EVALUATION OF THE SUPEROVULATORY EFFECT OF PREGNANT MARE'S SERUM GONADOTROPIN (PMSG) AND FOLLICLE STIMULATING HORMONE (FSH) IN THE NON-BREEDING SEASON OF RIVER BUFFALOES OF THE MIANKALEH PENINSULA, NORTHERN IRAN

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ABSTRACT

Ten primiparous river buffaloes of the Miankaleh peninsula, northern Iran in the non-breeding season from the native herds were selected. The buffaloes were divided into two groups (n=5) and were injected intramuscularly with gonadotropin releasing hormone (GnRH) and a controlled internal drug-release device (CIDR) inserted on the first day. Ten days later, administration of follicle stimulating hormone (FSH) in the first group and pregnant mare's serum gonadotropin (PMSG) in the second group based on determined protocols were done. Concurrent to prostaglandin administration, CIDRs were removed and followed by estrus detection. Artificial insemination was done twice a day in both groups. Ovarian activity was assayed by ultrasonography on the first and fifth day after the start of the superovulation program. Buffaloes in which no oocyte/embryo was recovered by uterine flushing were considered non responsive to the embryo collection and were not injected with $PGF_{2\alpha}$. Fifty days after insemination, pregnancy detection was performed by rectal ultrasonography. The mean (±standard deviation, SD) of follicles diameter on right ovaries in the first day in the FSH group (9.5±1.8 mm) was less than in the PMSG group (11.1±1.1 mm, P=0.006). The mean (±SD) number of follicles on both ovaries in the fifth day in the FSH group (7.4±0.8) was significantly greater than that in the PMSG group (5.0±2.0; P=0.032). However, no oocyte/embryo was recovered in either group. Pregnancy detection 50 days after insemination showed that one cow of the FSH group and four cows of the PMSG group were pregnant. In conclusion, the PMSG and the FSH regimes induced follicular growth, but embryo recovery was poor during the non-breeding season in river buffaloes of the Miankaleh peninsula.

Keywords: pregnant mare's serum gonadotropin, follicle stimulating hormone, superovulation, non-breeding season, river buffalo

INTRODUCTION

In cattle, superovulation and embryo transfer (ET) have become established procedures for achieving genetic improvement over the past decades. However, despite the fact that the first embryo transfer in buffalo was performed 30 years ago (Drost *et al.*, 1983), the utilization of

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reproductive biotechnologies in buffalo species is still limited to experimental trials (Gasparrini, 2002; Singh *et al.*, 2009; Neglia *et al.*, 2010). Water buffaloes are notorious for their poor superovulatory responses, as commonly reported (Manik *et al.*, 1998; Techakumphu *et al.*, 2001). The ovarian response of buffaloes to superovulatory treatment has been less than one third of that reported in cattle (Carvalho *et al.*, 2002; Drost, 2007), and in those that respond, only 2-4 ovulations are induced, producing only 0-2 transferable embryos (Anwar and Ullah, 1998). The reasons for low response to superovulatory treatments in buffalo are various (Misra and Tyagi, 2007). It has been reported that the number of primordial follicles in river (Danell, 1987) and swamp (Ty *et al.*, 1994) buffaloes (30% and 20%, respectively) are lower than that described in cattle (Erickson, 1966). However, different superovulation schedules resulted in the growth of high numbers of ovulatory follicles and relatively high ovulation rates (50-70%), although embryo/ova recovery rates (13-35%) were very low (Baruselli *et al.*, 1999; Carvalho *et al.*, 2002) in the breeding season.

During the non-breeding season, September to January, reproductive function of buffaloes is reduced by increasing day length (Perera, 2011). Season affects the reproductive process of buffaloes directly through environmental temperature and photoperiod and indirectly through the quality and quantity of feed, incidence of disease and management practices (Ahmad and Noakes, 2009). Several reports suggest a lower follicular population in buffalo ovaries (Madan, 1990; Totey *et al.*, 1991). It is essential to know the number of follicles recruited in buffaloes during a superovulation program. The objective of the present study was to compare the superovulatory effect and embryo recovery rate of follicle stimulating hormone (FSH) and pregnant mare's serum gonadotropin (PMSG) in river buffaloes of the Miankaleh peninsula, northern Iran during the non-breeding season.

MATERIALS AND METHODS

The Committee for Animal Experiments of Tehran University approved the experimental protocol. Ten primiparous river buffalo were selected during January and February, the early non-breeding season, from the native herds of Miankaleh peninsula (latitude of 50° 36′ N and longitude 53° 17′ E, 21-26 m below sea level), southeast of the Caspian Sea and northern Iran. The Miankaleh peninsula is located in the vicinity of the sea shore and is bush land commonly with pomegranates as the dominant vegetation. In the winter greens are scarce so the available nutrition is very weak and poor. The buffaloes of the Miankaleh peninsula graze freely on bush lands with no manual feeding. The buffaloes were milked twice daily.

Buffaloes were injected with 100 µg i.m. gonadotropin releasing hormone (GnRH, Gonabred, Parnell Laboratories, Australia). A controlled internal drug-release device (CIDR, 1.9 g progesterone, InterAg, Hamilton, New Zealand) was inserted on the first day. Ten days later, the buffaloes were randomly assigned to two groups (n=5). In the first group, follicle stimulating hormone (FSH, Folltropin-V, Bioniche Animal Health, Canada) was given i.m. twice a day (8:00 and 18:00 h) in divided doses (400 mg; 65×65, 55×55, 45×45, 35×35) and in the second group, pregnant mare's serum gonadotropin (PMSG, Folligon, Intervet, Canada) was injected 3000 IU i.m. once. Two days later, concurrent to a 250 µg i.m. prostaglandin ($PGF_{2\alpha}$, Estroplan, Parnell Laboratories, Australia) injection, the CIDR was

removed. Estrus was detected every four hours in the buffalo cows using a teaser buffalo bull. Artificial insemination was done twice a day (at a 12 h interval) in both groups. Ovarian follicular status was monitored using a real-time B-mode ultrasound scanner equipped with a 7.5 MHz linear-array transducer (500 V, ami, Canada) on the first and fifth day after the start of the superovulation program. The number and diameter of anovulatory follicles were recorded.

Six days after the first insemination, uterine flushing with Dulbecco's phosphate buffered saline (Sigma, St. Louis, MO, USA), supplemented with 1% fetal calf serum (Sigma, St. Louis, MO, USA), was carried out: each uterine horn was washed with at least 500 ml of solution. Embryos were immediately searched for by filtration (Neglia *et al.*, 2003). Buffaloes from which no oocyte/embryo was recovered, were considered non responsive to the embryo collection and did not receive $PGF_{2\alpha}$. Fifty days after insemination, pregnancy detection was performed by rectal ultrasonography.

Mean ± standard deviation (SD) of the number and diameter of follicles on the first and fifth day after the beginning of the superovulation program were analyzed between two superovulatory schedules using the Mann-Whitney test. Means (±SD) of the numbers and diameters of follicles between the first and fifth day after the start of the superovulation program of each superovulatory schedule were analyzed by the Wilcoxon signed ranks test. (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). Values of $P<0.05$ were considered significant.

RESULTS

Two buffaloes in the FSH group showed signs of endometritis during estrus detection and artificial insemination. The effect of FSH and PMSG administration on ovaries on different days of the superovulation programs is demonstrated in Figure 1.

The mean (±SD) of follicle diameter in the right ovary of the PMSG group was significantly greater than that of the FSH group in the first day (P=0.006; Table 1). On the fifth day, the mean (±SD) of number of follicles on both ovaries in the FSH group was significantly greater than that of the PMSG group (P=0.032). Mean (±SD) follicle diameters in the right (P=0.001), the left (P=0.003), and both (P=0.004) ovaries of the FSH group significantly increased from the first to the fifth day post treatment. However, in the PMSG group, mean (±SD) follicle diameter significantly increased from the first to the fifth day post treatment only in the right (P=0.029) and this resulted a decrease in both (P=0.021) ovaries. Moreover, mean (±SD) number of follicles on the right (P=0.034) and both (P=0.039) ovaries in the FSH group significantly increased after 4 days.

No oocyte/embryo was recovered after survey of flushing medium in either the FSH or the PMSG groups. Pregnancy detection examination showed that one buffalo cow in the FSH group and four buffalo cows in the PMSG group were pregnant.

DISCUSSION

The follicle diameter in the right ovary of the PMSG group was greater than that of the FSH group on the first day of superovulation treatment. After four days, follicle diameters in both ovaries of the FSH group increased. However, in the PMSG group, follicle diameters increased only in the right

Table 1. Mean (± standard deviation) of number and diameter of follicles on first day and fifth day after the start of two superovulatory schedules using follicle stimulating hormone (FSH) and pregnant mare's serum gonadotropin (PMSG) during the non-breeding season in river buffaloes (n=5) of the Miankaleh peninsula, northern Iran.

Group and day	Follicle number			Follicle diameter (mm)		
	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total
FSH						
Day 1	2.2±1.3[a]	2.4±0.8	4.6±1.9[a]	9.5±1.8*,[a]	9.9±2.8[a]	9.7±2.3[a]
Day 5	4.0±0.7[b]	3.4±0.5	7.4±0.8*,[b]	14.0±2.5 [b]	13.2±2.2[b]	13.6±2.3[b]
PMSG						
Day 1	3.0±2.0	2.6±1.6	5.6±3.5	11.1±1.1*,[a]	9.6±1.5	10.4±1.5[a]
Day 5	2.8±1.0	2.2±1.3	5.0±2.0*	12.2±4.4[b]	12.8±2.5	12.5±3.6[b]

Stars show significant differences in each column between two superovulatory schedules ($P<0.05$). Different superscript letters indicate significant differences in each column between the two days in each superovulatory schedule ($P<0.05$).

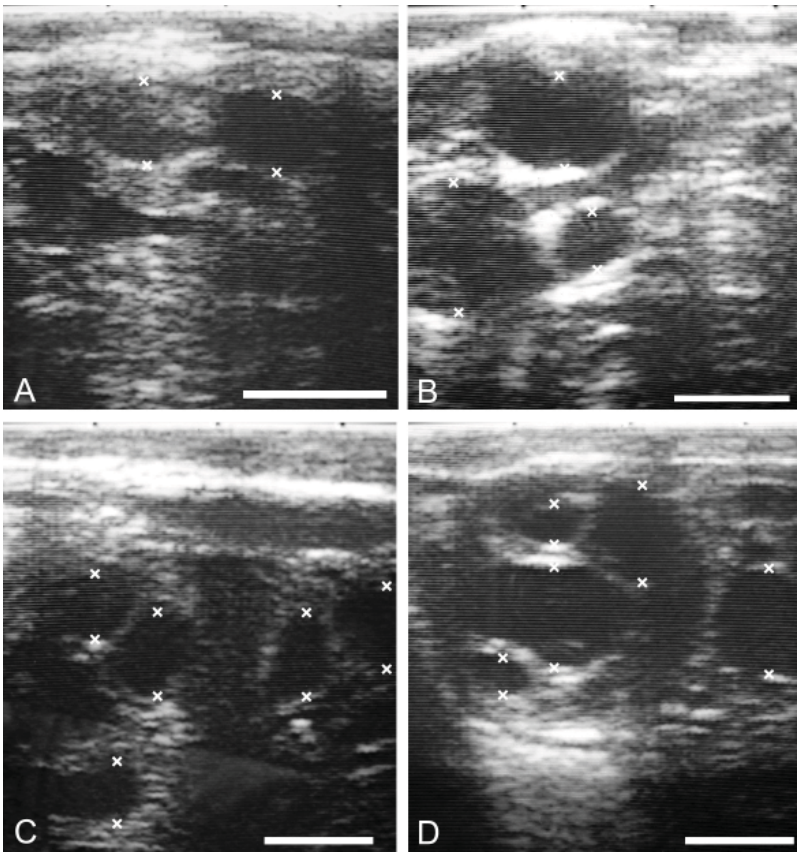

Figure 1. Sonograms (7.5 MHz) demonstrating the superovulatiory response of ovaries of Miankalleh buffaloes to the PMSG (A and B) and FSH (C and D) administration (Day 5). Crosses indicate follicle or corpus luteom diameters. Line indices are 10 mm.

ovaries. Furthermore, since number of follicles on both ovaries in the FSH group increased, on the fifth day, the number of follicles on both ovaries in the FSH group was greater than that in the PMSG group. Conversely with our findings in out of reproductive season, Karaivanov (1986) reported no substantial difference in the ovarian response had been established under PMSG and FSH regimens, but the percentage of the non-ovulating follicles following the PMSG treatment was considerably higher than that following FSH treatment in the spring and summer. The percentage of the non-ovulating follicles after FSH stimulation established by Karaivanov (1986) in the summer as well as in the spring experiments (15.2 and 7.7, respectively) was lower than that reported. Patel *et al.* (2009) showed that in Toda buffaloes, late ovulation and a lower number of recruited follicles during superovulation may be the reason for low superovulation response. In the present study, recruitment of follicles in PMSG treated ovaries in out of reproductive season buffaloes was less than in FSH treated ovaries.

In the present study, no oocyte/embryo was recovered after survey of the flushing medium in either the FSH or the PMSG groups. Since no embryo was recovered, PG treatment was not done and buffaloes were left to become pregnant. One buffalo cow in the FSH group and four buffalo cows in the PMSG group became pregnant. Chantaraprateep *et al.* (1988) showed that using FSH and PMSG for superovulation in swamp buffaloes, the recovery rate of embryos was 54.5% (6/11) while the recovery rates for normal embryos, degenerated and unfertilized eggs were 37.5% (6/16) and 25% (4/16), respectively. In buffalo species, the efficiency of superovulation treatment is still poor and 1.8-2.5 embryos/donor have been reported by several authors (Misra and Tyagi, 2007). In particular, according to Zicarelli (1997) the low number of embryos is mainly due to the low percentage of responsive subjects, which is around 55%. Zambrano-Varón and BonDurant (2007) showed failure in ovulation after the superovulation treatment with FSH in water buffalo during the non-breeding season, although the follicular recruitment and further development of ovulatory follicles was evident. Therefore, it has been supposed that the transport of ova and embryos within the oviduct may be compromised (Carvalho *et al.*, 2002). The relatively high concentration of circulating estradiol during superovulation and embryo transfer protocols in buffaloes (Beg *et al.*, 1997) may negatively affect the action of fimbriae and the recruitment of oocytes (Misra *et al.*, 1998). Moreover, Patel *et al.* (2010) observed that the total embryo recovery and viable embryo recovery were lower in buffalos with small corpora lutea . Other possible explanations that have been suggested for poor embryo recovery rates in buffalo following standard superovulation protocols include the smaller pool of primary oocytes available for recruitment, relative to bovine oocyte pools (Danell, 1987). However, given that even a smaller pool still contains tens of thousands of oocytes, this explanation by itself seems insufficient.

In conclusion, Although the PMSG and FSH regimes induced follicular growth and embryo recovery was poor, superovulation treatment with FSH caused a greater number of follicles but less pregnancy than treatment with PMSG during the non-breeding season in river buffaloes of the Miankaleh peninsula.

ACKNOWLEDGEMENTS

The authors thank Mr. J. Rahani

(Correspondent of Buffalo Genetic Improvement Project, Jihad e Keshavarzi Organization, Mazandaran, Iran) and the staff of the Miankaleh Buffalo Institute for their kind cooperation. This study was financially supported by a grant of the Babol Branch, Islamic Azad University.

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