SUPEROVULATORY RESPONSE AND EMBRYO RECOVERY IN SURTI BUFFALOES USING FSH AND DIFFERENT DOSES OF PMSG

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ABSTRACT

The experiment was conducted to study superovulatory response and embryo recovery rate in 18 Surti buffaloes of 2-6 parity during breeding season (August to February) using FSH and two doses of PMSG. Animals were divided into three groups. Group I (n=6) and Group II (n=6) donors were superovulated with single i/m dose of 2500 and 3000 IU PMSG, respectively, on day 10 of the estrous cycle, whereas Group III (n=6) donors were treated intramuscularly with 400 mg FSH per donor in 8 equal doses 12 hrs apart. Luteolysis was carried out on day 12 using $PGF_{2\alpha}$. All the animals were bred at superovulatory estrus. Almost all the animals responded to superovulation. Flushing was performed on day 6 after superestrus. The mean number of ovulations and embryo recovery rates in three groups (I, II and III) were 2.67, 3.50, 4.67 and 0.00, 0.17, 0.17, respectively. The mean numbers of unovulated follicles were 1.67, 2.17 and 1.00, respectively, in the three groups.

Key words: Buffalo, Superovlation, FSH, PMSG.

India is the world's largest milk producer with 104.9 million tonnes milk production per year. Milk production in India is growing at 4% per year, and at present India contributes 15% of the total global milk production. Out of this, 55% is contributed by buffalo (Ramteke, M., 2009). However, only 0.1 per cent of those milking buffaloes are capable of producing 3500-4000 liters of milk in 305 days of lactation (Taneja *et al.*, 1995). Initial reviews on the embryo transfer in buffaloes suggested low ovulation rate, low fertility, low embryo recovery and poor conception rate (Jainudeen, 1989) when the average number of embryos per donor was 1.16, transferable embryos 0.51, and the pregnancy rate 14 per cent (Kamonpatana, 1990). However, subsequent work reported improved results and viable embryo recovery up to 3.5 (Rao *et al.*, 1994) and 2.9 (Misra *et al.*, 1999) in farm and field conditions, respectively. In view of above facts, the present study was carried out to observe the superovulatory response and embryo recovery in Surti buffaloes using FSH and different doses of PMSG.

Eighteen donor buffaloes were selected from amongst the highest milk producers. They were in the age group of 6 to 14 years and of 2 to 6 lactations. The experiment was carried out during August to February, 2008. Two buffaloes in each group were previously used for similar superovulatory regimes. All the experimental animals were healthy and were kept under standard conditions of feeding and management. They were subdivided into group- I, II & III, having six buffaloes in each group.

Superovulatory treatment in donor animals was initiated on day 10 of the estrous cycle (day 0 = onset of estrus). Buffaloes of Group-I and II (n=6 each) were superovulated with PMSG (Folligon®, Intervet India Pvt. Ltd.) @ 2500 and 3000 IU/animal in single dose, respectively, whereas buffaloes of Group-III were superovulated with FSH (Follitropin®-V, Bioniche, Canada) @ 400 mg/animal as total dose given i/m at 12 hrs intervals in 8 equal divided doses for four days. The superovulatory estrus in donor animals was induced by i/m administration of 25 mg $PGF_{2\alpha}$ given at 48 hrs of

superovulatory treatment, i.e. on day 12 morning in all the groups. Donor animals were observed for signs of estrus every morning (6-8 AM) and evening (6-8 PM) starting 24 hrs after injection of $PGF_{2\alpha}$. At superovulatory estrus, donor buffaloes were bred three times at 12 hrs intervals by AI using fresh liquid semen. All the buffaloes of group I, II and III were injected with hCG (Chorulon®, Intervet India Pvt. Ltd., India) @ 1500 IU/ animal on the day of superovulatory estrus to improve ovulation rate and transport of embryos into the uterus. The number of corpora lutea (CL) were counted per rectum just prior to non-surgical embryo collection on day 6 of superovulatory estrus. Flushing was carries out using the Rusch catheter (16/ 18 Gauge, Rusch) and Dulbecco's phosphate buffered saline (DPBS, Sigma Aldrich, USA) as flushing medium. The recovered embryos were assessed under stereo zoom microscope. Data obtained were analyzed for mean, standard error in mean and percentage (Snedecor and Cochran, 1994).

All the six buffaloes treated with 2500 IU PMSG (Group-I) once i/m responded (> 2 CL) to superovulation. The number of CLs varied from 2 to 4 with a mean of 2.67 per donor. Out of the 16 total number of CLs, 9 (56.25%) were found on the left ovary and 7 (43.75%) on the right. Moreover, total 10 (1.67/donor) unovulated follicles were also palpated, of which 5 each (50%) were on right and left ovaries. However, no embryo could be recovered on flushing from this group. These findings are in agreement with the report of Singh and Madan (1989), who reported 2.50 mean number of ovulations with 2500 IU PMSG in Murrah buffaloes. Similarly, Hamam *et al.* (1992) observed 2.86 corpora lutea per donor with 1000 IU PMSG.

Almost all donor buffaloes treated with 3000 IU PMSG once i/m had 1 to 6 CLs at the time of embryo recovery. The mean number of CLs was found to be 3.50 per donor. Out of the total 21 CLs, 11(52.38%) were found on the left ovary and 10 (47.62%) on the right ovary. Moreover, 13 (2.17/donor) unovulated follicles were found, of which 6 (46.15%) were on the left ovary and 7 (53.85%) on the right ovary. Only one (0.17/donor) viable embryo was recovered from the donor No. 1 of this group. The quality of the embryo collected was good, of B grade, but upon transfer to the synchronized recipient did not result into pregnancy. The response to superovulatory treatment with 3000 IU dose was lower than that (6.2 CLs) reported by Karaivanov *et al.* (1990). Hamam *et al.* (1992) also observed higher superovulatory response (7.25 CLs/ donor). They observed better mean ovulations of 5.87 and 6.13 with 2000 IU and 4000 IU PMSG than the present study. Schallenberger *et al.* (1990) reported higher (2.00) mean embryo recovery rate as compared to the present result (0.17).

All the donors treated with 400 mg FSH over 4 days responded and had 3 to 7 CLs with a mean of 4.67. Out of the total 28CLs, 15 (53.57%) were on the left and 13 (46.43%) on the right ovary. Further, total 6 (1.00/donor) unovulated follicles were found equally distributed (50%) on the left and right ovaries. Out of six donors, only one degenerated embryo, D grade, was recovered, which was rejected. In this group, the mean ovulation rate (4.67) was found to be the highest while the mean non-ovulatory follicles (1.00) was the lowest among all the three groups. Songsasen *et al.* (1999) reported similar mean ovulations (4.3 and 4.13), but the embryo recovery rate (2.3) was higher than the present one (0.17). The mean number of ovulations and embryo recovery rate recorded in the present study are in accordance with Sahatpure *et al.* (2005) who found 4.13 and 3.46 ovulations and 0.13 and 0.31 embryo recovery rate in Nagpuri buffaloes treated with 450 and 500mg FSH respectively..

Comparatively greater response to multiple ovulation treatment (3.50 ovulations) and higher embryo recovery (0.17) were observed in buffaloes of Group II than in buffaloes of Group I (2.67 ovulations and 0.00 embryos). Similarly, higher response to multiple ovulation treatment (4.67 ovulations) was observed in buffaloes of Group III than in Group II (3.50 ovulations), whereas, mean embryo recovery rate was similar (0.17) in two groups. Further, the response to multiple ovulation treatment and embryo recovery were higher in buffaloes of Group III than in buffaloes of Group I. In spite of the presence of superovulatory response in donors the lower embryo recovery rate in this study may be due to several factors, such as breed, nutrition,

season, dominant follicle, repeated superovulation, effect of semen, interval between $PGF_{2\alpha}$ treatment and superovulatory estrus, estrogen: progesterone ratio etc. and mainly competence of technical person in recovery of embryo by using proper technique is important.

In conclusion, the results suggest that FSH had better superovulatory response in donors than both the doses of PMSG. There was no significant difference in the superovulatory response of donors superovulated with two doses of PMSG. It was observed that the numbers of unovulated follicles on the ovary were more with PMSG treatment than FSH. Higher numbers of unovulated follicles (i.e. high estradiol) affected the embryo transport within fallopian tube that indirectly affected the overall embryo recovery rate.

ACKNOWLEDGEMENT

We are grateful to Dean/Principal of the College for the facilities provided for this work. The financial support received from ICAR, New Delhi in the form of JRF to first author is also gratefully acknowledged.

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