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SOME POST-BREEDING TREATMENTS FOR IMPROVING FERTILITY IN MARES

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SUMMARY

A total numbers of 115 native breed mares were used in the present study. Uterine swabs were taken for bacteriological culture. The follicular growth and pregnancy were detected by rectal palpation and ultrasonography. There was a higher incidence of bacterial isolation from lactating mares (70.91%) than those in barren ones (43.33%).

The mares were randomly divided into 9 groups which subjected to the following treatment regimens: 1) no drugs (control), 2) intrauterine infusion with 120 ml homologus plasma, 3) intrauterine infusion with 120 ml leukocyte enriched homologus plasma, 4) intrauterine infusion with antibiotics, 5) intravenous injection with 20 I.U oxytocin giving 3 times post breeding and 6) intravenous injection with 20 I.U oxytocin giving once.

The animalís groups from 1-4 were treated with the corresponding regimens. Meanwhile, the 5th, 6th, 7th, 8th and 9th groups were treated with regimens 2 and 4; 3 and 4; 5; 2 and 6 and 3 and 6 respectively.

Our results indicated that irrespective of treatment regimens, a higher pregnancy rate (56.36%) was observed in lactating than in barren mares (50.0%). Regarding the effect of regimen irrespective of types of mares, a higher pregnancy rate was found in groups 3 (71.40%) and 9 (71.72%) which were treated with regimens 3 and 3 and 6 respectively. It is worthy to note that the lowest value (36.36%) was recorded in group 4 and 7 which were treated with antibiotics alone and oxytocin alone respectively.

In conclusion, the intrauterine infusion of leuckocyte enriched homologus plasma alone or with I.V injection of oxytocin increased the pregnancy rate in barren and lactating mares respectively.

INTRODUCTION

The uterine contamination is frequently observed in mares during breeding (Kenney and Ganjam, 1975 and Mattos et al., 1999). The uterus of most mares respond to the contaminant by eliminating the agent within few hours or days and removing the infection before entrance of embryo into the uterine cavity (Evans et al., 1986; Watson, 1988 and Asbury and Lyle, 1993). Meanwhile, some mares cannot eliminate the contaminant due to defective uterine contractility and lymphatic drainage and local cellular and humoral immunity (Troedsson and Lui, 1991; Troedsson et al., 1993 *Troedsson et al.* a & b and 1995 and LeBlane et al., 1994 and 1995). These contaminants results in endometritis which disrupts the uterine environment and is detrimental to fertility in mares (Mattos et al., 1999). However, streptococcus zooepidemicus is the most frequently bacterial agent causing endometritis in mares (Ferreiro et al., 1986).

Several uterine infusions are currently used for treatment of post-breeding endometritis in mares including uterine lavage with homologus plasma (Asbury, 1986; Pascoe, 1995 and Mattos et al., 1997); homologus plasma enriched with leukocytes (Mattos et al., 1997 and 1999); homologus plasma and antibiotics (Pascoe, 1995 and Mattos et al., 1997); homologus plasma and oxytocin injection (Asbury, 1986 and Pascoe, 1995); homologus plasma enriched with leukocyte and oxytocin injection (Mattos et al., 1999) and oxytocin injection only (Asbury, 1986; Pascoe, 1995 and Mattos et al., 1999).

The present work aimed to explore whether the post-breeding therapies using intrauterine infusion with homologus plasma; leukocyte enriched homologus plasma; antibiotics and oxytocin injection improve the fertility in native breed mares.

MATERIAL AND METHODS

A total of 115 native breed mares (60 Barren and 55 lactating) belonged to some owners at Menoufia and Gharbia Governorates were used in the present work. All females were examined for reproductive soundness at the onset of the first detected estrus for barren mares and at the first period after foaling for lactating mares. All mares were submitted to rectal palpation of the internal reproductive tract and vaginal examination with speculum. Perineal confirmation was evaluated and proper corrections performed when necessary. Uterine swabs were taken using double guarded swabbing techniques. Several uterine swabs were taken from mares before breeding (Mattos et al. 1984). Mares were teased daily for detection of estrous when the first day of heat was observed, the follicular growth was detected by rectal palpation and ultra-sonography (Figures 1, 2 & 3). Females in heat were bred by proven fertile stallions after detection of a pre-ovulatory follicle of more than 40 mm in diameter. Mares under experimental condition were randomly assigned into 9 groups according to the type of

postbreeding therapies as follows:

1- The 1st group (13 mares, 7 barren and 6 lactating) received no treatment and used as control.

2- The 2nd group (15 mares, 7 barren and 8 lactating) received an intra-uterine infusion with 120 ml homologus plasma.

3- The 3rd group (14 mares, 7 barren and 7 lactating) received an intra-uterine infusion with 120 ml leukocyte enriched homologus plasma.

4- The 4th group (11 mares, 6 barren and 5 lactating) received an intra-uterine infusion with antibiotics (5 x 10^6 IU procaine-penicillin and 2 g streptomycin sulphate).

5- The 5th group (12 mares, 6 barren and 6 lactating) received an intra-uterine infusion with 120 ml homologus plasma and antibiotics (5 x 10^6 IU procaine-penicillin and 2 g streptomycin sulphate).

6-The 6th group (13 mares, 7 barren and 6 lactating) received an intra-uterine infusion with 120 ml leukocyte enriched homologus plasma and antibiotics (5 x 10^6 IU procaine-penicillin and 2 g streptomycin sulphate).

7- The 7th group (11 mares, 6 barren and 5 lactating) received an intravenous injection with 20 IU oxytocin administered 3 times at 0, 12 and 16 hours after breeding.

8- The 8th group (12 mares, 6 barren and 6 lactating) received an intra-uterine infusion with 120 ml homologus plasma and an intravenous injection of 20 IU oxytocin at 0 hour after breeding.

9- The 9th group (14 mares, 8 barren and 6 lactating) received an intrauterine infusion with leukocytes enriched homologus plasma and an intravenous injection of 20 IU oxytocin at 0 hours after breeding.

Separation of homologus plasma:

The separation of homologus plasma was done according to the method adapted by Mattos et al. (1997). Briefly, 500 ml blood with Na-heparin (10 IU/ml of blood) were collected on the first or second day of estrus. The collected blood was placed in a refrigerator (5°C) for 2 hours to allow sedimentation and plasma separation. Plasma was split into 120 ml aliquots and frozen at -20°C until use. Thawing was performed in a 38°C water bath.

Separation of homologus plasma with leukocytes:

The separation of homologus plasma with leukocytes was done according to the method adapted

by Mattos et al. (1997 and 1999). Briefly, 270 ml of heparinized blood (10 IU/ml) were collected one hour before infusion. After plasma separation, 100 ml of a 6% dextrose solution were added. Leukocytes floatation occurred after 30 minutes. Enriched plasma (> 5000 leukocytes / mm3) was then aspirated and used immediately.

In all groups of animals under investigation, the treatments were given after mating. Breeding and treatments were repeated every 48 hours until ovulation was detected. Pregnancy diagnosis was performed by ultra-sonography 18 days after mating. Pregnancies were followed up until 45 days post-breeding (Figures 4 & 5).

RESULTS

Table 1 revealed the various types of bacteria isolated from uterine swabs of both barren and lactating mares. Bacteria were isolated from 43.33% of barren mares and 70.91% of lactating ones. The various types of bacteria including staphylococcus spp., streptococcus cpp., E.coli; proteus spp., pseudomonas spp., klebsilla spp., staphylococcus spp. and streptococcus spp.; staphylococcus spp. and E. coli; pseudomonas spp. and E.coli; staphylococcus spp.; streprococcus spp. and E. coli and staphylococcus spp.; streptococcus spp. and proteus spp. were isolated from 5.00

and 7.27; 3.33 and 5.55%; 6.67 and 10.91%; 3.33 and 5.55%; 5.00 and 5.55; 3.33 and 7.27%; 3.33 and 3.64% and 5.00 and 7.27, 3.33 and 10.9%; 1.67 and 1.82% and 3.33 and 5.55% of mucus collected from the uteri of both barren and lactating mares, respectively.

The accuracy of determining pregnancy using the ultrasonographic examination on day 18 post breeding was found to be 90% for barren mares and 94% for lactating ones. Collectively, it was recorded to be 92% in all mares under investigation.

Pregnancy rates in barren and lactating mares on days 45 post-breeding are presented in Table. 2. Regarding the effect of type of mare irrespective of treatment regimes, a higher pregnancy rate (56.36%) was observed in lactating than in barren mares (50.0%). In lactating mares the maximum rate of pregnancy was observed in females treated with leukocytes enriched homologus plasma and oxytocin (83.33%). Meanwhile, in barren mares, the maximum rate of pregnancy was recorded in females treated with leukocytes enriched homologus plasma (72.43%). The minimum rates of pregnancy were observed in other groups of both barren and lactating mares (28.57 - 50.0%).
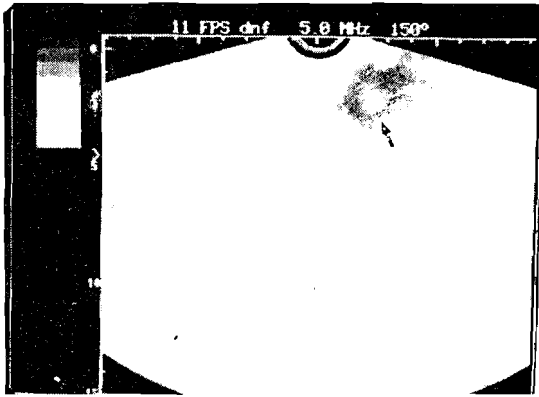
Fig. (1): Ultrasound image of right ovary showing a growing follicle (10 x 10 mm) appear as anechoic vesicle (arrow).
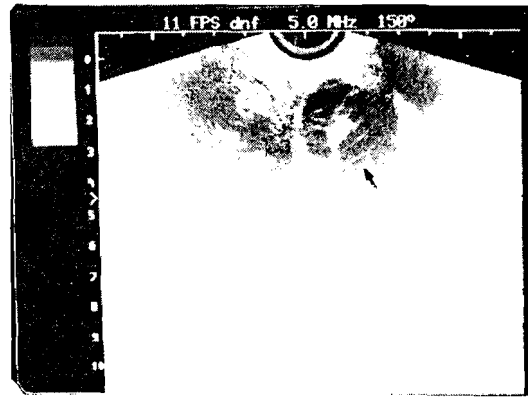

Fig. (2): Ultrasonogram of right ovary showing a mature large follicle (anechoic) just before ovulation (arrow).
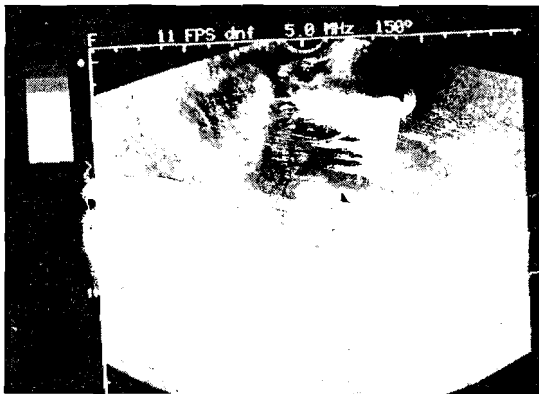

Fig. (3): Ultrasonogram of right ovary showing a single pre-ovulatory follicle (25 x 41 mm) the ultrasound images as black (anechoic) circumscribed area (arrow)
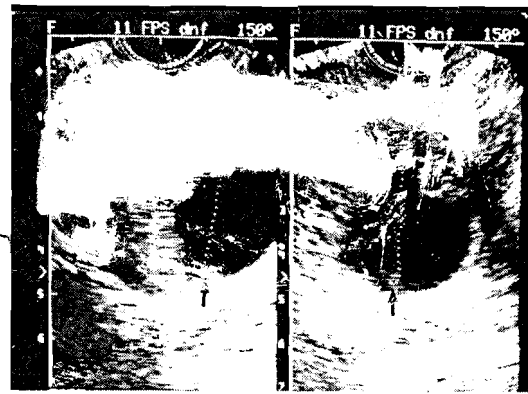

Fig. (4): Embryonic vesicle (18 days) charachterized by two artifactual hyperechogenic reflections located at the dorsal and ventral aspect of the vesicle (arrow).

Fig. (5): Embryonic vesicle (45 days) it is
irregular and the embryo proper
is obvious (arrow).

Table (1). The incidence of pre-breeding bacterial isolates recovered from the uteri of
both barren and lactating mares.

Types of bacterial isolates	Barren mares (n = 60)	Lactating mares (n = 55)
Staphylococcus spp.	3/60 (5.00%)	4/55 (7.27%)
Streptococcus spp.	2/60 (3.33%)	3/55 (5.55%)
E. coli	4/6 (6.67%)	6/55 (10.91%)
Proteus spp.	2/60 (3.33%)	3/55 (5.55%)
Pseudomonas spp.	3/60 (5.00%)	3/55 (5.55%)
Klebsilla spp.	2/60 (3.33%)	4/55 (7.27%)
Staphylococcus + Streptococcus	2/60 (3.33%)	2/55 (3.64%)
Staphylococcus + E. coli	3/60 (5.00%)	4/55 (7.27%)
Pseudomonas spp. + E. coli	2/60 (3.33%)	6/55 (10.91%)
Staphylococcus, Streptococcus + E. coli	1/60 (1.67%)	1/55 (1.82%)
Staphylococcus, Streptococcus + proteus	2/60 (3.33%)	3/55 (5.55%)
Total + ve mares	26/60 (43/33%)	39/55 (70.91%)
Total - ve mares	34/60 (56.67%)	16/55 (29.09%)

Table (2): Pregnancy rate on day 45 in barren and lactating mares after different post-breeding treatments.

Treatment group	Barren mares			Lactating mares			Total mares		
	No. of treated mares	Pregnant		No. of treated mares	Pregnant		No. of treated mares	Pregnant	
		n	%		n	%		n	%
G1: Control	7	2	28.57	6	2	33.33	13	4	30.70
G2 : H. plasma	7	3	42.86	8	4	50.00	15	7	46.60
G3: H. plasma + Leukocytes	7	5	72.43	7	5	71.43	14	10	71.40
G4: Antibiotics	6	2	33.33	5	2	40.00	11	4	36.36
G5: H. plasma + Antibiotics	6	3	50.00	6	3	50.00	12	6	50.00
G6: H. plasm + Leukocytes + Antibiotics	7	4	57.14	6	4	66.67	13	8	61.53
G7: Oxytocin	6	2	33.33	5	2	40.00	11	4	36.36
G8: H. plasma + Oxytocin	6	4	66.67	6	4	66.67	12	8	66.60
G9: H. plasma + Leukocytes + Oxytocin	8	5	62.50	6	5	83.33	14	10	71.72
Total	60	30	50.00	55	32	56.63	115	61	53.04

H = Homologous

DISCUSSION

The higher incidence of bacterial isolation from lactating mares (70.91%) than those recorded in case of barren ones (43.33%) may be attributed to the incomplete elimination of microbes during the early postpartum period in lactating females (Merket and Gunzel, 1979).

In the present work, the minimum pregnancy rate was recorded for the untreated barren mares (28.57%). A much higher values were observed for pregnancy rate in case of untreated lactating mares (33.33% on 45 days post-breeding). These findings are nearly similar to those of Mattos et al. (1999) concerning to barren and lactating mares. In general, the post-breeding treatment with oxytocin or antibiotics did not improve the pregnancy rate when compared to the untreated groups in both barren and lactating mares. In this respect, the use of oxytocin immediately after breeding of mares failed to improve pregnancy rate (Mattos et al., 1999 and Rigby et al., 1999). Others reported an improvement in pregnancy

rate after treatment of barren mares with oxytocin 4-8 hours after breeding (LeBlanc et al., 1994 and Rasch et al., 1996). Moreover, the failure in improving pregnancy rate after post-breeding treatment of mares with antibiotics may be returned to most females under investigation had no previous history of infertility.

The present study indicated a relative improvement in the pregnancy rate following the post-breeding treatment with homologus plasma in barren and lactating mares and with homologus plasma in combination with antibiotics in barren mares and not in lactating ones. However, the post-breeding treatment of mares with homologus plasma and antibiotics may be improved (Pascoe, 1995) or not improved (Mattos et al., 1997) their pregnancy rates.

Greater pregnancy rates were obtained in both barren and lactating mares after their post breeding treatment with homologus plasma and oxytocin. A more pronounced pregnancy rates were recorded in both barren and lactating mares received an intra-uterine infusion of leukocytes enriched homologus plasma. This may be due to the supplemental opsonins present in plasma may have allowed increased the opsonization of microorganisms by phagocytes already present in the uterus by those infused in the plasma (Mattos et al., 1999). Also, The presence of platelets in the infused plasma may have increased the phagocyt-

ic activity and inhibited leukocyte death by apoptosis (Zalavary et al., 1996 and Andonegui et al., 1997). Moreover, the better pregnancy rates may be attributed to the synergistic effect of the homologus plasma infusion added opsonizing factors (complement factors and immunoglobulins) to the uterine lumen promoting uterine contraction and elimination of contaminating materials, in addition to the fresh blood neutrophils allowed optimal phagocytosis, possibly enhanced the former step (Mattos et al., 1997).

The infusion of antibiotics with leukocytes enriched homologus plasma decrease pregnancy rates in both barren and lactating mares. In this respect the use of plasma with antibiotics did not improve pregnancy rate (Mattos et al., 1997). Meanwhile, the injection of oxytocin with leukocytes enriched homologus plasma improve the pregnancy rates in both barren and lactating mares. This improvement was more obvious in lactating females. This finding came in accordance with those of Mattos et al. (1999) in barren and lactating mares susceptible to endometritis. They also reported that the administration of oxytocin after breeding may be involved in improving the uterine contractility and eliminating the post breeding infection.

In a conclusion, the infusion of antibiotics with leukocytes enriched homologus plasma decrease pregnancy rates in both barren and lactating

mares. Meanwhile the injection of oxytocin with infusion of leukocytes enriched homologus plasma improve the pregnancy rates in both barren and lactating mares. This improvement was more obvious in lactating ones.

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