3,04 | IIbE | 9 | 16,95 | 4,03 |
| IIID | 5 | 13,06$^{b}$ | 3,37 | IIIE | 4 | 14,07 | 5,72 |

X= mean, SD= standard deviation, a-Group IIaD significantly different from group IIaE (p=0,0312), b-Group IIaD significantly different from group IIID (p=0,0433).

**Table 3**: Descriptive statistical analysis and analysis of variance of the histomorphometrical measurements of glandular epithelial height in endometrial biopsies from mares in diestrus (D) and estrus (E) classified according to Kenney and Doig

| Group | n | X(µm) | SD | Group | n | X(µm) | SD |
|---|---|---|---|---|---|---|---|
| ID | 10 | 13,81 | 2,93 | IE | 5 | 16,39$^{b}$ | 0,61 |
| IIaD | 10 | 15,02$^{a}$ | 2,03 | IIaE | 10 | 16,73$^{c}$ | 2,19 |
| IIbD | 10 | 14,38 | 2,43 | IIbE | 9 | 15,86$^{d}$ | 2,62 |
| IIID | 5 | 11,72$^{a}$ | 2,24 | IIIE | 5 | 11,72$^{bcd}$ | 1,95 |

X= mean, SD= standard deviation, a-Group IIaD significantly different from group IIID (p=0,0169), b-Group IE significantly different from group IIIE (p=0,0122), c-Group IIaE significantly different from group IIIE (p=0,0040), d-Group IIbE significantly different from group IIIE (p=0,0113).

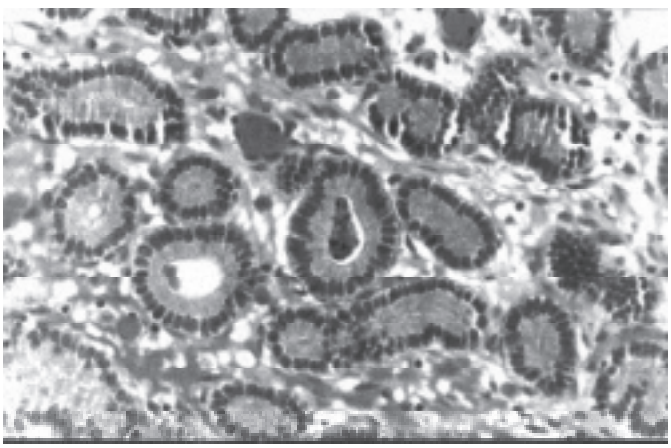

**Figure 2**: Equine endometrial biopsy (7IIaE). Tall columnar glandular epithelium. Glandular intraluminal secretion. Congestion and Hemorrage. H.E. 400x negative

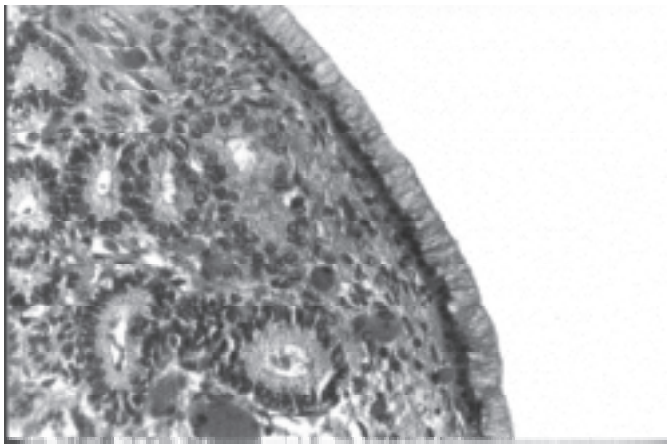

**Figure 3**: Equine endometrial biopsy (1-IIaE). Tall columnar laminal epithelium. H. E. 400x negative.

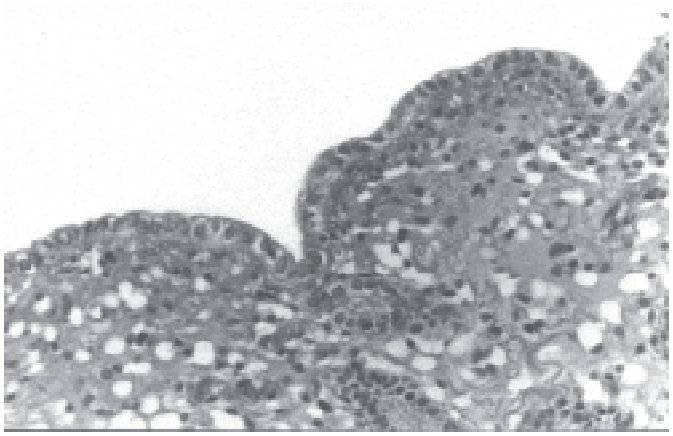

**Figure 4**: Equine endometrial biopsy (5-IIID). Cuboidal luminal epithelium. H.E. 400x negative.

Endometrial samples collected during estrus showed greater medium diameters of glandular lumina than those collected during diestrus (Table 4). These differences haven't been significant, though, except to I category.

**Table 4:** Descriptive statistical analysis and analysis of variance of the histomorphometrical measurements of glandular lumen diameter in endometrial biopsies from mares in diestrus (D) and estrus (E) classified according to Kenney and Doig

| Group | n | X(µm) | SD | Group | n | X(µm) | SD |
|---|---|---|---|---|---|---|---|
| ID | 9 | 10,19$^{abcd}$ | 0,94 | IE | 5 | 13,65$^{a}$ | 1,38 |
| IIaD | 10 | 13,29$^{b}$ | 1,33 | IIaE | 10 | 13,59 | 1,70 |
| IIbD | 10 | 13,15$^{c}$ | 1,91 | IIbE | 9 | 14,56 | 3,84 |
| IIID | 5 | 12,55$^{d}$ | 2,19 | IIIE | 5 | 22,35 | 13,13 |

X= mean, SD= standard deviation, a-Group ID significantly different from group IE (p=0,0034), b-Group ID significantly different from group IIaD (p=0,0003), c-Group ID significantly different from group IIbD (p=0,0048), d-Group ID significantly different from group IIID (p=0,0234).

Glandular density (Table 5) was greater in diestrus samples than estrus samples, but this difference was significant only in normal considered samples (I category) and in IIb category samples.

**Table 5**: Descriptive statistical analysis and analysis of variance of the glandular density in endometrial biopsies from mares in diestrus (D) and estrus (E) classified according to Kenney and Doig

| Group | n | X(gl/mm2) | SD | Group | n | X(gl/mm2) | SD |
|---|---|---|---|---|---|---|---|
| ID | 10 | 129,93$^{a}$ | 22,80 | IE | 5 | 95,97$^{a}$ | 30,16 |
| IIaD | 10 | 115,43 | 25,32 | IIaE | 10 | 101,18 | 34,42 |
| IIbD | 10 | 129,12$^{b}$ | 45,04 | IIbE | 9 | 80,99$^{b}$ | 19,13 |
| IIID | 5 | 151,35 | 55,63 | IIIE | 5 | 89,62 | 66,15 |

X= mean, SD= standard deviation, a-Group ID significantly different from group IE (p= 0,0433), b-Group IIbD significantly different from group IIbE (p=0,008).

A higher percentage of glands with apparent lumen (Table 6) and glands with intraluminal secretion (Table 7, Figure 2) occurred during estrus, except in IIa category samples. Hoewever, there was no significant difference between both phasis of the estral cycle, in relation to intraluminal secretion.

**Tabela 6**: Descriptive statistical analysis and analysis of variance of the percentage of glands with apparent lumen in endometrial biopsies from mares in diestrus (D) and estrus (E) classified according to Kenney and Doig

| | n | X(gl/mm2) | DP | Grupo | n | X(gl/mm2) | DP |
|---|---|---|---|---|---|---|---|
| ID | 10 | 25,37$^{acd}$ | 16,95 | IE | 5 | 49,68$^{a}$ | 19,41 |
| IIaD | 10 | 45,31$^{c}$ | 15,90 | IIaE | 10 | 44,48$^{e}$ | 8,83 |
| IIbD | 10 | 46,38$^{d}$ | 13,26 | IIbE | 9 | 52,22$^{f}$ | 17,74 |
| IIID | 5 | 44,62$^{b}$ | 18,35 | IIIE | 5 | 76,29$^{bef}$ | 13,44 |

X= mean, SD= standard deviation, a-Group ID significantly different from group IE (p= 0,0321), b-Group IIID significantly different from group IIIE (p= 0,0216), c-Group ID significantly different from group IIaD (p= 0,0211), d-Group ID significantly different from group IIbD (p= 0,0091), e-Group IIaE significantly different from group IIIE (p= 0,0027), f-Group IIbE significantly different from group IIIE (p= 0,0164).

**Table 7**: Descriptive statistical analysis and analysis of variance of the percentage of glands with intraluminal secretion in endometrial biopsies from mares in diestrus (D) and estrus (E) classified according to Kenney and Doig

| Grupo | n | X(gl/mm2) | DP | Grupo | n | X(gl/mm2) | DP |
|---|---|---|---|---|---|---|---|
| ID | 10 | 11,75$^{a}$ | 8,78 | IE | 5 | 17,94 | 11,83 |
| IIaD | 10 | 19,80$^{a}$ | 8,48 | IIaE | 10 | 15,52 | 8,14 |
| IIbD | 10 | 18,14 | 9,47 | IIbE | 9 | 19,78 | 14,60 |
| IIID | 5 | 21,49 | 12,57 | IIIE | 5 | 34,39 | 29,47 |

X= mean, SD= standard deviation, a-Group ID significantly different from group IIaD (p= 0,0376).

Pseudostratification in luminal epithelium occurred in estrus as often as in diestrus, but IIa category presented the highest numbers of samples with epithelial pseudostratification. There

was a higher appearance of basal citoplasmic vacuoles in luminal epithelium and mitotic figures in glandular epithelium (Figure 5) in samples collected during estrus. Category III samples haven't shown epithelial pseudostratification and mitotic figures and also presented the smaller number of samples with citoplasmic vacuoles in luminal epithelium.

Congestion (Figure 2) was observed in all endometrial samples evaluated. Hemorrage (Figure 2) was observed in a higher number of samples collected during estrus and more frequently in IIa and IIb categories. Edema has appeared in all categories, but it was more intense and more often in samples collected during estrus.

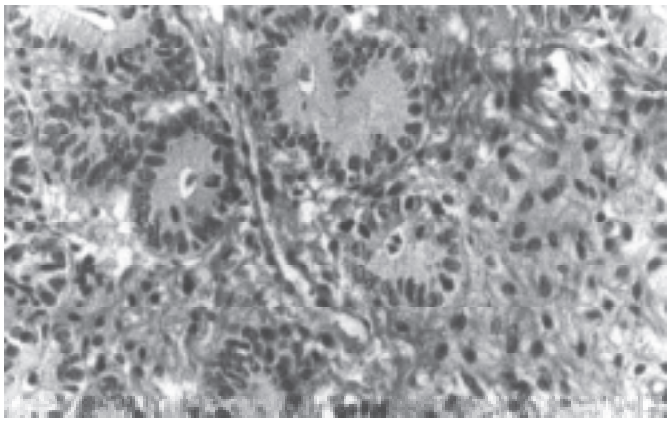

**Figure 5**: Equine endometrial biopsy (10-IIbE). Glandular epithelium showing mitotic figure. H.E. 400x negative

## Discussion

The height of mare's endometrial epithelium varies according to the ovarian cycle. The increase in height of the luminal (Kenney, 1978; Kenney and Doig, 1986; Van Camp, 1988; Ginther, 1992; Doig and Waelchi, 1993) and glandular (Van Camp, 1988; Doig and Waelchi 1993) epithelial cells during estrus is described as a normal variation related to estrous cycle.

Many authors have employed histomorphometry in the evaluation of luminal epithelial cell heights and the values found in this work were similar to theirs (Leishman et al., 1982; Arrot et al., 1994; Rasch et al., 1996). However, stabilizing an analogy between these values is difficult since the groups of mares utilized as well as the methodology employed were differents.

In both stages of the estrous cycle, IIa category had the greatest medium heights of luminal and glandular epithelium. Perhaps the increase in epithelial activity is one of the first endometrial responses in early inflamatory reaction. Indeed, in a work, following the trauma of indwelling catheterization, the height and staining reactions of luminal epithelial cells were greater than their appearance before (Freeman et al., 1990).

Glandular and luminal epithelium were comparable in height, although luminal epithelial cell heights were greater. It may be related to the fact that deep glands in lamina propria had shown less active epithelium when compared to upper glandular portions and uterine lumen. In fact, the percentage of glands related to a defined area, as well as the diameter of glands were found to be higher in stratum compactum than in stratum spongiosum (Rasch et al., 1996).

Few works refer to cyclic variations of the glandular luminal diameter. Neither have been found quantitative parameters to differentiate cystic glands and to compare to the wide range of values found in this work. Keenan et al. (1991) found that glandular lumina were more distended with secretion at day 12 of pregnancy than that observed during diestrus, at the same stage post ovulation. In addition, wider glandular lumina were found in mares with mucometra when compared to mares without intraluminal fluid, as indicated by morphometrical measurements (Rasch et al., 1996). Histomorphometric analysis of the glandular luminal diameter may be useful in the recognition of endometrial atrophy as well as cystic glands, which probably has an important prognostic value.

Gland density was higher during diestrus probably because of the diminished stromal edema, and the increased gland tortuosity (Kenney, 1978). The current study showed a great variability in gland density. It has already been suggested that it may reflect a significant individual variation, but for a group considered essentially normal reproductively (Leishman et al., 1982).

Pseudostratification of the uterine luminal epithelium is described during estrus (Ginther, 1992; Kainer, 1993), although it has been observed also during diestrus. Most of citoplasmic vacuoles was observed at the basal portion of the luminal epithelial cells, described as a common find in estrus (Kenney, 1978; Doig and Waelchi, 1993). Hypertrophy and cellular vacuolation during estrus were also noted through electron microscopy (Keenan et al., 1991). The greater appearance of mitotic figures in the glandular epithelium during estrus suggests that it is also an effect produced by estrogen. Thus, pseudostratification, citoplasmic vacuoles and mitotic figures, in addition to increased epithelial height, seem to be cellular activity signs, which higher intensity is an effect of estrogen.

The results suggests that vascular changes may be produced when the sample is taken. So they must be evaluated carefully because they can also be signs of inflamatory processes.

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