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The Effect of Uterine Lavage and Oxytocin Administration Before and After Breeding on Fertility in Mares in The First Postpartum Estrus

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Abstract

The objective of this study was to investigate the effects of the combination of uterine lavage and oxytocin administration before and after breeding during post-partum first estrus on the pregnancy rates of mares. Thirty mares whose fetal membranes were released within the first 3 h after parturition were divided into three groups - the control (n=10), pre-breeding (n=10) and post-breeding treatment group (n=10). The uterine lavage was performed 4 h before breeding in the pre-breeding group and 4 h after breeding in the post-breeding group. The oxytocin administration was performed twice in both treatment groups intravenously - immediately after and 12 h after the uterine lavage. A sterile NaCl solution (0.9%) was administrated intravenously in the control group. In the control group there was a longer interval between parturition and first ovulation (14.6 days) compared to treatment groups ($p<0.05$). The pregnancy rates in the control; pre-breeding and post-breeding treatment groups were calculated as 40%, 40%, and 60%, respectively. Although early embryonic loss was not observed in both the pre- and post-breeding treatment groups, this ratio was 25% for the control group. As a conclusion, the administration of a uterine lavage (1 liter of sterile 0.9% NaCl solution +4.000.000 IU crystallized penicillin +4g streptomycin sulfate) and 20 IU oxytocin 4 h before or after breeding mares at their first postpartum ovulation shortens the day interval between parturition and ovulation. It can be assumed that breeding during foal heat can be effective in reducing uterine involution, inflammatory reactions related to breeding and embryonic death.

Keywords: Foal heat, lavage, ovulation period, oxytocin, pregnancy rates

Introduction

Fertility problems lead to profitability problems in horse breeding. Racehorse breeding, especially, is a very expensive and time consuming profession due to financial considerations such as the commercial need to have foals regularly. In the racehorse industry, it is crucial to breed the mare in the post-partum first estrus right after foaling in order to gain competitive advantage in the races. This period, which is also called the foal heat, is characterized by physiological follicular development and ovulation of the mare. Mares should become pregnant within one month postpartum to continue producing foals each year. Breeding the mares in the first post-partum estrus is one of the methods used to improve the chance of maintaining yearly foal production. Successful pregnancy rates for breeding during foal heat has been indicated in various studies (Gündüz et al., 2008; Le Blanc, 2003).

One important factor in successful early postpartum breeding is uterine fluid load after foaling. Mares with a clinically normal uterus should not have a significant volume of fluid in the uterus. The presence of a moderate or large volume of fluid in the uterus visible on ultrasound suggests the presence of an active infection, a prolonged non-infectious inflammatory condition, inadequate uterine clearance mechanism or failure of normal cervical function (Dadarwala et al., 2004; Katila and Reilas, 2001;

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McKinnon et al., 1988; Sertich and Watson, 1992). The incidence of intrauterine fluid retention in mares was indicated as 11-39% (Reilas et al., 1997; Watson, 2000). The visible amount of intrauterine fluid in the estrus cycle may refer to endometritis and may decrease sperm mobility, which may cause failure of pregnancy (Pycock and Newcombe, 1996). The goal of oxytocin therapy is to stimulate uterine contractions that will expel the fluid out of the uterus through the cervix. Oxytocin administration may be recommended for a fluid volume more than 1 cm radius. If the radius is 2 cm or more, moderate examinations should be performed for diagnosis of uterine inflamation. In general, according to the findings of the examination, intrauterine lavage and administration of antibiotics are performed (Pycock, 2001).

Administration of intrauterine lavage, antibiotics, oxytocin and human Chorionic Gonadotropin (hCG) after breeding has shown to be positively effective on fertility (Azawi, 2008; Kılıçarslan, 2002; Kılıçarslan, 2013; Kılıçarslan et al., 1996).

The aim of the study was to investigate the possible effects of uterine lavage and oxytocin administration during foal heat on fertility parameters such as length of ovulation periods, pregnancy rates and rates of early embryonic death. The expected hypothesis was that the combination of uterine lavage and oxytocin administration performed 4 h before and 4 h after breeding in foal heat would shorten the interval between parturition and first ovulation, increase pregnancy rates and decrease early embryonic death rates.

Materials and Method

The study was conducted on 30 Thoroughbred mares in good body condition (6/9), weighing approximately 500 kg, with an average age of 13±2. All breeding activities were held between the years 2011 and 2012. All mares had normal clinical and gynecological characteristics according to history findings, general clinical examinations and gyneacological ultrasound examinations before entering the study. Breeding criteria at foal heat were accepted as physiological parturition process without abnormality, correct timing of placenta expulsion, no visible trauma at the vagina and perineum, no trace of infection, acceptable ultrasound and cytological examination results (Le Blanc, 2009). In the present study, all criteria except cytological examination were considered as animal selection criteria. Additionally, uterine biopsy sampling was not performed due to the absence of any suspicious findings such as endometrial cysts or intrauterin fluid accumulation with a diameter ≥2 cm at ultrasound examination. All mares used in the study delivered without any complication and released their fetal membrane within the first three h after parturition.

Mares were randomized into 3 groups-control (n=10), pre-breeding (n=10) and post-breeding treatment (n=10) groups.

Twenty IU intravenous oxytocin (Oksitosin, Vetaş, İstanbul, Turkey) and uterine lavage using a combination of 1 liter of sterile 0.9% NaCl solution (Izotonik Sodyum Klorür Solusyonu, Eczacıbası-Baxter, İstanbul, Turkey) at 40˚C; 4.000.000 IU crystallized penicillin and 4 g streptomycin sulfate (Clemipen-Strep, Topkim, İstanbul, Turkey) was administrated 4 h before breeding for the pre-breeding treatment group and four hours after mating for the post-breeding treatment group.

Oxytocin administration was performed intravenously immediately after the uterine lavage. Injections were done twice during the same day at 12 h intervals. Two ml of 0.9% NaCl solution was injected intravenously in the control group 12 h before and 12 h after breeding.

All mares were examined with the same ultrasound device (ALOKA SSD500, Mindray, Shenzhen, China) used by the same operator in the same room. The ultrasound examinations were performed daily from detection of a preovulatory follicle with a diameter ≥35 mm until the day of ovulation. The diameter of the preovulatory follicle, edema of endometrial folds and intrauterin fluid accumulation were evaluated ultrasonographically. The mean preovulatory follicle at foal heat was recorded as 48±2 mm while the volume of intrauterin fluid accumulation was below 1 cm in diameter for all mares.

Twelve stallions with known reproductive performance with progressive sperm motility of 40-60% were used during the study. Mares in foal heat were mated with stallions with an interval of 48 h. The mean breeding time was recorded as 1.8 per mare. Ovulation periods after last breeding were investigated with ultrasonography every two days. Pregnancy controls were done at day 16, 30 and 42, respectively. Neither any multiple ovulation nor twin pregnancy was detected in this study.

Statistical analysis

Statistical analysis were calculated using the The Statistical Package for the Social Sciences (SPSS) version 13.0 (SPSS Inc., Chicago, IL, USA). Statistical evaluation of the first ovulation periods after parturition in the control, pre-breeding and post-breeding treatment groups was performed using the "One-way ANOVA test" and the "Duncan test". Pregnancy rates and early embryonic death ratios were evaluated using the "Chi-square test". The statistically significant rate was set as $p<0.05$.

Results

The ovulation periods of all mares were determined ultrasonographically every two days. According to ultrasound inspections performed once every two days, the days interval from parturition to first ovulation - or foal heat as it is known - in the control, pre-breeding and post-breeding treatment groups were measured as 14.6 days, 12 days and 11.1 days, respectively (Table 1), which started on the postpartum 7.6th day. The days interval from parturation to first ovulation in the control groups

Table 1. Mean values and standard deviations for ovulation times after foaling

Parameter	Groups			Significance
	Control Group (n=10)	Pre-breeding Treatment Group (n=10)	Post-breeding Treatment Group (n=10)	
The days interval from parturation to first ovulation	$14.60^{a}\pm1.176$	$12.00^{b}\pm0.471$	$11.10^{b}\pm0.674$	*

*: $p<0.05$

[a,b]: Difference between mean values with different letters in the same line is significant.

Table 2. Pregnancy rates at first postpartum mating

Parameter	Groups			Significance
	Control Group (n=10)	Pre-breeding Treatment Group (n=10)	Post-breeding Treatment Group (n=10)	
Pregnancy rates	4 /10 40%	4 /10 40%	6 / 10 60%	NS

NS: Not significant.

Table 3. Early embryonic loss rates at first postpartum mating

Parameter	Groups			Significance
	Control Group (n=10)	Pre-breeding Treatment Group (n=10)	Post-breeding Treatment Group (n=10)	
Early embryonic loss rates	1/4 25%	0/4 0	0/6 0	NS

NS: Not significant.

was statistically higher ($p<0.05$) than both pre- and post-treatment groups, however there was no statistically significant difference between the two treatment groups.

The pregnancy rates calculated for the three groups are shown in Table 2. Although the pregnancy rate for the post-breeding treatment group was found to be slightly higher than the other two groups, no statistically significant difference was detected ($p>0.05$).

Early embryonic loss was not observed in either pre- or post-breeding treatment groups, however it was recorded as 25% in the control group ($p>0.05$) as shown in Table 3.

Discussion

Given the ideal requirement of producing one foal per year from each Thoroughbred mare, tracking reproductive efficiency is critical for stud managers. The mare is a seasonally polyestrous breeder. They have multiple estrus cycles during one part of the year, which is called the ovulatory season; and is followed by an anestrous period for the rest of the year. In early spring, the mare enters a transitional period between the anovulatory season and the first ovulation of the year. As the foals are accepted to be born in the first day of January, it is crucial for the stud manager to arrange the parturitions as early as possible, to allow foals maximum growth during the year in order to get an advantage in races, in the Thoroughbred breeding industry (Gündüz et al., 2008; Kılıçarslan and Uçar, 2015).

Pregnancy is a long period in mares. Therefore, it is also important to breed the mare in the closest date after parturition. Gündüz et al. (2008) reported that ovulation during the first postpartum estrus was seen at 6 to 12 days post partum. Another study performed by Keskintepe et al. (1988) showed that ovulation was seen between 8 to 42 days after foaling. When all study groups were assessed together, the first postpartum estrus was detected to start at 7.6 days after parturition in our study.

Keskintepe et al. (1988) stated that first ovulation at postpartum occurred between the 13th and 64th day. In another study the first ovulation was detected at approximately 12.2 days after foaling (Katilla et al., 1988). Loy (1980) observed the first ovulation at an average of 14.6 days. In another study, it was found that the first ovulation occurred earlier at postpartum during the year from spring to summer (Nagy et al., 2000). Le Blanc (2009) stated that the timing of first ovulation at postpartum tends to occur gradually closer to the parturition date from January to May. In the present study, findings indicated that the first ovulation in the control group occurs significantly later ($p<0.05$) than the ovulation in the pre-breeding treatment (12 days) and the post-breeding treatment (11.1 days) groups. The lack of significancy between the first postpartum ovulation

date of pre- and post-treatment groups suggests that the use of uterine lavage and oxytocin administration combination may not be effective in ovulation induction. However, the period between foaling and first ovulation of the season was found physiological for all study groups.

Uterine lavage is recommended in order to assist the uterus to physically clear the normal inflammatory byproducts, which occur as a response to breeding, and to increase uterus muscle tone (Brinsko, 2001). Brinsko et al. (1991) assumed that the timing of uterine lavage could have an effect on pregnancy rates. Intrauterine treatments that were performed just before breeding (Vanderwall and Woods, 2003) and also performed after 4 h to 4 days following breeding do not have a negative influence on fertility (Brinsko et al. 1990; Brinsko et al. 1991; Knutti et al. 2000). Additionally, intrauterine treatments including uterine lavage and antibiotic administration which are combined with oxytocin and hCG injections are reported to increase fertility (Azawi 2008; Kılıçarslan et al. 1996; Kılıçarslan 2002; Kılıçarslan 2013). Therefore, treatment protocols of uterine lavage and oxytocin administration were tested 4 h before and 4 h after breeding in the present study. However, no statistically significant difference was observed between the pre-breeding treatment and post-breeding treatment groups similar to previous studies (Brinsko, 2001; Malschitzky et al., 2002).

Oxytocin is widely accepted as an effective therapy in aiding mechanical clearance mechanisms and improving fertility. The endometrium shows irregularities because of incomplete involution during foal heat. Myometrial contractions help bacterial elimination and stimulate mucosal rejeneration (Katila and Reilas 2001), and oxytocin injections promote uterine involution especially when administered during estrus (Nikolakopoulos and Watson, 1999). Cadario et al. (1999) stated that mares weighing 450 kg could be given 10 IU or 20 IU of oxytocin. In another study, it was emphasized that 25 IU oxytocin applications 72 h after breeding does increase pregnancy rates by 7% (Pycock and Newcombe, 1996). In the present study, the results indicate that 20 IU oxytocin given after breeding was positively effective on pregnancy rates by showing a slightly higher pregnancy rate in the post-breeding treatment group, however, this difference was not significant.

It is known that the use of oxytocin and antibiotic combination is effective on fluid elimination and increasing pregnancy rates in mares (Pycock and Newcombe, 1996). The findings of the present study indicate that the combination of oxytocin and antibiotics could have an effect on increasing pregnancy rates when used after breeding.

Embryonic death rate for fertile mares was approximately calculated as 5-24% and the highest rate was seen at 10 to 14 days after detection of pregnancy via ultrasound (Ball, 1993). It was advocated that the embryonic death rate was higher in the mares bred at foal heat (Blanchard and Varner, 1993; Lowis and Hyland, 1991; McKinnon et al., 1988). In the present study, one embryonic death was noticed at the 30th day among the 4 mares in the control group.

Conclusion

Administration of uterine lavage (1 liter of sterile 0.9% NaCl solution + 4.000.000 IU crystallized penicillin + 4 g streptomycin sulfate) and 20 IU oxytocin 4 h before or after breeding mares at their first postpartum ovulation shortens the day interval between parturition and ovulation. It can be assumed that breeding in foal heat could be effective in reducing uterine involution, inflammatory reactions related to breeding and embryonic death.

Ethics Committee Approval: The study was approved by Istanbul University Local Committee on Animal Research Ethics (no: 2011/122) (29/09/2011).

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