Assessment of Endometritis in Arabian Mare

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Abstract. This study aimed to employ different methods for diagnosis of endometritis in Arabian mare. The study was conducted on 88 barren Arabian mares. After establishing the breeding history and completing the clinical examination, 50 of them were diagnosed as endometritis. Two swabs were obtained for bacteriological culture and cytological smears. Biopsy specimens were taken from the endometrium for histological examination. The results revealed that the ageing and the abnormal vulvar conformation were predisposing factors for endometritis. The number of mares affected with endometritis were 10, (20%) 13 (26%) and 27 (54%) in nullipara, primipara and pluripara mares respectively. Mares affected with endometritis were 6 (12%), 20 (40%), 18 (36%) and 6 (12%) in relation to body scoring 1, 2, 3 and 4 respectively. Bacteriological and mycological examinations of the uterine culture were positive in 41 (82%) and in 5 (10%) animals respectively. No growth was recorded in 4 swabs (8%). Streptococcus equi zooepidemicus were found to be the most common potential uterine bacterial pathogens (39.1%) followed by *E coli* (21.7%). Cytological examination was positive in 60% (n=30) and negative in 40 % (n=40). The histopathological investigation revealed that 10/50 cases (20%), 18/40 cases (45.0%), 11/40 cases (27.5%), 7/40 cases (17.5%) and 4/40 cases (10.0%) were diagnosed as normal endometrium (Grade I), mild endometritis (Grade II A), moderate endometritis (Grade II B), severe endometritis (Grade III) and uterine atrophy respectively.

Keywords: Arabian mare, biopsy, cytology, endometritis

Abstrak. Penelitian ini bertujuan menerapkan metode yang berbeda untuk diagnosa endometritis pada kuda betina Arab. Penelitian dilakukan pada 88 kuda betina Arab yang mandul. Setelah menetapkan sejarah peternakan dan melengkapi pemeriksaan kesehatan, 50 kuda didiagnosa endometritis. Dua swab diperoleh dari kultur bakteriologi dan *cytological smears.* Spesmen biopsy diambil dari endometrium untuk pemeriksaan histology. Hasil penelitian menunjukkan bahwa bentuk vulva yang menua dan abnormal merupakan faktor yang mempengaruhi endometritis. Jumlah kuda yang terkena endometritis adalah 10 (20%) kuda nullipara, 13 (26%) kuda primipara dan 27 (54%) kuda pluripara. Kuda yang terkena endometritis adalah 6 (12%), 20 (40%), 18 (36%) dan 6 (12%) menurut ukuran tubuh 1, 2, 3 and 4. Pemeriksaan bakteriologi dan mikologi pada kultur uterus menunjukkan nilai positif pada 41 (82%) dan 5 (10%) kuda. Tidak ada pertumbuhan dalam 4 swab (8%). *Streptococcus equi* zooepidemicus diketahui sebagai potensi bakteri pathogen di uterus (39.1%) diikuti oleh *E coli* (21.7%). Pemeriksaan cytology positif pada 60% (n=30) dan negatif pada 40% (n=40). Penelitian histopatologi menyatakan bahwa 10/50 kasus (20%), 18/40 kasus (45.0%), 11/40 kasus (27.5%), 7/40 kasus(17.5%) dan 4/40 kasus (10.0%) didiagnosa sebagai endometritis normal (Grade I), endometritis ringan (Grade II A), endometritis sedang (Grade II B), endometritis parah (Grade III) dan *uterine atrophy*.

Kata kunci: Kuda Arab, biopsy, cytology, endometritis

Introduction

Infertility in mare is a common and important clinical topic. Equines show a low reproductive performance due to anatomic abnormalities, physiological conditions, infectious agents and management factors. Various uterine disorders have been described in equine and may play an important role in reduced fertility in these species (Brendemuehl et al., 2002).

Endometritis is the most common cause of sub-fertility in mares (Nikolakopoulos et al., 1999). Endometritis in mares has large economic impact in the horse-breeding industry (Watson, 2000). The uterus is exposed to the

risk of infection, particularly at the time of breeding, following parturition and during reproductive examination (Ricketts, 2008). The vulva, vestibule, vagina and cervix normally act as physical barriers protecting the uterus from external contamination (Rambags, 2003). Based on the etiology and pathophysiology, endometritis can be divided into four groups: sexually transmitted diseases, chronic infectious endometritis, persistent mating-induced endometritis and endometrosis (Troedsson, 1999).

The present study was developed to employ different methods for definite diagnosis of endometritis in Arabian mare. An accurate diagnosis in veterinary medicine is always a pre-requisite to prognosis, treatment and successful management (Ricketts, 2008).

Materials and Methods

Animals . A total of 88 Arabian repeat breeding mares were used all over the study and housed on a private horse stud for Arabian Horses (Kingdom of Bahrain). Mares had a history of failure conception after more than two services. Mare's age ranged from 4 to 20 years. The investigations were carried out during one year. Fifty animals were diagnosed as endometeritis depending on history, rectal and vaginal examinations (Watson, 2000).

Examinations. The animals were subjected to breeding soundness examinations as follow:

Reproductive history of the animal. The full reproductive history of animals was recorded and comprised the age, breed, parity, date and circumstances of last foal, purperium, regularity of estrous cycle, characters of estrous secretion, number of services, date of last service and information on stallion(s) used during previous unsuccessful breeding.

General physical examinatio. The general physical examination was focused on body scoring (MeCue, 2002).

Evaluation of perineal conformation. The external genitalia (vulva) evaluated for conformation, slope and muscular tone (Pascoe, 1979).

Rectal palpation and ultrasonographic examination. Endometritis was suspected by rectal examination (Hughes and Loy, 1969). Ultrasonographic examination using a 7.5 MHz or 5 MHz linear transducer was used .The presence of two or more centimeters of intra-uterine fluid were taken as an indicator for endometritis (Bucca et al., 2008) (Figure 1).

Examination of the vagina and the external os of cervix. This examination was performed to evaluate vaginal fault and the external os of the cervix by sterile vaginoscope (kruuse VARTA 618).

Uterine cytology and culture. Two swabs were obtained for bacteriological culture and cytological smears. The average number of neutrophils per microscobical fields was calculated (Riddle et al., 2007). The swabs were cultured on Columbia agar (CM 331; Oxoid, Basingstoke, UK). Each bacteriological culture was inspected and bacterial growth was registered after 24 and 48 h incubation at 37°C. The swabs were also cultured on Sabouraud Dextrose agar (Oxoid CM 41) for mycological culture and incubated at 30°C for 5 days.

Endometrial biopsy. Endometrial biopsy samples were collected using sterilized biopsy punch instruments (Equi Vet® Kruuse) (Nielsen, 2005) (Figure 2). The specimen fixed in 10% formol saline and processed to paraffin wax, and 4 μm sections were cut and stained with haematoxylin and eosin (Bancroft and Stevens, 1996). Histopathological diagnosis and grading of endometritis were followed (Kenny and Doig, 1986).

Statistics. All comparisons were made by a Chi square test, and significance was considered at $P < 0.05$.

Results and Discussion

The rectal examination revealed that the ageing was a significant predisposing factor for endometrits ($P < 0.05$). 10% (n=5), 36% (n=18) and 54 % (n=27) of the animals diagnosed located in age group ≤ 4, 5-8 and > 8 years respectively. Direct relationship between age and endometritis has been well documented (Hurtgen, 2006).

The relation between parity and endometritis indicated that endometritis were 20% (n=10), 26% (n=13) and 54% (n=27) in nullipara, primipara and pluripara respectively. The differences were significant ($P < 0.05$). Age and parity are usually associated with delayed uterine clearance (Rigby et al., 2001).

A significant inverse relationship ($P < 0.05$) between body scoring and endometritis was recorded. The number of mares affected with endometritis were 40% (n=20), 36% (n=18), 12% (n=6) and 12% (n=6) in relation to body scoring 1, 2, 3 and 4 respectively. The pressure gradient from uterus through vagina to the exterior is found in mares with high body score whereas the case was reversed in mares with low body score. This will hinder the evacuation of uterine fluid leading to persistent uterine infection (Newcombe, 2011).

The frequency of abnormal vulvular conformation were 3.4% (n=1), 34.5% (n=10) and 62.1% (n=18) in age group≤ 4, 5- 8 and > 8 years respectively. There is a higher predisposition towards endometritis in mare with poor perineal conformation (Watson, 2000) and abnormal conformation of the vulva (Hemberg et al., 2005). The above abnormalities can all affect the barrier function, causing air, feces and urine to enter the reproductive tract (Hurtgen, 2006).

With aid of ultrasound, endometritis could be diagnosed in 82% (n=41) and could not be confirmed in 18% (n=9). The negative sonographic result may be explained on the base of not all uterine pathogens produce intrauterine fluid (Le Blanc, 2002). However, the detection of PMN-cells has been considered the most reliable diagnostic test for endometritis (Nielsen, 2005), the cytological examination was positive in 60% (n=30) and negative in 40 % (n=20).

Bacteriological and mycological examinations of the uterine culture were positive in 41 (82%) and in 5 (10%) animals respectively. No growth was recorded in 4 swabs (8%). Streptococcus equi zooepidemicus were found to be the most common potential uterine bacterial pathogens (39.1%) followed by *E coli* (21.7%). Reiswig et al., (1993) found that bacterial infection in the uterus is recognized as a major cause of reproductive failure in mares. Streptococcus equi was found to be the most common potential uterine bacterial pathogens (Szeredi et al., 2003). The histopathological investigation revealed that 20 % (n=10) and 80% (n=40) had normal and inflamed endometrium respectively. 18/40 cases (45%), 11/40 cases (27.5%), 7/40 cases (17.5%) and 4/40 cases (10%) were diagnosed as mild endometritis Grade IIA (Figure 3,4), moderate endometritis Grade II B (Figure 5), severe endometritis Grade III (Figure 6) and uterine atrophy respectively.

The coincidence between the histopathological and bacteriological results in 88% proved that most of the cases of endometritis was due to bacterial infection (Reiswig et al., 1993). Disagreement was reported in 12 % of mares. This could be due to the isolation of bacteria from the uterus did not prove the presence of endometritis (Troedsson et al., 1995).

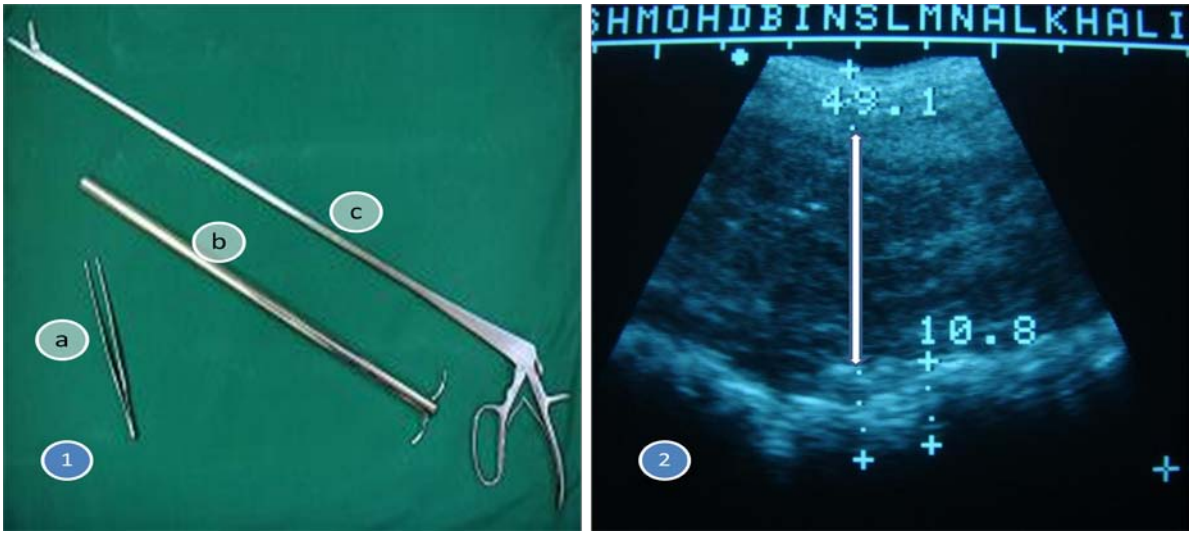

Figure 1. A forceps. b protection tube. c biopsy knife (KRUUSE) 62cmx4mmx115mm.
Figure 2. Ultrasound image for the presence of free fluid in uterine lumen (arrow).

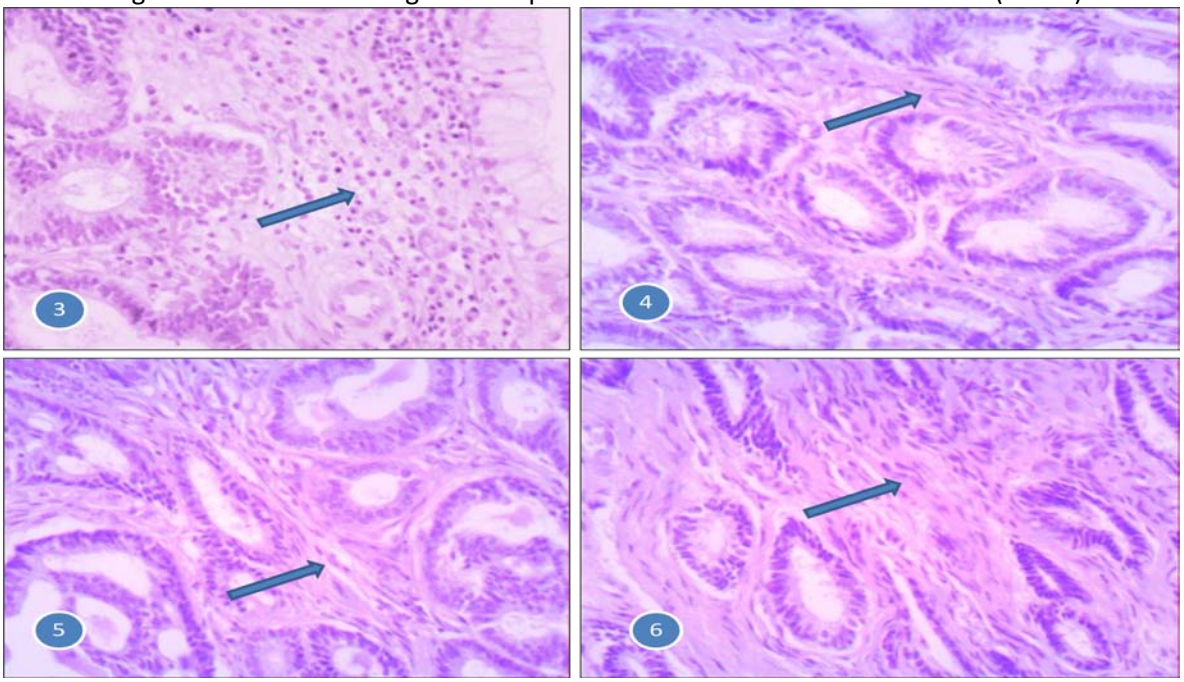

Figure 3. Stratum compactum showing mononuclear cells (arrow), Grade IIA. H&EX400.
Figure 4. Stratum spongiosum showing periglandular fibrosis 1-3 layers fibroblasts (arrow), Grade IIA. H&EX400.
Figure 5. Stratum spongiosum showing periglandular fibrosis 4 or more layers fibroblasts (arrow), Grade IIB. H&EX400.
Figure 6. Stratum spongiosum showing massive fibrosis around uterine glands (arrow), Grade III. H&EX400.

Conclusions

Infection is the principle trigger for endometritis in mares (46 out of 50 animals). Streptococcus equi and E coli are the main causative organisms for endometritis. Integration of the microbiological, cytological, ultrasonic and histopatholgical investigation are essential for precise diagnosis of the endometritis in mare.

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