

Treatment of Persistent Mating Induced Endometritis in Arabian Maiden Mares

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Abstract: The objective of the present study was to evaluate the treatment of Persistent Mating Induced Endometritis (PMIE) in Arabian maiden mares using uterine lavage containing antibiotics alone or with uterotonic agents. Maiden mares (n = 42) with PMIE were subjected to detailed clinical examinations including palpation per rectum, vaginoscopy and cytological examination. The mares were qualified as PMIE due to accumulation of uterine fluid 1-2 days post breeding. Uterine lavage containing crystalline penicillin and streptomycin sulphate was performed (n = 36) 6 h post-breeding. After 8 h of mating maiden mares were randomly divided into 3 groups, 12 animals in each group. The first group injected with 250 µg of cloprostenol (PGF2α) S.C. and the second group received oxytocin 20 I.U. I.M., while the third group with no hormonal treatment. Control maiden mares (n = 6) received neither uterine lavage nor hormonal preparations. Results of pregnancy rate revealed 83.3% pregnancy of maiden mares treated with uterine lavage and PGF2α and 66.6% in mares treated with uterine lavage and oxytocin. In conclusion, maiden mares with PMIE could be successfully treated with uterine lavage and PGF2α.

Key words: Treatment of persistent, induced endometritis, Arabian maiden, PMIE, mares

INTRODUCTION

Persistent Mating Induced Endometritis (PMIE) is still a major cause of economic loss in the equine industry. The incidence of intra uterine fluid accumulation 24 h after breeding has been reported to be 16% when natural mating was used (Watson *et al.*, 2001). Mares that accumulate fluid after breeding were found to have decreased pregnancy rate and increased embryonic loss rates (Newcombe, 1997). However, pregnancy rates were improved if the mares were treated to eliminate the fluid (Zent *et al.*, 1998). Treatment of PMIE is aimed at assisting the uterus to physically eliminate contaminants and inflammatory products. Uterine lavage and uterotonic agents are used for this purpose alone or combined (Hurtgen, 2006). Removing contaminants and inflammatory products as soon as possible reduces the time available for bacteria to replicate and become established and reduces the time of contact of the endometrium with inflammatory products and chemoattractants (Knutti *et al.*, 2000; Brinsko *et al.*, 1991). Uterotonic agents used in the treatment of PMIE include oxytocin and prostaglandin F2α (Le Blanc *et al.*, 1994; Troedsson *et al.*, 1995; Allen, 1991; Pycoc, 1994; Campbell and England, 2002; Paccamonti *et al.*, 1999). Both drugs increase uterine clearance by inducing myometrial contractions. The objective of this study was to evaluate using uterine lavage containing antibiotics

alone or with uterotonic agents as oxytocin or PGF2α on the pregnancy rates in Arabian maiden mares affected with PMIE.

MATERIALS AND METHODS

Animals: The study was conducted from June 2004 to September 2005. Arabian maiden mares (N = 42) were part of the racing club herd kept in Nineveh province and were treated as clinical cases of PMIE on racing club herd consulted to the clinicians in the Department of surgery and obstetrics, College of Veterinary Medicine, University of Mosul. All mares included in this study were Arabian breed, aged 4-8 years. Animals that are eligible for the study were required to meet the following criteria's that were used to diagnose PMIE; 1- History of repeat breeding of more than 3 natural services without any signs of pregnancy. 2- Incidence of intrauterine fluid exist one to two days after natural breeding. 3- Detailed clinical examination including rectal palpation, vaginoscopy and cytological examination of the endometrium ($\geq 5\%$ polymorphonuclear cells).

Design: Mares considered being in estrus when posturing, frequent urination, tail raising and clitoral wink in response to stallion were seen. Ovarian activity was monitored by palpation per rectum every other day until a follicle ≥ 3 cm in diameter was identified, then mares were

mated by a known fertile stallion. The mares were restrained and tail wrapped and secured and perineum was cleaned and scrubbed with povidine iodine. Uterine lavage with 500 mL N-saline containing 4 million IU of crystalline penicillin and 4 g of streptomycin sulphate was performed 6 h after breeding, 6 mares did not received any treatment and served as a control group. Cervical opening was examined per vagina on performing uterine lavage by finger manipulation and classified as partial opened if cervical opening is less than one finger and complete opened when one or more fingers passes easily. The 500 mL lavage volumes are massaged throughout the uterus and are retrieved from the uterus by gravity flow. Immediately after uterine lavage, fluid was recovered into 50 mL sterile conical tubes. Samples were then centrifuged at 5000 rpm for 10 min. The supernatant was removed and a smear was prepared from the pellet for cytological examination. Smears were fixed with absolute methyl alcohol and then stained with Wright's-Giemsa stain and 100 cells were counted and classified neutrophils, lymphocytes, macrophages, eosinophils and endometrial cells. The concentration of nucleated cells in the recovered fluid was counted in the sample and expressed as number of cells per ml of fluid. Uterine lavage pellet was resuspended in 1 mL of PBS and leukocytes were counted by hemocytometer (25 µL sample+475 µL of 1% acetic acid). The differential count was used to calculate the concentration of leukocytes in the fluid. Calculations were done as follows:

$$\text{Conc. of leukocytes/mL} = \text{Conc. of nucleated cells/mL} \times \text{percentage of leukocytes}$$

The severity of endometritis was graded using the following classification: 0-4% of PMNs no inflammation (excluded from the study); 5-10% slight inflammation; 11-20% moderate inflammation; ≥21% severe inflammation.

Treatment methods: After 8 h of mating the 36 mares received uterine lavage with antibiotic were randomly divided into 3 groups 12 animals in each. The first group injected with cloprostenol 250 µg (Veteglan, Laboratories calier, P.O. Box 150, 08520 Les Franqueses del Valles, Barcellona, Spain) S.C. and the second group received

oxytocin 20 I.U. (Intervet International, B.V. Boxmeer, Holland) I.V., while the third group with no hormonal treatment. The mares that were returned to estrus received a second dose of the same treatment. Pregnancy diagnosis was performed per rectum after 2 months of treatment and pregnant mares were recorded as clinical cure, while maiden mares failed to be pregnant after 2 months of the second treatment were recorded as clinical failure.

Statistical analysis: Statistical analyses were performed with the software (Sigma sat, 2004). The differential leukocyte concentration was tested by the Analysis of Variance (ANOVA) and Least Significance Differences (LSD). The chi square test was performed for the difference between the percent of pregnancy rates of the different groups.

RESULTS

Results of clinical examination revealed that 52.4% of PMIE mares were having slight endometritis and 26.2% with severe endometritis as shown in Table 1. High proportion ($p < 0.01$) of maiden mares having partially opened cervix was founded in mares affected with severe and moderate endometritis, 16.66 and 14.29%, respectively. High percent ($p < 0.01$) of completely opened cervix was observed in maiden mares with slight endometritis. There was no significant difference found in the total concentration of leukocytes in slight and moderate forms of endometritis, while a significant ($p < 0.01$) high percent of PMN was founded in mares with severe endometritis and a significant ($p < 0.05$) increase in total concentration of leukocytes.

High proportion ($p < 0.01$) of pregnancy rate 58.3% was founded in maiden mares treated with uterine lavage and PGF2α. Lower pregnancy rate 25% was observed in mares treated with uterine lavage and 5 mares were pregnant (41.6%) using uterine lavage and oxytocin, as shown in Table 2. The mares returned to estrus and received a second dose of the same treatment regimens showed high pregnancy rate (60%) in the treatment group using uterine lavage and PGF2α, as shown in Table 2. A total 24 mares (57.1%) were pregnant after the first and

Table 1: Types of endometritis, percent PMN, total concentration of eukocytes and kind of cervical opening in Arabian maiden mares affected with PMIE

| Types of endometritis | No. mares (%) | PMN (%) | Total conc. of Leukocytes (million mL ⁻¹) | Lymphocytes (%) | Macrophages (%) | Cervical opening | |
|-----------------------|--------------------------|-----------|---|--------------------------|--------------------------|--------------------------|--------------|
| | | | | | | Partial (%) | Complete (%) |
| Slight | 22 (52.4 ^{**}) | 13.7±2.68 | 7.352±0.0753 | 19.87±1.73 | 16.45±1.05 | 2 (4.7) | 20 (47.08) |
| Moderate | 9 (21.4) | 29.2±5.25 | 11.561±0.0935 | 47.52±2.08 ^{**} | 12.28±3.88 6 | (14.29 ^{**}) | 3 (7.14) |
| Severe | 11 (26.2) | 43.9±8.09 | 15.372±0.0596 [*] | 32.26±8.06 | 15.62±4.22 ^{**} | 7 (16.66 ^{**}) | 4 (9.59) |
| Total | 42 (100.0) | | | | | | |

* $(p < 0.05)$, ** $(p < 0.01)$

Table 2: Pregnancy rate of maiden mares in different treatment groups after first and second treatment regimen

| Groups | First treatment regimen | | Second treatment regimen | |
|------------------------------|-------------------------|--------------|--------------------------|--------------|
| | No. of mares | Pregnant (%) | No. of mares | Pregnant (%) |
| Uterine lavage | 12 | 3 (25) | 9 | 3 (33.3) |
| Uterine lavage+PGF2 α | 12 | 7 (58.3**) | 5 | 3 (60**) |
| Uterine lavage+oxytocin | 12 | 5 (41.6) | | |
| Control (untreated) | 6 | 0 (0) | 7 | 3 (42.8) |
| Total | 42 | 15 (35.7) | 21 | 9 (42.8) |

Table 3: Total pregnancy rate of Arabian mares in different treatment groups

| Groups | No. of mares | Pregnant (%) |
|--------------------------------|--------------|--------------|
| Uterine lavage. | 12 | 6 (50) |
| Uterine lavage+PGF2 α . | 12 | 10 (83.3**) |
| Uterine lavage+oxytocin. | 12 | 8 (66.6) |
| Control (untreated) | 6 | 0 (0) |
| Total | 42 | 24 (57.1) |

second treatments regimens. Higher pregnancy rate (83.3%) was achieved using uterine lavage and PGF2 α after first and second treatments, as shown in Table 3. Total percent of pregnancy in maiden mares treated with uterine lavage alone and uterine lavage and oxytocin were 50 and 66.6%, respectively.

DISCUSSION

It is believed that persistent mating induced endometritis is accompanied by intrauterine fluid accumulation (Paccamonti *et al.*, 1999; Pycock and Newcombe, 1996). The deficiency in myometrial contractility responsible for this delay in uterine clearance after breeding may also account for the deficient sperm transport to the oviducts in PMIE mares (Scott *et al.*, 1995). The primary mechanism involved in persistence of fluid in the uterus after mating is an impaired physical clearance (Nikolakopoulos and Watson, 1999). Myometrial contractions, cervical relaxation and lymphatic drainage are known to influence the ability of the uterus to physically clear inflammatory products.

Results of clinical examination of Arabian maiden mares affected with PMIE indicate high proportion of mares with constricted cervical opening. Adequate cervical dilatation is necessary to allow fluid expulsion from the uterus (Brinsko *et al.*, 1991). Steroid hormone environment is known to affect uterine clearance by influencing both uterine contractility and cervical dilation. Estrogens are known to decrease cervical tone and increase uterine motility, facilitating uterine drainage during preovulatory period (Hayes and Ginther, 1986). The presence of macrophages and lymphocytes in PMIE maiden mares was unexpected. Acute endometritis is characterized by the high level of PMN. Macrophages clear the debris generated by the PMN. Clinical experience

would show that mares which are attested PMIE initially in the breeding season can develop into mares with chronic uterine infection. The chronic endometritis might have been resulted from repeated breeding during consecutive estrus cycles and may have been exacerbated by unrelaxed constricted cervical opening observed in studied maiden mares. Constriction of the cervix reduced the clearance rate of the uterus (Hurtgen, 2006). Not only has evacuation of the uterus after mating been shown to be defective in mare with PMIE, but sperm transport to oviduct is also affected, mares with endometritis had fewer sperm in the caudal isthmus than normal mares and very few of these sperm were motile (Bowen *et al.*, 1987; Scott and Liu, 1997).

Treatment of Arabian maiden mares included in this study indicated that uterine lavage containing antibiotics performed 6 h after natural mating alone, or with injection of oxytocin or PGF2 α 8 h post-breeding improve fertility of maiden mares, when compared to the pregnancy rate of maiden mares in control group. This finding is clinically important, because performing uterine lavage alone/with hormones to maiden nulliparous mares that have PMIE may be necessary, as an accumulation of fluids and contaminants adversely affect equine spermatozoa (Troedsson, 1999) and fertility (Hurtgen, 2006). This situation typically arisen in untreated maiden mares (control group) as non of the maiden mares founded pregnant in this study. Uterine clearance is affected by circulating steroids hormone concentration and differed during pre-ovulatory and post-ovulatory (Evans *et al.*, 1986; Le Blanc *et al.*, 1989). As all the treatments were performed during the pre-ovulatory period, the endocrine environment was not likely to contribute to the variability observed in this study.

High pregnancy rate resulted from treatment with uterine lavage and PGF2 α when compared to uterine lavage alone or with oxytocin might be contributed to the improvement of the uterine clearance mechanism (Scott and Liu, 1997) by increasing myometrial contractions and relaxation of the cervix (Hurtgen, 2006) as high percentage of studied maiden mares were having elongated, fibrous cervix during estrus. This may be a

mechanism that expels debris and microorganisms that contaminate the uterine lumen. Also, PGF2 α may have a stimulating effect on the phagocytic activity of uterine PMNs (Ley *et al.*, 1986). This observation confirmed by (Scott and Liu, 1997). In addition Paccamonti *et al.* (1999) concluded that treatment of PMIE with uterine lavage and PGF2 α improve mare fertility. Low pregnancy rate of maiden mares founded in using uterine lavage and oxytocin differ from studies by (Brinsko *et al.*, 1991; LeBlanc *et al.*, 1994; Campbell and England, 2002). In the previous studies, mares treated with oxytocin were multiparous and their age was more than 10-15 years, while in the present study nulliparous maiden mares were treated. Also, in the present study a single injection of oxytocin was performed while others used multiple low doses of injections. It was thought that oxytocin has no beneficial effect on cervical dilatation of mares (Hurtgen, 2006) as well as, oxytocin half life is very short to use as a single injection (Hurtgen, 2006; Campbell and England, 2002).

CONCLUSION

It could be concluded that maiden mares that do not conceive after being bred during three consecutive estrus cycles should not rebred into the following estrus unless endometrial cytology is done and uterine lavage containing crystalline penicillin and streptomycin sulphate performed 6 h after breeding and injection of PGF2 α 8 h post-breeding is an appropriate treatment for PMIE in maiden mares.

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