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Efficacy of Amoxicillin-Clavulanic Acid, Ceftiofur Sodium and Florphenicol in Purebred Arabian Mares Susceptible to Post-Breeding Endometritis

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Abstract

Post-breeding endometritis is among the most important causes of infertility in purebred Arabian mares. In this study, it was aimed to evaluate the diagnosis of post-breeding endometritis by various intrauterine antibiotics administrations and to determine the most successful treatment protocol in terms of achieving pregnancy. For this purpose, a total of 80 purebred Arabian mares, that had no reproductive problems in the previous season but were susceptible to post-breeding endometritis in the season of the study were included. During the ovulation follow up performed 24 and 48 hours after the insemination, mares with an intrauterine fluid accumulation larger than 2 cm in diameter were considered susceptible to post-breeding endometritis. These mares were divided into 3 different intrauterine treatment groups: Group I (Amoxicillin-Clavulanic Acid, n=20), Group II (Ceftiofur Sodium, n=20) and Group III (Florfenicol, n=20). The mares in the control group were not given any treatment other than placebo (0.09% NaCl) (Group IV, n=20). Oxytocin (IM) was administered to all mares in the treatment and control groups 2-3 hours after the application, in 3 doses at 6 hour intervals. An examination was performed at the 24th and 48th hours following the ovulation to determine ovulation. Pregnancy examinations were carried out by ultrasound on the 15th, 30th and 45th days after the ovulation. As a result of the treatments, pregnancy rates among the groups were found as 40%, 60%, 55% and 20%, respectively. As a result, it was concluded that Ceftiofur sodium and Florfenicol applications are effective ($p<0.05$) in terms of pregnancy rates in mares susceptible to post-breeding endometritis.

Keywords: Antibiotics, Endometritis, Infertility, Mare, Post-breeding, Treatment.

INTRODUCTION

Post-breeding endometritis, one of the most important causes of infertility in mares, leads to embryonal resorption, abortion, and reduced pregnancy rates (Mary and Richard, 1986; Bozkurt, 2007; Canisso et al., 2016). Fluid containing excessive spermatozoa and contaminated bacteria entering into the uterus after insemination is considered pathological if it cannot be effectively removed within 12 hours following insemination (Alaçam, 1997; Intas and Cetin, 2015). Inflammatory reactions develop and trigger the inflammatory cycle, causing damage to mucous membranes and spermatozoa and pose a risk for pregnancy. In this cycle, anatomical structure, excessive mucus production, failure in myometrial contractility, insufficient lymphatic drainage, vascular degeneration, aging effects or the combination of all these factors can result in post-breeding endometritis (Mary and Richard, 1986; Timoney and Powell, 1988; Aurich, 2011; Intas and Cetin, 2015). While the use of oxytocin alone is sufficient when the fluid accumulation in the uterus is below 1 cm, a fluid accumulation of 2 cm or more is considered as possible inflammation and infection (Pycock, 2001). In the treatment of post-breeding endometritis, local and systemic antibiotics, chemical antiseptics, antimycotics and hormones are widely used (LeBlanc, 2010; Woodward and Troedsson, 2013).

In this study, it was aimed to evaluate the diagnosis of post-breeding endometritis, which causes significant economic losses in horse breeding and considered as one of the most important causes of infertility, by gynecological examinations and assessment of treatment attempts with various intrauterine antibiotics administrations and to determine the most successful treatment protocol in terms of achieving pregnancy.

MATERIALS AND METHODS

Mares

The study material consisted of 80 purebred Arabian mares between ages of 4 and 24 years that were brought to Harran University Veterinary Faculty Obstetrics and Gynecology Clinic during the breeding season (15 February-30 June). Located in southeast Turkey, province of Sanliurfa, has an altitude of 518 m and latitude of 37° 20'48"N and 39°02'06" E.

Records and reproductive management

Gynecological examinations of the all mares (n=80), mating criteria and the processes related to diagnosing pregnancy, i.e. history taking, inspection, palpation and ultrasound examination were obtained were recorded in the follow-up form. The mares with a follicle diameter greater than 30 mm and responding positively to the trial stallion examination were mated with stallions with normal fertility parameters every other day until ovulation. A single dose of oxytocin (Oxytocin, Teknovet® 5 ml, IM) was administered to all mares 4-5 hours after insemination. An examination was performed at the 24th and 48th hours following the ovulation to determine ovulation. In this study, estimation of ovulation time was calculated by measuring diameter of the dominant follicle, echogenicity

of follicular fluid, growth rate of follicle (mm/6-hr), irregular follicle form, longest axis of follicle wall with ultrasonography (SIUI, CTS-800, linear probe, 5 MHZ, Guangdong, China) (Figure 1.) (Pycock, 2001; Dolezel et al., 2012). During the examination, ovulation was assumed to take place by the absence of the preovulatory follicle, the determination of the corpus luteum, the absence of uterine edema and presence of closed uterine cervix (Figure 2.).

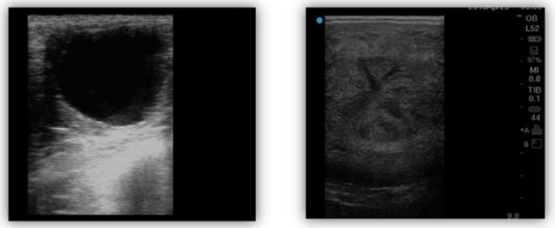

Figure1. Transrectal ultrasonographic image of preovulatory follicle and intrauterine edema.

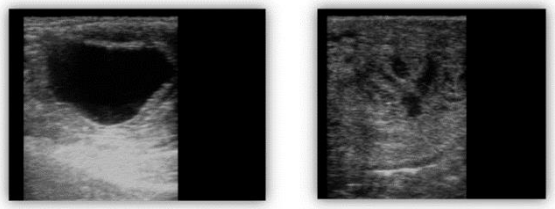

Figure 2. Transrectal ultrasonographic image of ovary and uterus during ovulation.

Study design and grouping

During the ultrasound examination of the uterus performed in the 24-48th hour after the mates. When endometrial fluid accumulation of 2 cm and above were evaluated as mares with suspected inflammation and infection (n=80) included in the study (Figure 3.) (Pycock, 2001). Three groups were formed by randomly dividing the mares (n=60) in the three treatment group and control group (n=20), regardless of fluid density.

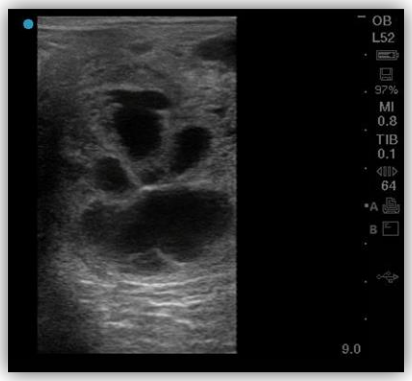

Figure 3. Transrectal ultrasonographic image of intrauterine fluid accumulation after insemination (>2 cm).

Intrauterine treatments

Before intrauterine treatment, the perineal area was washed with soapy water and dried. Disposable sterile lavage catheters were used for intrauterine treatment (Ley, 1994; Pycock, 2001). Amoxicillin-Clavulanic Acid (20 ml) (Synulox, Pfizer®) was used in Group I (n=20), Ceftiofur sodium (20 ml) in Group II (n=20) (Excenel, Zoetis®) and in Group III (n=20) Florfenicol (20 ml) (Armaflor, Arma®) via intrauterine route. The mares who did not receive any treatment in the study were evaluated as the control group (Group IV) (n=20) and only intrauterine placebo (20 ml, 0.09% NaCl) was administered to these mares. Oxytocin (Oxytocin, Teknovet® 5 ml, IM) was administered to all mares in the treatment and control groups 2-3 hours after the application, in 3 doses at 6 hours intervals. Mares in all groups were re-evaluated for the presence of endometrial fluid 2 days after intrauterine treatment administration.

Pregnancy evolution

Transrectal ultrasound examination was performed on the 15th, 30th and 45th days in all groups in cases in which ovulation was determined after the insemination. The mares that were found to be pregnant in the first examination were recorded as pregnant and these data were evaluated in the statistical evaluation. The mares with embryonic and fetal deaths in the following pregnancy examinations were recorded in the follow-up form.

Ethics approval

This study was conducted upon the permission of HRU-HADYEK dated 24/12/2018 and numbered 2018/008/01-07.

Statistical analysis

Analyzes of the results obtained in the study were performed with the Statistic Packet of Social Sciense (SPSS 24.0 Inc. Chicago, IL, America) package program. The significance of difference between groups in terms of follicle diameter was determined by one-way analysis of variance. Presence or absence of a difference between groups in terms of pregnancy rates was compared using Chi-square or Fisher's tests. Findings where the P value was less than 0.05 were considered to be statistically significant.

RESULTS

Follicle diameter findings: In this study, the mean preovulatory follicle diameter was 42.50±1.24 mm in Group I; 41.45±1.28 mm in Group II; 44.45±1.01 mm in Group III; and 39.06 ± 1.19 mm in group IV. The overall follicle size in the groups varied between 30 mm and 55 mm (Table 1).

Age and lactation findings

Among these mares in groups of twenty, the mean age was 12 in Group I; 9.8 in Group II; 11 in Group III; and 11 in Group IV. Eight mares in Group I, 13 mares in Group II, 12 mares in Group III and 15 mares in Group IV were in lactation (Table 1).

Table 1. Preovulatory follicle diameter measurements, age distribution and ratio of lactating mares in groups.

Group	n	Follicle diameter		Age distribution		Mare in lactation (n)
		Average ($\bar{x} \pm S_{\bar{x}}$)	Min-Max (mm)	Average (n)	Min-Max (n)	
G I	20	42,50 ± 1,24 [a,b]	30,00-50,00	12	6-24	8
G II	20	41,45 ± 1,28 [a,b]	30,00-52,00	9,8	4-18	13
G III	20	44,45 ± 1,01 [b]	38,00-55,00	11	6-15	12
G IV	20	39,06 ± 1,19 [a]	31,00-46,00	11	5-20	15
Total	80	42,01 ± 0,62	30,00-55,00	10,85	4-24	48

* (a, b) there is a statistically significant difference between the values (P<0.05).

Pregnancy findings

In the study, the highest pregnancy rate was achieved in G II (n=12), mares that were given intrauterine Ceftiofur sodium (P <0.05). In the other groups, pregnancy rates decreased by this order: G III (Florfenicol group), G I (Amoxicillin-Clavulanic Acid group) and the lowest pregnancy rate was obtained in the control group (GIV). It was observed that intrauterine administration of Ceftiofur sodium (G II) and Florfenicol (G III) in the presence of possible uterine infection after insemination could improve the pregnancy rate by improving fertility (p<0.05). No statistically significant difference was found between GI and GII in terms of pregnancy rates (Table 2). In pregnancy examinations on the 15th day after the insemination; 8 (40%) pregnancies were encountered in Group I, 12 (60%) in Group II, 11 (55%) in Group III and 4 (20%) in Group IV (Table 2). On the 30th day examination of the mares evaluated as pregnancy-positive, it was found that early embryonic death (EED) occurred in 8 pregnancies; 2 in Group I, 2 in Group III, and 4 in Group IV. No embryonic death was encountered in the 45th day pregnancy examinations.

Table 2. Evaluation of pregnancy rates in study groups.

Group	n	Pregnancy positive (15 days)		Pregnancy positive (30 days)		EED	P
		Number	Rate (%)	Number	Rate (%)	Number	
G I	20	8 [a]	40	6[a]	30	2	0.126
G II	20	12 [b]	60	12[b]	60	-	0.856
G III	20	11 [b]	55	9[b]	45	2	0.159
G IV	20	4 [a]	20	0[a]	0	4	0.041
Total	80	35	43,75	27	33,75	8	

* (a, b) there is a statistically significant difference between the values (P<0.05).

DISCUSSION AND CONCLUSION

Post-breeding endometritis in mares is a major problem that leads to delayed uterine clearance after mating, significantly affecting pregnancy rates and resulting in economic losses in mares (Zent et al., 1998; Gutjahr et al., 2000; Watson, 2000). In this study, it was aimed to evaluate post-breeding endometritis in purebred Arabian mares under field conditions and to explicate statistically the success of various intrauterine antibiotic treatment methods.

In addition to classical treatment methods such as mechanical curettage and uterine lavage with irritants (kerosene, collagenase, povidone iodine, dimethylsulfoxide), positive results were achieved using the intrauterine application of mesenchymal stem cells produced from the multipotent adipose tissue of horse with the in vivo method (Neuhauser and Handler, 2017). Studies have indicated that corticosteroid treatment alters the protein profile of the endometrial fluid and its use would be beneficial in mares susceptible to endometritis (Bucca et al., 2008; Wolf et al., 2012). However, it has also been reported that administration of dexamethasone (10-20 mg IM) 6-12 hours after insemination does not have a positive effect on pregnancy rate (Vandaele et al., 2010). Furthermore, it was stated that intrauterine colostrum (120 ml colostrum+380 ml saline) can be used as an option in the treatment of endometritis (Mary and Richard, 1986). Oxytocin (10-25 IU) or chlorprostenol (250 µg) is commonly used in the treatment of post-breeding endometritis to clear uterine fluid and prevent uterine irritation (LeBlanc, 2010; Troedsson, 2011). However, it is also argued that the treatment may not be successful in hormone applications alone due to uterine exudates such as biofilm or mucus produced by some bacteria and fungi (Buczkowska et al., 2015).

Although the intrauterine use of broadspectrum antibiotics in post-breeding endometritis in mares is controversial (Pycock and Newcombe, 1996; Woodward and Troedsson, 2013), it is routinely treated with antibiotics and it is reported that there is no side effect on pregnancy (Pycock and Newcombe, 1996; Le Blanc and Causey, 2009). Penicillin K, chloramphenicol, oxytetracyclines and gentamicin are most commonly used types of antibiotics in the treatment of endometritis in mares. It is reported that daily administration of enrofloxacin for 3 days is beneficial in endometritis (Maria et al., 2015). In his study where he applied penicillin+neomycin and penicillin+platelet rich plazma (PRP) combinations, Pascoe (1995), reported that although there was no difference in pregnancy rates in maiden mares, the treatment with PRP combination was more successful in lactating and infertile mares. In several purebred horse breeding in the UK and the USA, it is reported that all mares are given an intrauterine antibiotic mixture infusion within 24 and 48 hours after mating (Cuervo-Arango and Newcombe, 2010). A study in which ceftiofur sodium was administered intravenously (2.2 mg/kg) for the treatment of post-breeding endometritis, it

was found that endometritis did not develop in the mares in the follow-ups (Witte et al., 2010). In a comprehensive field study comparing oxytocin, a single dose of intrauterine antibiotic and oxytocin+intrauterine antibiotic combination in mares with intrauterine fluid within 72 hours after insemination, and a control group without any treatment, it was found that the best pregnancy rate was achieved from the combination of oxytocin and intrauterine antibiotics (72%) (Pycock and Newcombe, 1996). In our study, the best pregnancy rate was obtained in G II (60%) that was given intrauterine Ceftiofur sodium. In other groups, pregnancy rates were as follows: 55% in Florfenicol group, 40% in Amoxicillin-Clavulanic Acid group, and 20% in the control group. In addition, early embryonic death was observed in 8 pregnancies; 2 in Group I, 2 in Group III and 4 in Group IV of mares that were evaluated as pregnancy positive. In the presence of possible uterine infection after breeding, the difference in administration of intrauterine Ceftiofur sodium (G II) and Florfenicol (G III) in terms of pregnancy rate compared to the control group was statistically significant ($p<0.05$). This favorable difference is thought to be due to the fact that antibacterial therapy reduces bacterial counts and suppresses subsequent fluid production. However, treatment failure is thought to be caused by resistance to antibiotics used for prophylaxis and the presence of bacterial biofilms. In this study, it is thought that the pregnancy rate in Group I was low due to these reasons. Furthermore, the methodology of the study raised the suspicion that low pregnancy rates and high embryonic losses were observed in the study groups due to the potential spermicidal and embryocidal effects of uterine fluid in mares with insufficient uterine clearance.

With advancing age, especially in multiparous mares, fluid accumulation in the uterus may occur due to defects in the genital canal, failure in myometrial contraction, poor lymphatic drainage, as well as hormonal and neurological disorders. It is stated that the accumulation of fluid over 18 hours decreases the pregnancy rate and repeated inseminations in these mares lead to development of bacterial and fungal infections (Alacam, 1997; Knutti et al., 2000; Woodward and Troedsson, 2013; Intas and Cetin, 2015). In a study conducted by Pycock (1994) in purebred Arabian mares, it was found that higher rates of pregnancies were achieved after administration of a single dose of antibiotics (with/without oxytocin) especially in older mares (>12 years old) mating in the first estrus after foaling. When deciding on treatment in post-breeding endometritis, the age of the mare should be taken into account along with the amount of intrauterine fluid observed in ultrasound examination. In one study, delays in the early breeding season, a reduction in the number of ovulation, failure to attain ovulation or persistence of anestrus state despite being in the breeding season were also associated with age (Pycock, 2001). In their study, Intas and Cetin, (2015) found that the rate of breeding live foals decreases as the mare gets older; the rate of conception in four-year-old mares is 75%, however this rate decreases to 50% in the 20s. Korkmaz et al, (2020) report that the reproductive performance and pregnancy rate of older mares (<18 years) are much lower than young and middle-aged Arabian mares. In our study, the average age of the mares in study groups ranged between 9.8 and 12, and because of the higher mean age compared to other studies, it may affect the pregnancy rate in a similar way. It was concluded that the difference in treatment results between the groups in our study was due to the older age of the mares, the lactation status, the time of the reproductive season in which the treatment was administered, the amount of the intrauterine fluid, hormonal applications, the severity of the inflammatory response, and the clinical history. However, Korkmaz et al, (2020) emphasized that the reduced reproductive performance and low pregnancy rates in older mares as a result of some ovarian hormones decreasing in parallel with increasing age should be considered as a complex geriatric problem, not just due to intrauterine fluid.

In this study, it was found that the lowest pregnancy rate was seen in mares susceptible to post-breeding endometritis and all pregnancies in the control group, which received no antibiotic treatment protocol, resulted in embryonic death. It is thought that high rate of pregnancy loss in the control group in our study is also due to the fact that this group included highest number of lactating mares. In other groups, it was found that the number of mares in lactation varied between 8 and 13. Durmaz et al. (2020) reported that embryonic deaths were higher in lactating mares compared to non-lactating mares, supporting this suspicion. Similarly, it was reported that embryonic mortality rate that takes place in between the 10-45th day of pregnancy in healthy mares, varied between 5-30% (Immegart, 1997) and this rate increases especially in infertile mares (Ball, 1986). It has been found that lactating mares may undergo anestrus or exhibit abnormal estrus behavior following foaling (Pycock, 2001; Heidleret al., 2004). The reason for these abnormalities in lactation is thought to be the result of suppression of gonadotropins by prolactin as well as its nature of being seasonal (Loy, 1980; Nagy et al., 1998). Polat et al. (2020) reported that the levels of estrogen, progesterone, Anti-mullerian hormone and insulin-like growth factor-I in lactating mares were significantly lower.

The preovulatory follicle diameter was reported as 30-100 mm by Bozkurt (2007), 34-70 mm by Ginter (1995) and 22-70 mm by Davies et al. (2010). Korkmaz et al. (2020) however reported an average preovulatory follicle diameter of 42.72 ± 0.80 mm in young purebred Arabian mares and 44.12 ± 0.65 mm in older purebred Arabian mares. In this study, the preovulatory follicle diameters varied between minimum 30 mm and the maximum 55 mm; the smallest value was 39.06 ± 1.19 measured in control group and the mean was $42,01 \pm 0,62$ mm in groups. In order to achieve a successful pregnancy, the time of ovulation should be determined precisely and the time of insemination should be decided accordingly. Although the most important criterion in determining the ovulation time in mares is the size of the dominant follicle, age, season and the presence of other preovulatory follicles (multiple ovulation) also affect ovulation. In mares at the end of the breeding season, especially in older mares, a large number of preovulatory follicles may result in the formation of smaller-diameter preovulatory follicles, a decrease in oocyte quality and lower pregnancy rate.

In conclusion, it has been observed that uterine fluid accumulation significantly affects pregnancy rates and constitutes an important problem. It has been concluded that Ceftiour sodium and Florfenicol applications are effective with regard to pregnancy rates in mares susceptible to post-breeding endometritis. Although antibiotics are widely used in post-breeding endometritis, review of literature did not yield sufficient number of articles on this issue. However, it was seen that conception and reproductive performance diminished as the age progressed. Considering the prevalence and economic

importance of the subject, it is believed that further comprehensive studies are needed in this field.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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