

The Importance of Endometrial Biopsy in the Evaluation of Fertility Potential of Mares in Raise Horses in Turkey

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Summary

The aim of this study was to detect pathological changes in the endometrium of mares with the endometrial biopsy technique and to classify these changes according to distribution and severity, and finally to investigate the relation of these changes to age and foaling rates. Biopsies were obtained from 53 mares which had not foaled for at least two years who were at different stages of the estrous cycles. Pathological endometrial changes were observed in 92.3 percent of mares with the majority having fibrosis (73.6%) and chronic infiltrative changes (50.9%). All categories included mares of different ages. Four mares were classified as category-I, 7 as category IIa, 10 as category-IIb and 32 as category-III. The foaling rate obtained for categories-I, IIa, IIb and III were 75, 42.8, 10 and 0% respectively for the breeding season following the collection of endometrial biopsy. It is apparent from the data that there is a meaningful association between histological categories and foaling rate indicating that endometrial biopsy should be used as a diagnostic technique in the evaluation of fertility potential of mares.

Keywords: Mare, Endometrial biopsy, Degenerative changes, Infertility

Türkiyedeki Yarış Atlarında Fertilitenin Değerlendirilmesinde Endometrial Biopsinin Önemi

Özet

Çalışmanın amacı, kısraklarda biopsi yöntemi ile endometrial patolojik değişimleri belirlemek, yaygınlığına ve şiddetine göre kategorize etmek, ve bu değişimlerin yaş ve fertilitenin ile olan ilişkisini belirlemektir. Biopsiler en az 2 yıldır doğuramayan 53 kısraktan siklusun farklı dönemlerinde alındı. Fibrosis (%73.6) ve kronik infiltratif değişimler (%50.9) en yaygın olarak üzere kısrakların %92.3'ünde çeşitli endometrial patolojik değişimlere rastlandı. Kategorilerde her yaşta kısrağın mevcuttu. Endometrial patolojik değişimlere göre 4 kısrağın kategori-I, 7 kısrağın kategori-IIa, 10 kısrağın kategori-IIb ve 32 kısrağın kategori-III grubuna dahil edildi. Biopsi alınımından sonraki üreme sezonunda kategori I, IIa, IIb ve III grubu kısraklar için elde edilen doğum oranları sırası ile %75, 42.6, 10 ve 0 olarak belirlendi. Sonuçlar, histolojik kategorilerle doğum oranları arasında belirgin bir ilişki olduğunu göstermektedir ve bu nedenle fertilitenin değerlendirilmesinde endometrial biopsinin klinik olarak kullanımının önem taşıdığını göstermektedir.

Anahtar sözcükler: Kısrağın, Endometrial biopsi, Dejeneratif değişimler, İnfertilite

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INTRODUCTION

Diagnostic techniques are of great importance in the determination of the causes and prognosis of infertility¹. A normal functional uterus, especially the endometrium, is of significant importance in the fertility potential of mares. While the size, shape and consistency of the uterus can be assessed with rectal palpation, ultrasound and hysteroscopy, endometrial secretions can also be assessed with endometrial swabs for pathogen microorganisms. However, despite all these accessory diagnostic techniques, it is yet impossible to determine endometrial degenerative (fibrosis, nonseasonal hypoplasia, atrophy or dilation of glands) and inflammatory changes that dramatically reduce fertility^{2,3}. The biopsy technique has been used for a long time in determining the estrous period in the sexual cycle, the existence of active or chronic endometrial inflammation, gestation chance of the mare and the possibility of continuance of the gestation^{4,5}. Changes determined to be related to fertility can also be evaluated with the results obtained from a second biopsy after the intrauterine treatment⁶. An important conclusion can be drawn about the fertility potential of mares when endometrial biopsy results are combined with other gynecological examination methods such as anamnesis, ultrasound and laboratory tests⁷⁻¹⁰.

The aim of the study was to detect pathological changes in the endometrium of infertile mares and to investigate the relation of these categorized changes with age and foaling rates.

MATERIAL and METHODS

The study was carried out using English and Arabic race horses that had not given birth to a foal for at least 2 years and those with a fertility problem, raised under different management conditions on various farms, mainly in Izmit Boarding Houses of the Jockey Club in Turkey during the year 1997 to 2000. In this study, which was completed in 3 years, 53 mares with an average age of 14.9 ranging from 7 to 22, were used.

A thorough gynaecological examination was performed on each mare prior to endometrial biopsy. In the absence of palpable uterine abnormalities, the

biopsies were taken from the floor or medial wall of either horn by inserting a basket jawed biopsy instrument (60 cm in length) through the cervix following putting on a plastic obstetrical sleeve soaked in an antiseptic solution¹¹. Each specimen of 10-20x3x3 mm was placed in 10% formalin just after collection by using a biopsy punch, and fixed, embedded in paraffin, sectioned at 6 microns and stained with Hematoxyline - Eosin (H.E) and Triple in the Laboratory of Pathology Department at the Faculty of Veterinary medicine, University of Istanbul¹¹.

Inflammatory and chronic degenerative changes such as fibrosis, dilation of glands, lymph lacunas, atrophy and hypoplasia were classified according to their prevalence and severity using the grading system of Kennedy and Doig¹². This system contains category-I: (Mares with normal endometrium), category-IIa: (Mares with mild endometrial change) category-IIb: (Mares with moderate endometrial changes, category-III: (Mares with severe endometrial changes). The expected foaling ratio for category I, IIa, IIb and III are approximately 80-90, 50-80, 10-50 and 10%, respectively according to this 4-category rating system.

In the case where two pathological changes were observed concurrently, the mare was assessed in a sub-category. Categorizations of the mares were only made according to the histopathological changes in the endometrium and were not influenced by the past history of the mare. Changes associated with surface epithelium, artifacts due to stromal edema, and changes such as perivascular hemorrhages were not taken into consideration.

Subsequent to the prebreeding examination uterine treatment was applied when indicated. The need for treatment was based on findings of anamnesis, clinical, ultrasonographic, and antibiogram tests. Therapy consisted mainly of an intrauterine infusion with antibiotics, one dose of Rifaximina (Fatroximine Spray can, 100 mg Vetaş, Turkey), was preferred for infusion due to its easy usage combined with Caslick's operation if necessary. The breeding of mares is not supervised and all mares were bred on different farms with considerable variation in management practice. Fertility data was determined only by the number of live foal and confined to one

breeding season following the biopsy.

The influence of endometrial changes on fertility was analyzed using a chi-square test. Probability values of $P < 0.05$ were considered significant.

RESULTS

The category distribution on the basis of uterine biopsy of 53 mares is given in the table. There were 4, 7, 10, and 32 mares in categories-I, IIa, IIb and III respectively. The mean age of the mares showed an

increase from category-I to III. It appeared that older mares tended to be classified into category-III, the category of lower expected fertility. The foaling rates of mares in category-I, IIa and IIb were 75, 42.8 and 10 % respectively. Thirty two mares classified in category-III failed to produce live foal. The difference in obtained foaling rates between category III and I, IIa, IIb, and between I and IIb were statistically significant ($p < 0.05$) (Table 1).

Only 4 mares included in category-I did not show evidence of endometrial histopathology (Fig. 1).

Table 1. Shows the number of mares and foaling mares, the mean age of mares and the number of mares with pathological endometrial changes in each category established according to the severity and prevalence of endometrial pathological changes.

Tablo 1. Patolojik değişimlerin yaygınlığını ve şiddetini gösteren kategorilere göre kısrakların dağılımı, yaş ortalamaları, doğum oranları ve patolojik değişimlere göre kısraak sayısı ve oranları görülmektedir.

Categories	Mares n (%)	Mean age	Foaling mares n (%)	Mares with pathological changes (n) and (%)						
				Acute endometriti	Chronic endometriti	Fibrosis	Lenf lakunae	Atrophy	Glandular dilatatioi	Hypoplasia
I	4 (7.5)	10.5	3 (75 ^a)	-	-	-	-	-	-	-
IIa	7 (13.2)	11.2	3 (42.8)	1	2	3	-	1	-	1
IIb	10 (18.9)	13	1 (10 ^a)	-	3	6	1	2	-	1
III	32 (60.4)	16.8	0 (0 ^b)	6	22	31	4	8	9	3
Total n (%)	53	-	7 (13.2)	7 (13.2)	27 (50.9)	40 (73.62)	5 (9.4)	11 (20.8)	9 (17)	5 (9.4)

a-b Percentage of foaling mares for each category within the same column with common superscript differ significantly ($p < 0.05$).

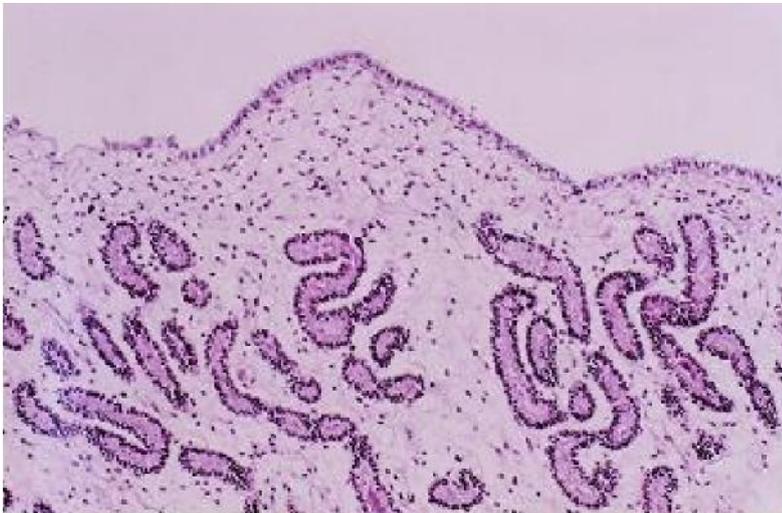


Fig 1. Category-I. Endometrial section with normal gland distribution and frequency, and infiltration of a few inflammatory cells in a normal endometrium. H.E (x100)

Şekil 1 . Katategori-I. Normal uterus endometriumundaki glandüler dağılımı ve çok az sayıda infiltratif yanğı hücrelerini gösteren bir endometrial doku kesiti. H.E (x100)

Pathological endometrial changes were observed in 49 mares (92.5%), either alone or mixed in some degree of slight to severe. Fibrosis and chronic infiltrative endometritis were the most widespread changes. Demonstrable endometrial fibrosis was

found in 73.6 % of mares with a very high ratio in category I II (Fig.4). Mononuclear cell infiltrations (lymphocyte and plasma cell) indicating chronic infiltrative endometritis were found in 50.9% of mares. Acute endometritis was less common

Fig 2. Category-IIa. Mild acute endometritis characterised with polymorphonuclear cell infiltration of epithelial and stromal tissue of uterus. H.E (x100)

Şekil 2. Kategori-IIa. Endometriumun stromal ve epitelyal katında polimorf nükleer lökosit infiltrasyonu ile karakterize hafif endometrial yangı. H.E (100)

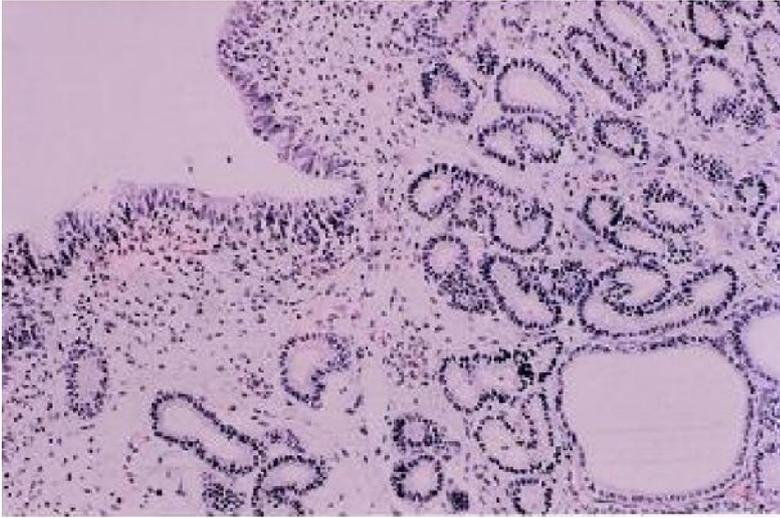
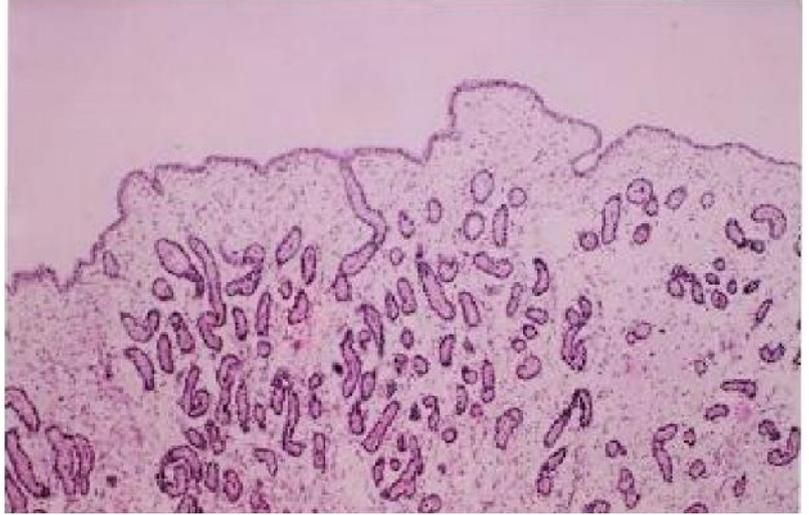
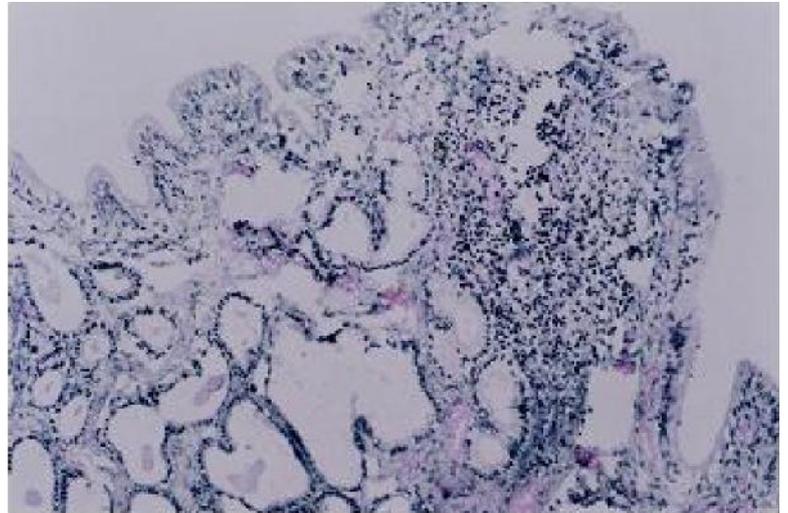


Fig 3. Category-IIb. Moderate infiltration of lymphocyte and mononuclear macrophage, periglandular and stromal fibrosis characterised with dilatation of glands in the stratum compactum. H.E (x 100)

Şekil 3. Kategori-IIb. Uterusun endometrial stratum kompaktum katında glandüler dilatasyon, lenfosit ve mononükleer makrofaj infiltrasyonu ve periglandüler ve stromal fibrosis ile karakterize orta derecede yangı olarak tanımlanan endometritis. H.E (x100)

Fig 4. Category-III. Severe chronic degenerative changes characterised with dilatation of glands, focal infiltration of inflammatory cells (lymphocyte and macrophage), and stromal fibrosis in the endometrium. H.E (x200)

Şekil 4. Kategori-III. Şiddetli kronik dejeneratif değişimlerle karakterize endometrial stromal fibrosis, odaksal infiltratif yangı hücreleri (makrofaj ve lenfosit) ve glandüler dilatasyon. H.E (x100)



pared to atrophy and the dilation of glands, and found in 13.2% of mares. In 5 mares with endometrial hyperplasia, the lamina propria was thin, containing few inactive and rudimentary glands. We had no opportunity to carry out chromosomal analyses for a certain diagnosis (Table).

The types and prevalence of various histopathological lesions in the endometrium of mares were given as Figure 1, 2, 3 and 4 for each Category-I, IIa, IIb and III.

DISCUSSION

Chronic infiltrative endometritis (CIE) and chronic degenerative endometritis (CDE) are unavoidable, when repeated acute endometritis, pregnancies, and post-parturient involutions combine with the normal results of aging¹. The findings in this study support previous observations that endometrial biopsy is a valuable diagnostic and prognostic aid in the evaluation of infertile mares^{2,13-14}. The type and incidence of various pathological changes are similar to those reported by Ricketts² and Doig et al.¹⁴. In both studies it has been reported that evidence of endometrial histopathology was observed in over 90% of subfertile mares, and that chronic infiltrative endometritis (CIE) and chronic degenerative endometritis (CDE), mainly fibrosis were the most common histopathological changes observed individually or sometimes associated with acute endometritis. In the study presented, except for 4 mares considered as normal in category-I, pathological endometrial changes were determined in 49 (92.4%) mares to some degree of slight to severe. Attention has been drawn to the relation between severity of chronic degenerative changes and the capability of the mare to maintain pregnancy. In the study of Kenney¹, it has been suggested that periglandular fibrosis may be the most common reason for early embryonic deaths (EED) seen before the 90th days of pregnancy. Results of field trials support this suggestion^{15,16}. In the presented study, endometrial fibrosis was the most common change observed individually or associated with other pathological lesions in 73.6% of mares. Acute infiltrative endometritis was observed in fewer mares (13.2%) while chronic infiltrative endometritis was observed in 50.9% of mares. When comparing the most common changes, fibrosis and chronic infiltrative endometritis ratios, with the results of Doig et al.¹⁴ (88.3 and 51%) and Ricketts² (45

and 43.1% respectively) a parallelism was observed among the ratios. The low acute infiltrative endometritis ratio was attributed to the small number of mares showing clinical symptoms although they were infertile.

The relation between the age of the mare and prognosis is interpreted differently by various authors. According to Shideler et al.¹⁷, a statistically significant relation is not found. However, when older mares were examined, they were included in groups with worse fertilization prognoses. On the contrary, Doig et al.¹⁴ related average mare age to degree of endometrial fibrosis and reported that the age range in each prognosis group can be rather wide. Many researchers point out that there is a correlation between the age of an animal and fibrotic changes, and that the degree of degenerative changes is more important than inflammatory changes^{14,18}. Although mares of all ages were present in the prognosis groups in this study, endometrial changes, mainly the chronic degenerative and cellular infiltration, showed an increase with aging of the mare. The same relation was observed between categories showing the fertility prognosis of mares and the average age of the mares. As the categories decreased, the mean age of the mares showed an increase. Some mares also had an excellent endometrial characteristic regardless of age.

Kenney¹⁹ has suggested that management of mares (follicular monitoring, appropriate mounting time, fertility potential of the stallion, veterinary care and other mare related factors) have an effect on the birth ratio obtained from category-I and category-II prognosis groups and that treatment and management with special care would not change the result in category-III prognosis group, and that births obtained depend on chance. Kenney and Doig¹² have modified the 3-category rating system due to the importance of fibrotic changes and have suggested the expected birth ratios for category I, IIa, IIb and III to be approximately 80-90%, 50-80%, 10-50% and 10%, respectively according to the 4-category rating system. Asbury²⁰ has suggested that fibrotic changes have a more significant effect on fertility than inflammatory changes, and has reported 81%, 24% and 6% birth ratios for category-I, II and III respectively. Ricketts and Alonsó⁶ have suggested that endometrial changes would first have to be demonstrated by means of biopsy specimens and then a second biopsy after the treatment would

lead to a more accurate assessment with regard to the fertility prognosis. The researchers, having used their own classification system in his study, have reported that they have obtained 77%, 57%, 43% and 0% birth ratios for category IB, 2B, 3B and 4B, respectively. In this study, at the end of the breeding season following the endometrial biopsy, the birth ratios obtained for categories-I, IIa, IIb and III were found to be 75, 42.8, 10 and 0%, respectively. According to the results of the study, it was observed that birth ratios in category-I prognosis group were better than those of category-II (a and b), such ratios being better in category-IIa than in IIb. In category-III, none of the mares gave birth.

In order to be able to compare with the results of authors using the 3-category assessment system, the combined birth ratios obtained from category-IIa and IIb was 23.5%. Birth ratios from each category, demonstrating fertility prognosis, are generally parallel to the results of other authors^{6,12,20} who used both 3-category and 4-category assessment systems, except for the results of Gordon and Sartin²¹, who used the 4-category assessment system and determined that the birth ratio in the category-II prognosis (%62.3) group was slightly higher than that in the category-I (%60) prognosis group.

In conclusion, when chronic degenerative and infiltrative changes in the endometrium of the mares are categorized according to their severity and prevalence, their relation with fertility can be seen clearly. As the technique was found to be useful in obtaining important information regarding the fertility potential of mares, its routine use for endometrial histopathological investigations is of great importance.

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