Assiut University web-site: www.aun.edu.eg

DIAGNOSIS OF ENDOMETRITIS IN INFERTILE ARABIAN MARES USING ULTRASONOGRAPHY AND BACTERIOLOGICAL EXAMINATION

M.K. DERBALA[1]; FADEL, M.S.[1]; HANAA A.E. ASFOUR [2] and ELSAYED M.M ABDEL GAWAD [3]

[1] Ultrasonography Unit, Animal Reproduction Research Institute (ARRI), Egypt, Giza
[2] Mastitis and Neonatal Diseases Department, Animal Reproduction Research Institute (ARRI), Egypt, Giza
[3] Fac. Vet. Med., Beni-Suef University, Theriogenology Depart.

Received: 7 December 2017; **Accepted:** 31 January 2018

ABSTRACT

Endometritis is one of the biggest problems affecting horses after colic and lameness. The current study was carried out on 26 mares (4-19 years old) of Straight Egyptian Arabian mares located in different 4 private farms. Ultrasonography revealed that most cases of endometritis were showing intraluminal fluid accumulation, although there was certain cases did not show fluid accumulation yet they suffered from endometritis and subfertility. The degree and severity of fluid accumulation was increased with age of mares. Additionally, there was a relationship between the fluid accumulation and echogenicity and the type of isolated microorganism whereas isolation of Staph and Strept associated with fluid formation and observation of clinical endometritis while in contrast with E.coli, it produced little amount of fluid only high degree of uterine edema was evident. Concerning bacterial isolation there were many incriminated bacteria for example Staphylococcus spp (35.1%), E.coli (18.9%), Proteus vulgaris (13.5%), Streptococcus spp (10.8%), Klebsiella pneumoniae (5.5%), and Pseudomonas aeriogenosa (2.7%) and there was 13.5% bacteriologically negative cases.

Key words: *Endometritis, ultrasonography, fluid, uterine, Arabian mares.*

INTRODUCTION

The horse can be a companion, a work animal, a performance animal or simply something ethereal for its owners (McKinnon *et al*., 2011).

Inflammatory conditions of the uterus, collectively known as endometritis, can be classified as acute, chronic, active, or subclinical. This condition causes substantial reductions in mare fertility (Hurtgen, 2006).

Breeding-induced endometritis is a physiological reaction to semen. It is an important part of normal sperm transport to the oviduct as well as elimination of excess sperm from the uterus. In 10-15% of brood mares, the normal clearance mechanism fails resulting in a persistent breeding-induced endometritis with severe consequences on fertility. The cause of persistent breeding-induce endometritis is believed to be impaired myometrial activity in response to uterine inflammation (Troedsson *et al*., 2008).

Corresponding author: Dr. M.K. DERBALA
E-mail address: mohamed_equine@yahoo.com
Present address: Ultrasonography Unit, Animal Reproduction Research Institute (ARRI), Egypt, Giza

The incidence of postbreeding fluid accumulation is approximately 15% in a normal population of Thoroughbreds. Both the incidence and severity of intraluminal fluid accumulation increase with age (Zent *et al*., 1998).

Pycock and Newcombe (1996) reported that ultrasound detection of uterine luminal fluid has proved useful in identifying mares with a clearance problem. The presence of free intraluminal fluid before breeding strongly suggests susceptibility to persistent endometritis.

Intrauterine fluid during diestrus is indicative of inflammation and associated with subfertility because of early embryonic death and a shortened luteal phase (Brinsko *et al*., 1991).

Previous studies demonstrated that the most common bacterial causes of uterine infections include *Streptococcus equi* subsp. *zooepidemicus, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacteroides fragilis* and *Bacteroides ureolyticus* (Dhingra and Sandhu, 1987; Ricketts and Mackintosh, 1987; Fodor *et al*., 1995; Langoni *et al*., 1997; LeBlanc, 1999).

The most frequently isolated micro-organism from culture swabs was *β-hemolytic Streptococcus* (39%),

with *E. coli* being second most common (16%). Together, the two organisms represented 55% of the positive culture swabs. Two or more organisms were recovered from culture swab in <2.3% of the positive cultures (LeBlanc *et al*., 2007).

Thoroughbred mares from which the bacterial organisms, *E. coli*, *Staphylococcus aureus*, *Pseudomonas* spp., or bacillus were isolated had intra-uterine fluid in less than 40% of the ultrasonographic examinations (17-39% depending on organism) conducted immediately before a uterine culture was obtained. While mares with β-hemolytic *Streptococcus*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, or yeast isolated from their uterus had intra-uterine fluid in 45-55% of the ultrasonographic examinations (Burleson *et al*., 2010).

Even when guarded uterine culture swabs are used, disagreement between culture and cytology or biopsy findings is possible. This disagreement means that positive culture results can be obtained from mares without endometritis and negative culture results can be obtained from mares with endometritis (Brinsko *et al*., 2011).

MATERIALS AND METHODS

Animals and nutrition:
The current study was carried out on 26 mares (4-19 years old). All mares were Straight Egyptian Arabian mares located in 4 farms in the pyramids area and Cairo-Alexandria desert road. The study was carried out during 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.

Ultrasound scanning:
Ultrasonography was performed using 2 different real time B-mode scanners (Esaote Mylab30-Netherlands) equipped with 5-7.5 MHz frequency LV513 linear-array rectal transducer, and (Shenzhen Well. D -China) equipped with 5-6.5- 7.5 MHz Linear Vet, transrectal probe (Fig. 2, A and B). The scanners have a built-in electronic caliper system for measuring distance, area and circumference, angle and auto follow measurements. Ultrasonic gel (Carboxymethylcellulose) was used as a lubricant during scanning.

Endometrial swabbing:
A double-guarded swab (Mimitube Company-Germany) was used to collect an endometrial sample. The perineal area was thoroughly washed with water and antiseptic. The end of the double guarded swab was kept covered and free from lubricant as it is introduced into the reproductive tract. The double-guarded swab is advanced through the cervix into the uterus and the inner sheath is pushed through the outer sheath. The sample swab is then moved forward to sample the endometrial cells, using a rolling and pushing motion in a time span of 1 min. The examiner's hand in the vagina is used to redirect the double guarded swab into different areas of the uterus. The sample swab is then retracted back into the inner sheath, which is pulled back into the outer sheath, and the entire unit is then removed. The sample swab was placed into a transport container for microbiology (Card, 2005).

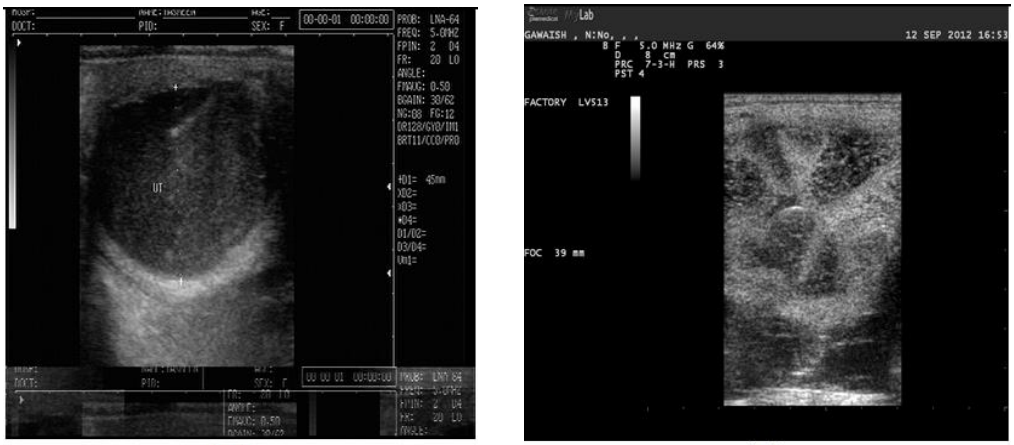
RESULTS

1- Ultrasonographic diagnosis:
The results of current study via ultrasound scanning revealed that 65.4% of mares (17/26) showed intraluminal fluid accumulation and 15.4% of mares (4/26) showed post mating fluid accumulation. There was one mare (3.8%) of cases in which fluid was detected using ultrasound during diestrus phase. In addition there were 15.4 % of cases (4/26) that did not produce intraluminal fluid although they have showed subfertility.

The relationship between the age of the mare and intrauterine fluid accumulation during estrous phase shown in Table (1), in old mares (mean 14.4 y old) incidence of intrauterine fluid during estrous phase was higher 75% (9/12). In young mares (mean 6.7 y old) the incidence of intrauterine fluid accumulation was lower 57% (8/14).

	Intraluminal fluid formation			
Average age	Positive fluid formation	Negative fluid formation	*Total*	**%**
6.7 y	8	6	14	57%
14.4 y	9	3	12	75%
	17	9	26	

The obtained results showed that there was a relationship between the isolation of some bacterial agents and the severity of fluid accumulation that detected by ultrasononography, for example 69.2% (9/13) of cases infected with *Staphylococcus species* showed turbid fluid accumulation that appeared as heterogeneous hypoechoic materials inside the uterus. The fluid accumulation was greater in cases which infected with *Staph* and *Strept* as a mixed infection. Therefore, infection with *Staph* and *Strept* was clearly associated with clinical endometritis. In contrast, only 25% of cases infected with *E.coli* (1/4) showed less evidence of fluid accumulation during ultrasound scanning, simply no more than high degree of uterine edema, therefore the infection with *E.coli* was not associated with clinical endometritis Fig. (1).



(A) (B)

Fig. (1): A - Heterogeneous hypoechoic fluid accumulation inside the uterus in case of Staph. and Strept. infection. High degree of uterine edema pattern in case of E.coli infection B.

2- Bacteriological diagnosis:

The isolated microorganisms are shown in table (2). The majority of endometritis cases were caused *Staphylococcus spp.* (35.1%) and the lowest incidence was *Pseudomonas aeriogenosa* (2.7%).

Table 2: Showing the incidence of bacterial isolates in mares suffering from endometritis.

Bacterial isolates	Number of cases	Percent
Staphylococcus spp.	13	35.1%
E.coli	7	18.9%
Proteus vulgaris	5	13.5%
Streptococcus spp.	4	10.8%
Klebsiella pneumoniae	2	5.5%
Pseudomonas aeriogenosa	1	2.7%
Bacteriologically negative	5	13.5%
Total	37*	100%

* The number 37 in the table is on consideration that the bacterial isolate is sole causative agent not mixed with other bacteria.

DISSCUSION

In the current study transrectal ultrasound scanning was used for diagnosis of endometritis in mares. The most prominent observation was intraluminal fluid accumulation during estrous phase whereas 65.4% (17/26) of mares showed fluid formation and these results supported by Pycock and Newcombe (1996). Furthermore, there were 3.8% of mares showed intraluminal fluid accumulation during diestrus phase and these cases were characterized by long period of infertility and these results comply with Brinsko *et al*. (1991) who stated that intrauterine fluid accumulation during diestrus is indication of inflammation and associated with subfertility and shortened Luteal phase.

In the current study there were a number of cases that suffer from endometritis although there was no fluid accumulation inside the uterus and this result was not recorded in the literatures describing the ultrasonographic picture of uterus of mares suffering from endometritis. These cases were suffering from subclinical endometritis.

Additionally, Zent *et al*. (1998) reported that both incidence and severity of intraluminal fluid accumulation increase with age and the current study revealed similar results as the incidence was 57% in mares with average age 6.7 years old while as the incidence was 75% in mares with average age 14.4 years old.

The obtained results showed that there was a relationship between the isolation of some bacterial agents and severity of fluid accumulation that detected by ultrasonography. The fluid accumulation was greater in mares infected with Staph and Strept, therefore infection with Staph and Strept was clearly associated with clinical endometritis. In contrast only 25% of cases infected with E.coli showed less evidence of fluid formation and these results are quite similar to those obtained by Burleson *et al*. (2010).

Concerning bacterial isolation through using long guarded endometrial swabbing, there were many

bacterial isolates detected through culture which was *Staphylococcus spp* (35.1%), *E.coli* (18.9%), *Proteus vulgaris* (13.5%), *Streptococcus spp* (10.8%), *Klebsiella pneumoniae* (5.5%), and *Pseudomonas aeriogenosa* (2.7%), all these bacterial isolates were mentioned by (Dhingra and Sandhu, 1987; Ricketts and Mackintosh, 1987; Fodor *et al*., 1995; Langoni *et al*., 1997; LeBlanc, 1999) As a major bacterial causes of endometritis in mares.

From observations in the current study there were many cases with negative bacterial culture although theses cases were suffering from endometritis and it is occasionally recorded in many cases as similarly recorded by Brinsko *et al*. (2011).

REFERENCES

Brinsko, P.S.; Blanchard, L.; Varner, D.V.; Schumacher, J.; Love C.L.; Hinrichs, K. and Hartman, D. (2011): Breeding soundness examination of the mare. Manual of equine reproduction Textbook, Third edition, Elsevier, 4: 40-53.

Brinsko, P.S.; Varner, D.D. and Blanchard, T.L. (1991): The effect of uterine lavage performed four hours post-insemination on pregnancy rates in mares. Theriogenology, 35:1111–1119.

Burleson, M.D.; LeBlanc, M.M.; Riddle, W.T. and Hendricks, K.E.M. (2010): Endometrial microbial isolates are associated with different ultrasonographic and endometrial cytology findings in Thoroughbred mares. In: Equine Reproduction X Proceedings International Symposium on Equine Reproduction, Ed: M. Evans, Lexington, KY. p S103.

Card, E.C. (2005): Post-breeding inflammation and endometrial cytology in mares. Theriogenology, 64: 580–588.

Dhingra, P.N. and Sandhu, K.S. (1987): Culture and sensitivity of aerobic bacteria isolated from mares suffering from reproductive disorders. Indian J. Anim. Sci., 57: 525–527.

Fodor, L.; Szenci, O.; Peters, M.; Varga, J.; Szemeredi, G. and Wyszoczky, F. (1995): Isolation of Bacteroides ureolyticus from vaginal discharge of mares. Zentralbl. Veterinarmed, B 42: 415–420.

Hurtgen, P.J. (2006): Pathogenesis and treatment of endometritis in the mare: A review. Theriogenology, 66: (560-566).

Langoni, H.; Alvarenga, M.A.; Papa, F.O.; Sakamoto, C.; Baldini S. and Listoni, F.J.P. (1997): Aerobic, microaerobic and anaerobic bacteria in equine endometritis. Pferdeheilkunde 13: 548.

LeBlanc, M.M. (1999): Diseases of the uterus. In: Colahan, P.T., Merrit, M., Moore, J.N., Mayhew, I.G.J. (Eds.), Equine Medicine and Surgery. W.B. Saunders Company, Philadelphia, pp. 1165–1173

LeBlanc, M.M.; Magsig, J. and Stromberg, A.J. (2007): Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. Theriogenology, 68:403–412.

McKinnon, O.A.; Squires, L.E.; Vaala, E.W. and Varner, D.D. (2011): Preface to the second edition. Equine reproduction 2nd edition, Wiley-Blackwell. A John Wiley and Sons, Ltd., Publication.

Pycock, J.F. and Newcombe, J.R. (1996): Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. Vet. Rec., 138: 320–323.

Ricketts, S.W. and Mackintosh, M.E. (1987): Role of anaerobic bacteria in equine endometritis. J. Reprod. Fertil., 35: 343–351.

Troedsson, H.T.M.; Desvousges, A.; Macpherson, L.M. and Pozor, P.M. (2008): Persistent breeding- induced endometritis. Pferdeheilkunde, 24(1-2): 56-60.

Zent, W.W.; Troedsson, H.T.M. and Xue, J.L. (1998): Postbreeding Uterine Fluid Accumulation in a Normal Population of Thoroughbred Mares: A Field Study. Proceedings of the Annual Convention of the AAEP, 44.

تشخيص التهاب بطانة الرحم في الأفراس العربية باستخدام الموجات فوق الصوتية والفحص البكتريولوجي

محمد كمال دربالة ، مصطفى سعيد فاضل ، هناء عصفور ، السيد عبد الجواد

E-mail: mohamed_equine@yahoo.com Assiut University web-site: www.aun.edu.eg

إن التهاب بطانة الرحم في الأفراس من أكبر المشاكل التي تؤثر على الخيول بعد المغص والعرج، وقد أجريت هذه الدراسة على عدد ٢٦ فرس عربي مصري أصيل (٤-١٩ سنة) كانت في أربع مزارع خاصة بمنطقة الهرم. وباستخدام الموجات فوق الصوتية تبين أن معظم الحالات التي تعاني من التهاب بطانة الرحم يحدث فيها تجمع للسوائل داخل تجويف الرحم، وبالرغم من ذلك وجد عدد من الحالات تعاني من التهاب بطانة الرحم ولا يحدث فيها تجمع للسوائل، بالإضافة إلى وجود علاقة بين تجمع السوائل ونوع الميكروب المعزول ، على سبيل المثال في حالة عزل الميكروب العقدي والميكروب العنقودي أدي الى تجمع سوائل، أما في حالة الأيكولاي فوجد سوائل قليلة أو زيادة في درجة استسقاء بطانة الرحم. وفي هذه الدراسة تم استخدام المسحة الرحمية وذلك لأخذ عينات من الرحم وزرع الميكروبات وعزلها لتشخيص الميكروبات التي تسبب التهاب بطانة الرحم في الأفراس، ومن الميكروبات التي تم عزلها هي المكورات العنقودية (٣٥,١%) ،الأيكولاي (١٨,٩%)، البروتياس فالجاريس (١٣,٥%) ، المكورات العقدية (١٠,٨%) ، الكلبسيلة الرئوية (٥,٥%)، والزائفة الزنجارية (٢,٧%) على الترتيب. وبالرغم من ذلك كانت هناك حالات مصابة ولم يتم عزل مكروبات منها (١٣,٥%).