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Endoscopic monitoring the estrous phase of Arabian mares with assaying its biopsy

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ABSTRACT

Objective: To evaluate the changes occur in the vagina, cervix and endometrium during the pre-ovulatory period in Arabian mares by using endoscope as a diagnostic tool as well as the availability of taking an endoscopic biopsy during this stage. **Methods:** Ten cyclic and healthy arabian mares were subjected to ultrasound scanning of their ovaries to estimate the diameter of the preovulatory follicles, collection of blood samples for estimating the hormonal levels (Estradiol-17 β and progesterone) for confirming the estrous phase, endoscopic monitoring their vagina, cervix and endometrium, the availability of taking biopsical samples and histological examination of the obtained samples. **Results:** The obtained data revealed that, the mean diameter of the preovulatory follicles was (3.95 \pm 0.34) cm, the plasma estradiol and progesterone levels were (85.98 \pm 5.26) pg/mL and (0.73 \pm 0.23) ng/mL, respectively, endoscopic monitoring the vagina, cervix and endometrium revealed hyperemic vaginal mucous membrane, soft and opened cervical ostium with abundant estrous mucous and folding, hyperemic and edematous appearance of the endometrium, respectively and the histological features of the biopsical endometrial samples revealed that, The lamina propria-sub mucosa was packed with simple or branched tubular glands lined with simple columnar epithelium showing oval or rounded dark nuclei with different degrees of secretory activities of the glands. **Conclusions:** The endoscope proved to be a reliable tool for confirming the estrous phase in mares and monitoring the gross features of the vagina, the cervix and the endometrium, in addition to the availability of taking biopsical smears.

1. Introduction

Sufficient gynaecological examination should be done for all barren mares to reach the maximum breeding efficiency.

Detection of the accurate time of the estrous phase in mares in which ovulation occurs is a critical point concerned with right reproductive management for mare breeding. The changes occur during the estrous phase can be easily observed using the endoscopic monitoring of the vagina, cervix and endometrial folds[1].

Hysteroscopy has been used to determine the healthy endometrium, facilitates the artificial insemination in mares, identify foreign bodies, evaluate the nature and condition of tumors or hematomas,

explore anomalies such as adhesions, and identify congenital abnormalities [2-4]. Hysteroscopic guidance may be used to obtain biopsies or cultures of affected and non-affected endometrial tissue[5].

Recent reports of biofilms and focal lesions in sub-fertile mares indicate that, it is desirable to obtain endometrial biopsies from affected areas of the endometrium in mares[4,6].

The aim of this study is to evaluate the criteria of the changes occur in the vagina, cervix and endometrium using endoscope

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during the pre-ovulatory period as well as the availability of taking endoscopic biopsy during this phase.

2. Materials and methods

2.1. Animals and diets

Animal care measures followed the ethical protocols approved by the Ethics Committee for Animal Use at Cairo University.

The animals were fed with well-formulated commercial grain mix, Egyptian clover (*Trifolium alexandrinum*) and had free access to concentrated salts and water.

A total number of 10 healthy and cyclic Arabian mares that aged 7-12 years and weighing 370-420 kg were exposed to examination of their genital tracts during the estrous phase at Theriogenology service, Faculty of Veterinary Medicine, Cairo University. Detection of the estrous mares based on the common estrous signs (dripping of urine, whinny and winking of clitoris) and with using a high libido stallion.

2.2. Ultrasonic examination

All of mares were subjected to ultrasonographic examination of the echo graphic follicular appearance in the ovary as described by[7]. Scanning was carried out using transrectal transducer of 6 MHz connected to ultrasonographic machine (Sonoscape, A5, China), the diameter of the non-echogenic preovulatory follicle was recorded.

2.3. Hormonal assay

The hormonal profiles during the preovulatory period (Estradiol; E17- β (pg/mL)), progesterone, and P₄ (ng/mL) were also measured using Radio Immune Assay (RIA) available commercial kits after collection of 10 mL blood from the jugular vein in heparinized tubes then centrifuged at 3 000 r/min for 15 min, the obtained plasma samples were stored at -20 °C until be used for confirming the preovulatory period[8].

2.4. Endoscopic examination

Preparation of the mares for endoscopic examination was done via securing the animal in the stanchion. The tail of the mare was wrapped using a non-sterile palpation sleeve and bandage tape, and tied around the neck to give clear access to the vulva.

Feces were back raked from the rectum and the perineum of the mare was aseptically cleansed. Each mare was sedated using xylazine 10% (1 mg/kg).

The endoscopic images were captured for mare genital passage using Eickemeyer© (Germany) video-endoscope unit supplied with halogen light source (Vet Lux-150 Watt) and insertion tube (8.5 mm diameter, 1.5 meter length and 2 mm working channel). A video and several still frame images of mares' vagina, cervix and uterus were collected[4].

The sides of the endoscope were lubricated using sterile lubricant, the clinician wearing a rectal sleeve and sterile glove passed the tip of the endoscope through the vagina, cervical canal, and into the

uterus.

The cervix was held closed around the endoscope. Insufflation was used to view the uterine body and the uterine horns, and low or intermittent air flow was used to maintain insufflation during the examination.

The body and both uterine horns might be evaluated for areas of interest by passing the endoscope into the various regions of the uterus[4].

2.5. Histological examination

Endometrial biopsy samples were fixed in 10% neutral buffered formalin for 24 h then processed into Paraplast tissue embedding media, 3-5 μ sections were cut by rotatory microtome. For demonstration of general tissue architecture, biopsy tissue sections were stained with Harris Hematoxylin and Eosin (H & E) as a general staining method as outlined by[9].

Tissue sections were examined under light microscope equipped with full HD microscopic camera (Leica Microsystems, Germany).

2.6. Statistical analysis

The differences between size of the pre-ovulatory follicles and the levels of hormones were done with the help of computer software SPSS[10], simple one way ANOVA was used and data were presented as mean \pm SEM.

3. Results

The conventional methods for confirming the estrous phases were that presence of estrous signs including (whinny, dripping of urine and winking of clitoris) and the reaction of the stallion toward the estrous mares.

3.1. Ultrasonic scanning

Rectal ultrasonic examination of the pre-ovulatory follicles showed that, the mean of the preovulatory follicle (dominant) diameter was (3.95 \pm 0.34) cm, (Figure 1).

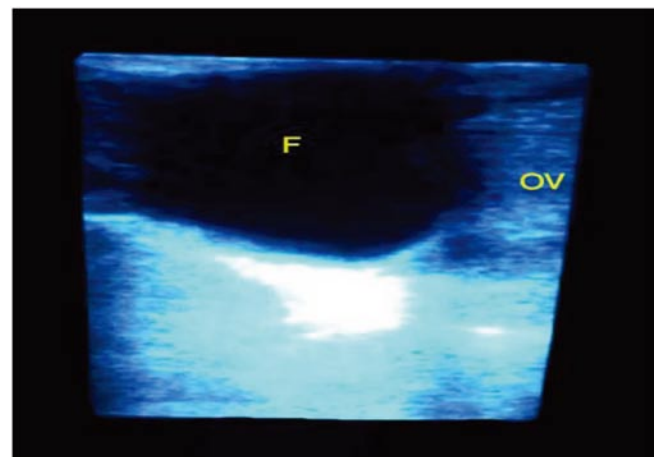


Figure 1. Ultrasonographic view of mature follicle on the ovary of estrous mare.

3.2. Hormonal profile

Hormonal analysis revealed that the mean values of both E17- β and P₄ were (85.98 \pm 5.26) pg/mL and (0.73 \pm 0.23) ng/mL, respectively during the preovulatory period in which the mentioned dominant follicle was found.

3.3. Histological findings of the biopsical samples

Histological examination showed that biopsy samples were missing the luminal epithelium as artifact during sampling procedures. However, the lamina propria-sub mucosa was formed of highly fibrous connective tissue rich in blood vessels of different sizes, fibroblasts, lymphocytes and few lymphocytic aggregations were observed. The lamina propria-sub mucosa was packed with simple or branched tubular glands lined with simple columnar epithelium showing oval or rounded dark nuclei. The glands showed different degrees of secretory activities (Figure 2).

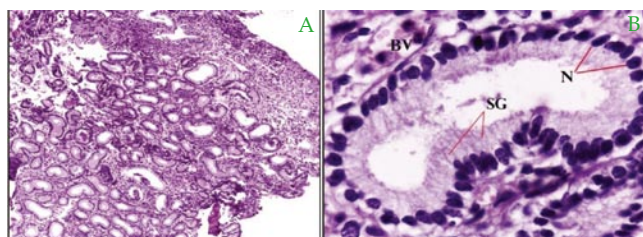


Figure 2. Micro graph of equine endometrial biopsy demonstrating densely .

(A) packed propria sub mucosa with simple or branched tubular glands with artifact of missing luminal epithelium (\times 100 H&E stain). (B) Photomicrograph of endometrial gland lined with simple columnar epithelium showing oval or rounded dark nuclei (N) and apical cytoplasmic secretory granules (SG). Notice presence of small blood vessel (BV) in the peri glandular stroma (\times 1 000 H&E stain).

3.4. Endoscopic imaging

The endoscopic evaluation of the vagina revealed hyperemic mucous membrane of longitudinal folds (Figure 3), the cervix appeared soft, opened cervical ostium (external uterine orifice) with abundant estrous mucous (Figure 4), and the endometrial changes showed folding, hyperemic and edematous appearance which covered with slight mucus secretion (Figure 5).

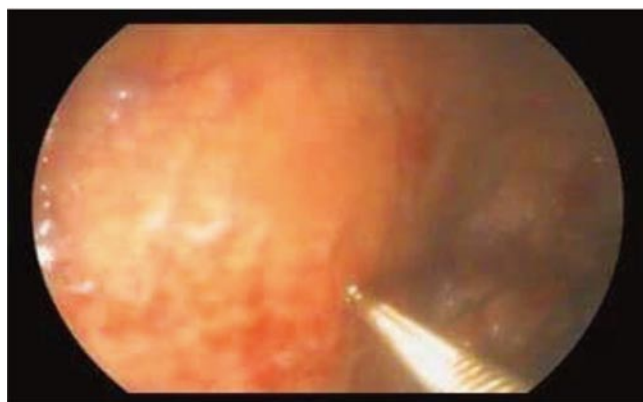


Figure 3. Endoscopic view showing hyperemic vaginal mucous membrane with biopsy sampling.

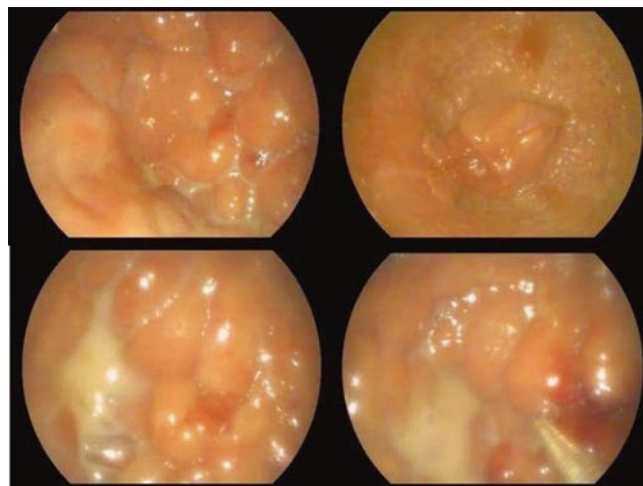


Figure 4. Endoscopic view showing the portio vaginalis uteri, estrous mucous and cervical mucosal biopsy.

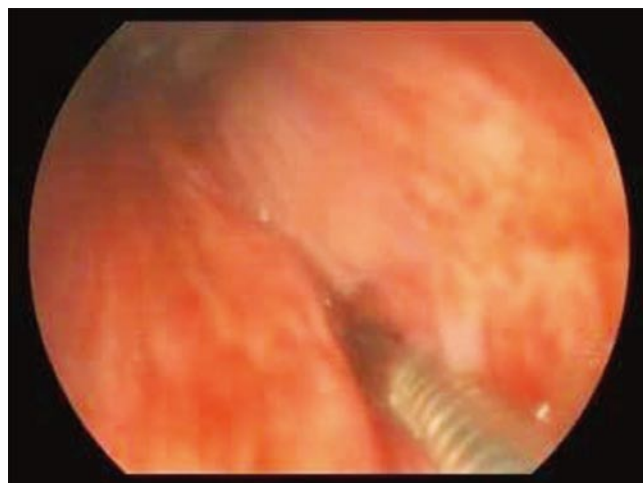


Figure 5. Endoscopic view showing biopsy from the uterine endometrium.

4. Discussion

Endoscopic examination of the uterine lumen allowed an evaluation of the extent and the significance of the lesions. However, targeted biopsy is a relatively new idea in Arabian mares.

According to the ultrasonic scanning and the hormonal profile results, it is demonstrated that the average diameter of the preovulatory follicle was (39.50 \pm 0.34) mm. A slightly higher value 44 mm was obtained by Dolezel *et al.*[11] who found that. This may be attributed to using of hCG treatment or the different breed of mares rather than the arabian mares. Arbal *et al.*[12] found that the mean diameter of the preovulatory follicle at day 9 post foaling was (39.25 \pm 4.24) mm in Thoroughbred mares. It was recorded that the largest (dominant) follicle during estrous phase was 35.86 mm in diameter[13], which may be attributed to the method of scanning or the seasonal variation. Also Abo-El maaty *et al.*[13] recorded that, the

levels of Estradiol-17 beta and progesterone were (83.21 pg/mL and 0.80 ng/mL, respectively), these values were nearly similar to our reported values.

From the obtained data concerned with the endoscopic findings, the endometrium of the fertile mare was pale pink and edematous endometrial folds covered with glistening mucus and a homogenous texture, it might contain endometrial glandular cysts and endometrial lymphatic cysts, these findings were in the same line with [2].

It was reported that a high rate of false negatives in uterine swab cultures, and improved methods of pathogen detection are needed [2,5,6]. Hysteroscopically guided biopsies showed statistically worse diagnosis compared with blind biopsies [5]. In addition of being a diagnostic tool, others concluded that the endoscope can be used as a therapeutic tool such as flushing of the uterus, application of drugs and minor surgeries can be done as removal of the endometrial cysts [14].

On the same hand, it was concluded that the endoscopy could be considered a screening method for monitoring the equine reproductive tract and had applicability to other species [15].

Although hysteroscopy can't replace the other diagnostic tools especially the ultrasonography, certain conditions as small endometrial cysts, adhesions, focal aplasia and congenital abnormalities can't be confirmed by other tools as reported in [16] and added that, the endoscopic examination must be accompanied by tissue biopsies.

The histopathological findings revealed densely packed propria sub mucosa with simple or branched tubular glands with different degrees of secretory activities with negligible artifact of missing luminal epithelium. Mansour GD *et al.* [17] found that the collected endometrial samples during estrous phase showed greater heights of luminal and glandular epithelial cells with pseudostratification.

The endoscope proved to be a reliable tool for determining the estrous phase in mares and a very useful instrument to monitor the gross anatomical features of the vagina, the cervix and the endometrium, in addition to the availability of taking a smear from the endometrium using endoscopic biopsy for histopathological examination.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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