



**The role of endometrial biopsy in diagnosis of equine endometritis**

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**Abstract**

Endometritis is one of the biggest problems affecting horses after colic and lameness. The current study was carried out on 15 mares (4-19 years old) of Straight Egyptian Arabian mares located in different 4 private farms. The study emphasized on the diagnosis of endometritis using endometrial biopsy interpretation. The results of the study showed endometrial changes that were graded into 4 grades, GI, GIIA, GIIB and GIII. GI showed minimal endometrial changes such as edema, slight inflammatory cells infiltration. GIIA showed mild endometrial changes in the lamina propria such as moderate edema, mononuclear cells infiltration, congested blood vessels and areas of sloughed epithelium of the endometrium. GIIB showed moderate endometrial changes in the lamina propria such as marked edema, focal areas of hemorrhage, eosinophilic and mononuclear cells infiltration, cystic dilatation of endometrial glands and periglandular fibrosis. GIII showed severe chronic degenerative changes such as focal infiltration of mononuclear cells (lymphocytes and macrophages), glandular cystic dilatation, marked periglandular fibrosis, glandular nesting, and vacuolar degeneration of the lining epithelium of endometrial glands. The conception rate for the 4 groups was 100%, 75%, 33.3% and 0%, respectively. In the current study there was a positive relationship between the progress of the mare age and the degree of endometrial changes i.e the degree increase with the age and a negative relationship with conception rate.

**Key words:** endometrial, biopsy, equine, uterine

**Introduction**

The endometrial biopsy sample can provide histologic evidence of uterine inflammation and provide information regarding the chronicity, severity and distribution of the endometritis. This information is useful in developing a therapeutic plan for managing the endometritis. Histologic evaluation of the endometrial biopsy sample can also reveal the presence of periglandular fibrosis, cystic glandular distension and lymphatic lacunae. In the mare, there is a correlation between the character of endometrial glands and the ability of the endometrium to carry a foal to term (Kenney and Doig, 1986).

Sertich (2007) documented that the endometrial histologic changes can be divided into four categories; **Category I:** The endometrium has a little or no pathologic changes and it is neither hypoplastic nor atrophic. Endometrium would be expected to carry a foal to term at a rate of 80% to 90%. **Category IIA:** The inflammatory changes are slight to moderate, diffuse infiltration of the stratum compactum or scattered but frequent focal in the stratum compactum and stratum spongiosum. Endometrium would be expected to carry a foal to term at a rate of 50% to 80%. **Category IIB:**

Inflammation that is widespread, diffuse, moderately severe, and focal would be included. Fibrotic changes are more extensive, with up to four or more layers of fibrosis surrounding individual gland branches and two to four fibrotic nests per 5.5 mm linear field in an average of four or more fields. These changes are moderate and this endometrium would be expected to carry a foal to term at a rate of 10% to 50%. **Category III:** Inflammatory changes are widespread, diffuse, and severe. This category also includes other changes that not interfere with the ability of the endometrium to carry a foal to term but also can not be improved by uterine therapy. Uniformly, widespread periglandular fibrosis or greater than an average of five fibrotic gland nests per 5.5 mm linear field in an average of four or more fields will automatically relegate the endometrium to category III. These changes are severe and the endometrium would be expected to carry to term at a rate of only 10%.

Furthermore, Waelchli (1990) stated that endometrial biopsy plays an important role in fertility examination of the mare. There were significant associations between categories and fertility. Mean pregnancy rates in the four categories were 79, 49, 33

and 0 % and mean foaling rates were 70, 42, 18 and 0 %, respectively.

Evaluation of an endometrial biopsy is probably the single most important mean of assessing the mare's potential as a broodmare. In other words, biopsy results can be categorized according to the prognosis for the mare to become pregnant and carry a foal to term: **Category I:** 80% to 100% chance, **Category IIa:** 50% to 80% chance, **Category IIb:** 10% to 50% chance and **Category III:** Less than 10% chance (Brinsko *et al.*, 2011).

In a study on endometrial biopsy 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 (Guvenc *et al.*, 2007).

### Materials And Methods

#### Animals and nutrition:

The current study was carried out on 15 straight Egyptian Arabian mares (4-19 years old) suffered from infertility, 5 mares of them were bacteriologically negative. Mares were located in 4 farms in the pyramids area and Cairo-Alexandria desert road. The study was carried out during the period from January, 2012 to February, 2013. Following up of these mares was carried out through regular visits to each farm 3-5 times per week. All mares were kept indoors where sufficient and balanced feed and water were given to each mare separately.

#### Endometrial biopsy:

Mares were restrained in an examination stock during all procedures. The vulva and perineal region were rinsed with warm water and antiseptic then dried with paper. Endometrial biopsies were obtained using sterilized biopsy punch instruments. The endometrial biopsy (Equi-Vet R, Kruuse)

### Results

The grading system for interpretation of endometrial biopsy depended on the changes of the epithelial lining in the endometrium, inflammatory cells infiltration, presence of hemorrhage, edema, lymphatic dilatation, and fibrosis around the endometrial glands. Accordingly, there were 4 grades for the endometrial changes recorded in the current results, GI, G IIA, G IIB and G III.

instrument has an overall length of 70 cm with an alligator forceps type of sample basket of 20× 43 mm (Nielsen, 2005).

Biopsy was taken using a clean and sterile shoulder-length examination sleeve covered by a sterile surgical glove. Sterile water soluble lubricant was placed on the dorsum of the operator's positioning hand and lower arm. The closed sampling end of the sterile biopsy instrument was carried through the caudal genital tract and, using the forefinger of the gloved hand as a guide, was passed through the cervix and into the uterine body. While holding the closed biopsy instrument stable and inside the uterus with the outside opposite hand, the gloved hand was withdrawn from the genital tract and placed into the rectum. By manipulations per rectum, the sample basket was positioned on the ventral aspect at the base of one of the uterine horns. The forceps was opened and an endometrial fold is pushed into the sides of the basket jaws per rectum. While the forceps was held closed, the instrument was withdrawn from the genital tract. Typically one can feel the biopsy instrument pulled away from the uterine wall when the sample is incised. (Sertich, 2007). Endometrial biopsies were taken and fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4-5 µm, stained with hematoxylin and eosin (H&E) and examined by light microscopy (Bancroft and Gamble, 2002).

#### Bacteriological examination:

These mares were subjected to bacteriological culture of the endometrial swab. 10 mares were bacteriologically positive and 5 mares were bacteriologically negative.

#### 1. Grade I endometrial changes (2 mares):

The endometrial changes in GI were minimal, and ranged from slight to marked edema with slight inflammatory cells infiltration, (Fig. 1). these endometrial changes were found in 2 mares. The prognosis was very good in this category and the conception rate was 100% (2/2).

#### 2. Grade IIA endometrial changes (4 mares):

The endometrial changes were mild and included moderate edema in the lamina propria with moderate mononuclear cells infiltration and congested blood vessels and occasionally presence of areas of sloughed endometrial epithelium (Fig. 2). The prognosis was good in this category and conception rate was 75% (3/4).

**3. Grade IIB endometrial changes (6 mares):**

The endometrial changes were moderate and characterized by presence of marked edema in the lamina propria, with focal areas of hemorrhage, congested blood vessels, eosinophilic and mononuclear cells infiltration, distension of lymphatic lacunae, cystic dilatation of the endometrial glands and periglandular fibrosis (Fig. 3). These changes were

found in 6 cases. The prognosis was fair and the conception rate was 33.3% (2/6).

**4. Grade III endometrial changes (3 mares):**

The endometrial changes were severe chronic degenerative changes which were focal infiltrations of mononuclear inflammatory cells (lymphocytes and macrophages), glandular cystic dilatation, marked periglandular fibrosis and glandular nesting, vacuolar degeneration of the lining epithelium of the endometrial glands and destruction of some endometrial glands (Fig. 4). The prognosis was very poor and conception rate was 0% (0/3).

The results of endometrial biopsy examination and their correlation with age and conception rate are listed in table (1).

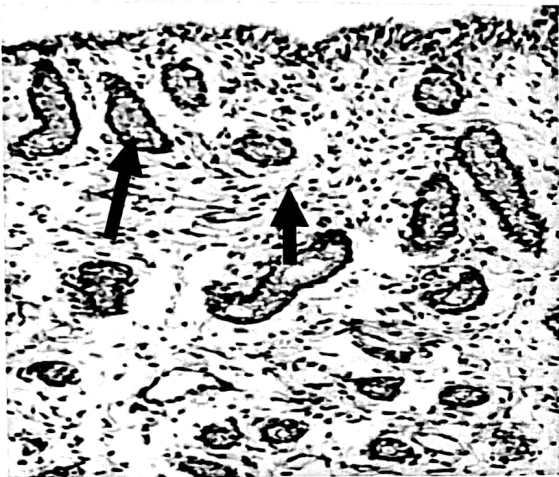


Fig. (1): Grade I: Endometrial section with normal glandular distribution (arrow) and frequency with marked edema and few inflammatory cells in the lamina propria. (X4)

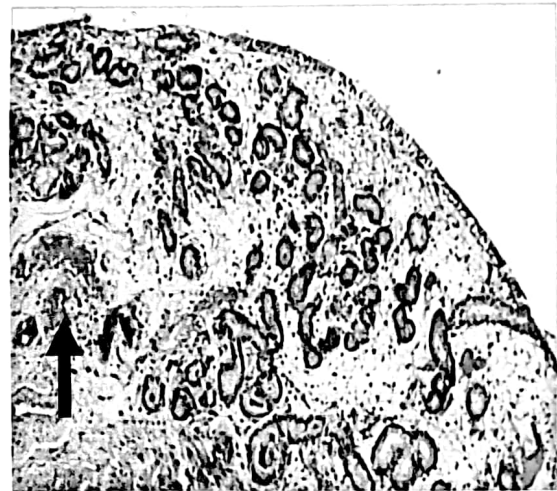


Fig.(2): Grade IIA: Vacuolar degeneration of the endometrial lining epithelium and moderate edema in the lamina propria with moderate mononuclear cells infiltration and congested blood vessels (arrow). (X4)



Fig. (3): Grade IIB: Marked edema in the lamina propria, focal areas of hemorrhage, congested blood vessels, eosinophilic and mononuclear cells infiltration, distension of lymphatic lacunae, cystic dilatation of few endometrial glands (white arrow) and periglandular fibrosis (blue arrow). (X4)

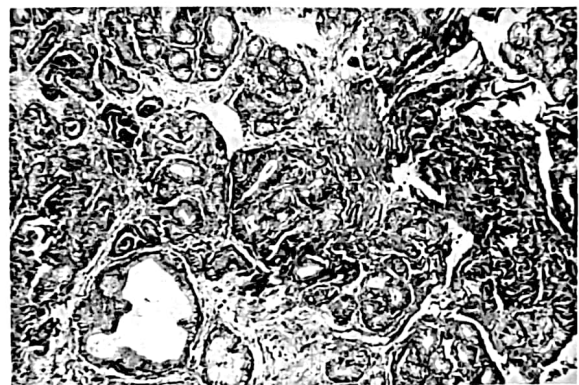


Fig. (4): Grade III: Focal infiltration of inflammatory cells (lymphocytes and macrophages), glandular cystic dilatation, marked periglandular fibrosis and glandular nesting, vacuolar degeneration of the lining epithelium of the endometrial glands and destruction of some endometrial glands. (X4)

**Table (1):** Grades of endometrial changes, average age and the corresponding conception rate after treatment:

Average age (y)	Biopsy grade	Conception
4.5	GI	100% (2/2)
10.5	GIIA	75% (3/4)
13.5	GIIB	33.3% (2/6)
15.4	GIII	0% (0/3)

From the results shown in table (1), GI of the endometrial changes was observed in young mares aged 4.5 y, while GIIA was observed in mares aged 10.5 y and GIIB was observed in mares aged 13.5 y and GIII was observed in older mares aged with average 15.4 y. Accordingly, there is a direct relationship between the age of the mare and the severity of the endometrial changes.

### Discussion

According to the endometrial changes that have been reported in our study, there was no to minimal changes observed in the endometrium such as slight edema and few inflammatory cells infiltration and the glands characterized by normal glandular distribution, this group was categorized as GI, followed by mares that showed mild endometrial changes such as moderate edema in the lamina propria with moderate mononuclear cells infiltration and congested blood vessels, this group of mares was categorized as GIIA. The third group of mares showed marked edema in the lamina propria, focal areas of hemorrhage, congested blood vessels, eosinophilic and mononuclear cells infiltration, distension of lymphatic lacunae and cystic glandular dilatation with periglandular fibrosis. This group of mares was categorized as GIIB. Ultimately, there were mares which showed sever endometrial changes such as focal infiltration of inflammatory cells (lymphocytes and macrophages), glandular cystic dilatation, marked periglandular fibrosis and glandular nesting, vacuolar degeneration of the lining epithelium of the endometrial glands. This group of mares was categorized as GIII. All the above mentioned results agree with the same categorization that pointed out by **Kenny and Doig, (1986)**; **Sertich (2007)**; **Brinsko et al., (2011)**; **Guvenc et al (2007)**.

There were 5 mares showed no bacterial isolation on culture but with microscopical examination of endometrial biopsy of 4 mares of them, they showed endometrial changes (1 mare with GIIA and 3 mares with GIIB), and these results indicated the presence of endometritis that could not be detected by ordinary uterine swabbing.

The endometrial biopsy samples provided histological evidence of uterine inflammation and provided information regarding the chronicity, severity, and distribution of the endometritis. In the current study 5 mares were bacteriologically negative whilst by applying endometrial biopsy analysis, they showed endometritis ranging from GIIA to GIIB, and accordingly, endometrial biopsy is an accurate tool in diagnosis of endometritis and plays an important role in fertility examination of mares. These results were consistent with those mentioned by **Kenny and Doig (1986)** and **Waelchi (1990)**.

The results of the present study revealed a negative relationship between the degree of endometritis and conception rates were 100%, 75%, 33.3% and 0% in mares with GI, GIIA, GIIB and GIII respectively, thus results come in agreement with that reported by **Brinsko et al., (2011)**.

From our results we have concluded that there was a correlation between the mare age and the severity of the endometrial change. This finding agreed with that mentioned by **Waelchli (1990)**, but we are differ in opinion concerning the association between the mare's age and fertility in the same category, as in our results the conception rate in the same category did not depend on mare age rather than depending on the duration of infertility and methods of treatment.

Data obtained from the current study on the endometrial biopsy were very useful in

diagnosis as well as in prognosis of endometritis in mares for future fertility Therefore the endometrial biopsy is

considered as an accurate method for detection and prognosis of mares suffering from endometritis.

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### المخلص العربي

#### دور الفحص النسيجي في تشخيص التهاب بطانة الرحم في الأفراس

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معهد بحوث التناسليات الحيوانية بالهرم- كلية الطب البيطري- جامعة بني سويف - قسم الولادة والتلقيح الإصطناع

تعد مشكلة التهاب بطانة الرحم في الأفراس من المشاكل التي تؤثر على الخيول بعد مشكلة المغص والعرج، أجريت هذه الدراسة على عدد 15 فرس (عمر 4-19 سنة) من الأفراس العربية المصرية الأصيلة في 4 مزارع مختلفة خاصة بمصر. كانت هذه الدراسة تعتمد على تشخيص التهاب بطانة الرحم عن طريق فحص الخزعة الرحمية ، وقد أظهرت هذه الدراسة تغيرات باثولوجية في بطانة الرحم وتم تقسيمها إلى أربع درجات وهي GI, GIIA, GIIB, GIII ، الدرجة GI ظهر فيها تغيرات طفيفة مثل أوديما وارتشاح بعض الخلايا الإنتهاجية و GIIA ظهر فيها تغيرات متوسطة في الطبقة الصفحية المخصوصة مثل الأوديما المتوسطة وارتشاح خلايا الدم البيضاء وحيدة النواه واحتقان الأوعية الدموية مع تآكل في الخلايا الطلانية لبطانة الرحم. أما الدرجة GIIB وفيها ظهرت تغيرات في بطانة الرحم والتي شملت الطبقة الصفحية المخصوصة مثل الأوديما ويقع نزيفية وارتشاح خلايا الدم البيضاء وحيدة النواه وخلايا الأزينوفيل بالإضافة الى تكيس غدد بطانة الرحم والتليفات المتكونة حول هذه الغدد. أما الدرجة GIII فظهر فيها تغيرات تنكسية مزمنة شديدة مثل ارتشاح خلايا وحيدة النواه وتمدد تكيسي لغدد بطانة الرحم وانتشار التليفات حول غدد بطانة الرحم. معدل الخصوبة للدرجات الأربع كانت 100% و 75% و 33.3% و 0% على التوالي، وتوصلت هذه الدراسة إلى أن هناك علاقة بين تقدم الفرس في العمر والتغيرات السلبية على جدار بطانة الرحم مع تقدم العمر ومن ثم تنخفض نسبة الخصوبة مع تقدم السن.

الكلمات الدالة: رحم، التهاب ، أفراس،خيول