

The index for assessing and predicting fertility in mares

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Abstract. The evaluation of reproductive qualities of mares is an important element of horse breeding technology. Histological analysis of the endometrium provides information about the mare's ability to conceive and carry a pregnancy to term. The article presents the results of histological studies of the endometrium in mares of <10 (n=6) and ≥ 10 (n=13) years old (yo). Two morphometric parameters were used for the analysis: diameter (GDm) and density (GDs) of endometrial glands in diestrus phase of the cycle. There was noted an increase in GDm (20.5 - 62.9 μm , $p < 0.001$) and a decrease in GDs (16.6 - 10.9 units per field of view (ufov), $p < 0.1$) in the endometrium of mares older 10 yo. An endometrial index EI (GDm / GDs, $\mu\text{m}/\text{ufov}$) characterizing the functional state of the endometrium has been proposed. The results of pregnancy rate and the level of life foal birth in mares after histological studies were analyzed. A significant relationship EI with age ($p = 0.0133$) and fertility ($p = 0.0059$) of mares was established. Among mares with $\text{EI} < 3 \mu\text{m}/\text{ufov}$ only one mare was older than 10 years and all the mares had foaled successfully. The mares with $\text{EI} > 3 \mu\text{m}/\text{fov}$ were older than 10 years and had problems with conception and gestation. In our studies, EI in the range from 3 to 6 $\mu\text{m}/\text{ufov}$ could be interpreted as reversible state of the endometrium and after correct treatment it is possible to increase the fertility of mares.

1 Introduction

One of the main tasks in the horse breeding is to increase the fertility of mares, which is based on their reproductive qualities. Different methods are used for evaluation functional state and identification of pathological changes in the reproductive organs that reduce fertility of mares. The most common of them in Russia are: rectal, vaginal, ultrasound diagnostics, cytological and bacteriological analysis. But there are some mares in which it could not be possible to identify the causes of reproductive dysfunction by these methods. In such cases, the histological analysis of the endometrium of mares is used as an additional, effective diagnostic method. It can help to reveal structural changes in the endometrium, which can indicate the presence and degree of destructive processes in it, and will serve as an explanation for the reasons of subfertility. In more rare cases, a comprehensive examination is supplemented with hysteroscopy, hormonal and chromosomal analysis [1-3].

Many authors have studied the relationship between fertility and specific pathologies in the endometrium of the mare [4-7]. In 1978, R. M. Kenny was the first to propose a classification

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of mares based on the quantitative determination of pathological changes within the endometrium, distinguishing three categories, according to which the prognosis of obtaining foals for category I mares was 70–92%; for category II - 50 - 67%; and for category III - <10% [4]. Later P.A. Doig (1981) additionally proposed to divide category II into two subgroups A and B depending on the degree of fibrotic changes in the endometrium, with a predicted fertility of 50-80% and 10-50%, respectively [8].

Indications for endometrium biopsy in mares are: neoplasms, diseases that are not amenable to standard therapy, subfertility, persistent infertility, habitual abortion, the birth of unviable and weakened foals. Many scientists studied the structure and functions of the endometrium of mares [5,9,10-14]. The main structural elements of the endometrial tissue were identified: the lining (covering the lumen of the uterus) and glandular epithelium, glands and vessels of the endometrium, stroma. It has been shown that the histological structure of the endometrium changes in different phases of the oestrus cycle. So, in the estrus phase, diffuse hyperplasia and stromal edema of the endometrium are observed. According to R. M. Kenney, during the period of early estrus, the surface epithelium usually reaches its maximum size. At this time, its height is 20-30 μm , but can reach 50 μm . In some mares in late estrus, the height of the epithelium decreased to low cylindrical form (15 μm), in other mares this decrease occurs no earlier than a few days after the disappearance of behavioral signs of the heat [4]. G.D. Mansour et al. (2015) with usage of histochemical methods reported that surface epithelial cells show higher proliferation activity during estrus, and glandular epithelial cells - during diestrus [15]. The regulatory role of sex hormones in this process was demonstrated by Aupperle, H. et al., who revealed a cyclic relationship between the stimulating effect of estradiol and the inhibitory effect of progesterone on protein expression in the endometrium during oestrus cycle [16]. S.W. Ricketts and S. Alonso (1991) established a close relationship between the severity of chronic degenerative endometrial disease (endometrosis) and the age of mares [17]. Endometrosis is recognized as a multifactorial disease, and comprehensive studies have shown that the progressive age is one of the main predisposing causes of this [4, 8, 17, 18, 19].

Particular attention was paid to the change in the cellular structure of the uterine mucosa under the influence of various damaging factors (birth trauma, gynecological diseases, chronic infections and fibrous processes in the endometrium). Characteristic signs of different endometrium damages were described (stretching and nesting of the glands, fibrosis, lymphatic lacunae, etc.) [1, 4, 12, 19]. To assess the severity of disorders in the endometrium, morphometric indicators were proposed – the height of the surface and glandular epithelium, the diameter and density of the glands [14]. Authors suggested the existence of a relationship between the morphometric characteristics of endometrium and the susceptibility/resistance to post- breeding endometritis in mares. An important sign characterizing the severity of an infectious lesion of the endometrium is its infiltration with neutrophils and lymphocytes. These indicators are directly related to the fertility of mares and, consequently, to the economic side of the horse industry [20].

According to many experts, the occurrence of degenerative changes in the endometrium is preceded by repeated inflammatory processes leading to the deposition of fibrin in the extracellular matrix [21-23]. Loss of elasticity of the walls of the lymphatic vessels is another pathology that affects the functioning of the endometrium. In the endometrium of mares, non-glandular cysts or lymphatic lacunae may occur, which are diffusely dilated folds filled with lymphoid fluid. Lymphatic lacunae are formed from dilated lymphatic vessels and are usually located in the endometrial lamina propria [12]. This pathology indicates poor drainage of the uterus. In cases of impaired lymphatic drainage, the lacunae become quite extensive, forming “pockets” on the walls of the uterus, resembling gelatinous fields, which is the basis for classifying this type of endometrium to category III, according to the classification of R.M. Kenney [4].

Another cause of pathological changes in the endometrium is a failure of arterial blood supply and venous outflow, due to angiogenesis or angiopathy of the uterine vessels, which in turn leads to a decrease in uterine drainage and is closely related to the onset and progression of endometrial fibrosis and atrophy. The latter may be the cause of hypofunction of the myometrium in the preimplantation or postpartum periods [19]. Thus, the fertility of mares, the ability to endure pregnancy and to give birth to a normal foal are directly related to the structural characteristics of the endometrium. So a further study of this aspect with usage of morphometric methods represents scientific and practical interest.

The aim of this work was to study the informative value of the histological analysis of the endometrium using morphometric parameters and to search for new diagnostic and prognostic criteria for assessing the fertility of mares.

2 Material and methods

The studies were carried out on nineteen 3-20 years old mares of saddle and draft breeds in 3 horse breeding farms located in central Russia (Ryazan region). Mares were divided in 2 age groups: 1) <10 years old (yo) and 2) ≥10 yo. For each mare, information was collected about the previous reproductive history and a comprehensive gynecological examination was performed (rectal and ultrasound exams of ovaries and uterus, vaginoscopy and manual examination of the vagina, cytological and bacteriological (if indicated) analysis of cervical and endometrial smears). Endometrial samples for histological analyze were obtained from mares in the diestrus phase using biopsy forceps (Jackson Uterine Biopsy Forceps, USA), 60 cm, with basket 4 x 28 x 3 mm). Samples were fixed in 10% formalin. Histological specimens were prepared in the pathoanatomical laboratory of the Ryazan city clinical hospital by the method of dehydration and pouring of histological material into paraffin using isopropyl alcohol and staining with hematoxylin-eosin. Histosections were examined using the microscopes “Nikon Eclipse 50i” and “Optitech XSP-128-30” at magnification (40x).

Morphometric analysis was carried out using of two indicators (Fig. 1): diameter (GDm) and density (GDs) of glands in the Stratum Spongiosum layer of the endometrium, where, according to our earlier studies [24], the differences in these indicators in mares are more significant than in the Stratum Compactum layer of the endometrium and are more evidence in the diestrus phase. The measurements were conducted using a WF10XDIN/18 mm micrometer eyepiece. Gland diameter was measured as halfsum of the largest and smallest distances between opposite apical edges of glandular epithelial cells (i.e., the lumen of the gland). In extended glands of irregular shape, total measurements of all parts of the gland were summarized. Each indicator was calculated on average over 10 fields of view. The endometrial index EI was calculated by the formula (1):

$$EI = \frac{GDm}{GDs}, \mu m/ufov \quad (1)$$

EI – endometrial index, GDm – the average diameter of the glands, μm , GDs – the average density of the glands, ufov - units per field of view.

After the biopsy procedure one oestrus cycle in mares was skipped. Problem mares were treated, according to the vet indications, based on the results of a comprehensive gynecological examination. Intact mares were mated with fertility stallions, and treated mares were inseminated closed to ovulation with quality-tested stallion semen. The results of mating/insemination were controlled from day 14 after ovulation by ultrasound examinations (pregnant/nonpregnant and foaled/aborted). The results (live foal, no live foal, or live foal after treatment) were compared with the endometrial index EI. Statistical data was analyzed by usage of Excel 2010 and Statistica 10 programs with assessment by Student's t-test and Fisher's test, by Factor analysis with the Principal Component method.

3 Results and Discussion

Histological analysis of endometrial samples showed a high variability of two morphometric parameters (GDm and GDs) in mares (Table 1). On average, gland diameter varied from 11.3 to 107.9 μm , and gland density from 5.6 to 20.2 units per field of view (ufov). At the same time, the predominance of distended glands with an increased diameter was noted in older mares (≥ 10 yo, $n=13$) (Fig.1). Progressive fibrotic processes in the endometrium contributed to a decrease in the number of glands in the field of view, which was also more relevant to older mares. In addition, various pathological changes were present on histological slides from older mares with gynecological problems: cystic transformation and nesting of the glands, fibrotic rings around the glands and blood vessels, hemorrhagic impregnation of the stroma, lymphatic lacunae.

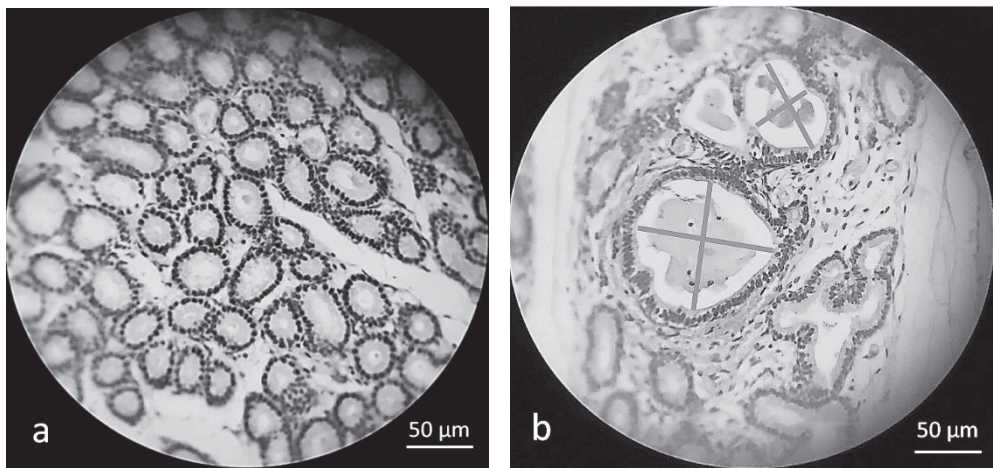


Fig.1. Histological section of the endometrium in a 4 yo healthy mare (a) and a 20 yo subfertile mare (b). Method for measuring the diameter of glands (40x).

The noted tendency gave the idea that in young healthy mares with good function of endometrium the average diameter of the endometrial glands in histological sections in diestrus should be small, and the gland's density should be high, while in subfertile older mares this ratio is reversed. Thus, the parameter value of GDm : GDs in young fertile mares will be less than in the old mares, and the value of this index (EI) will increase with the age and progressive gynecological problems in mares.

Indeed the endometrial index in mares had a wide range (0.56-18.64 $\mu\text{m}/\text{u}$), but the higher EI values were observed in mares older than 10 years ($p<0,002$) (Table 1). The results of mares insemination after histological analysis of the endometrium showed (Fig. 2) that 11 of 19 mares successfully foaled, including 8 pregnancies, obtained in the next cycle after the biopsy procedure, and 3 pregnancies after gynecological treatment of the mares. The range of EI in these mares was 0.56-4.10 $\mu\text{m}/\text{ufov}$ and 3.98-6.04 $\mu\text{m}/\text{ufov}$, respectively. All nonpregnant and aborted mares ($n=8$) were in group with EI of 3.33-18.64 $\mu\text{m}/\text{ufov}$. There were only one mare older than 10 yo and no one without life foals among mares with $\text{EC} < 3 \mu\text{m}/\text{ufov}$. In contrast, all mares with $\text{EC} > 3 \mu\text{m}/\text{ufov}$ were older than 10 years and had problems with fertilization and pregnancy. In this group, 11 yo mare №12 with $\text{EC}=4.1$ (Table 1) had embryonic death 2 months before biopsy procedure. Gynecological treatment was not carried out in this case; the mare became pregnant in the next cycle after the biopsy and gave birth to a healthy foal.

Table 1. Morphometric parameters of the endometrium of mares

№ of the mare	The age of the mare	Glandula diameter (GDm), μm	Glandula density (GDs), ufov*	Endometrial index (EI), $\mu\text{m} / \text{ufov}$
		1	2	1 : 2
Mares age <10 yo				
1	3	11.3	20.2	0.56
2	4	14	19.1	0.73
3	4	21.5	16	1.34
4	6	23.3	15.7	1.48
5	8	24.6	14.6	1.68
6	7	28.3	14.1	2.01
M\pmm		20.5\pm2.67¹	16.6\pm1.0³	1.3\pm0.2⁵
Mares age \geq 10 yo				
7	12	30.1	12.5	2.41
8	13	50	15	3.33
9	16	63.5	18.2	3.49
10	13	50.4	12.7	3.97
11	16	37	9.3	3.98
12	11	51.3	12.5	4.10
13	19	36.5	8.6	4.24
14	16	64	12.2	5.25
15	10	41.1	6.8	6.04
16	17	88.9	9.8	9.07
17	20	107.9	11	9.81
18	19	92.2	7.2	12.81
19	18	104.4	5.6	18.64
M\pmm		62.9\pm7.47²	10.9\pm3.0⁴	6.7\pm1.9⁶

^{1,2}p<0.001, ^{3,4}p<0.1, ^{5,6}p<0.002

*units per field of view (fov)

Thus, the EI in the range from 3 to 6 $\mu\text{m}/\text{ufov}$ in our studies characterizes the state of the endometrium as reversible or borderline, in which it is possible, through timely and correct treatment, to increase the pregnancy rate and the level of successful foaling in mares. Factor analysis using the Principal Components method confirmed the significant relationship between the endometrial index (EI) and two other indicators - fertility (p=0.0059) and age (p=0.0133) of mares. This confirms the important prognostic value of EI for reproduction process in horse breeding. However, the noted trend of decreasing fertility with age in mares does not exclude possibility to receive healthy foals from them after 10 yo if they have normal endometrial function (Fig. 2, mares No.7, 12 yo and No.12, 11 yo).

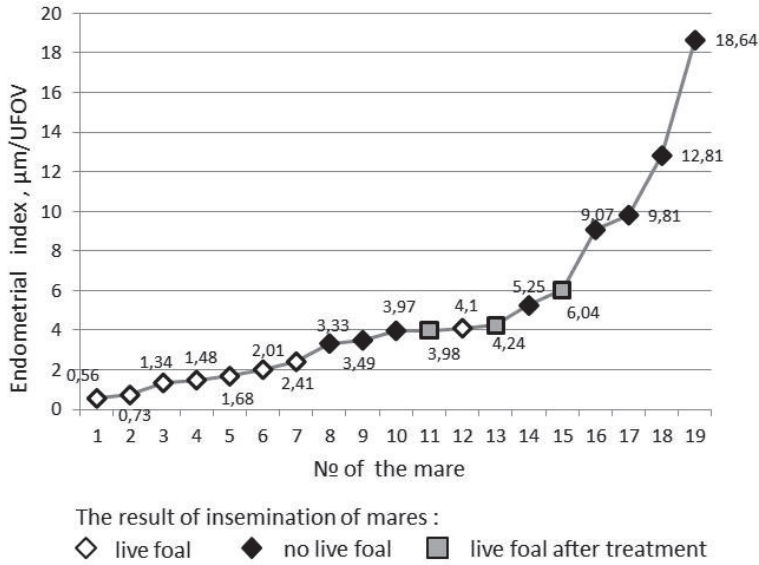


Fig. 2. Fertility of mares depending on the value of the endometrial index (EI)

4 Conclusion

The results of the studies have shown that the morphometric parameters of the endometrium can be useful tool for assessing the functional activity of the endometrial tissue and are significantly associated with the fertility level of mares. The proposed method for calculating the endometrial index (EI), as the ratio of the average diameter to the average density of the glands (GDm :GDs) in histological samples of the endometrium, obtained in the diestrus phase, can be used to assess and predict the fertility of mares. In healthy, potentially fertile mares, the EI value, according to our data, does not exceed 3 µm/ufov.

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