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Effect of repeated dual superovulation using FSH and PMSG+FSH on ovulatory response in Murrah buffalo

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Abstract

The current experiment was conducted to determine the ovulatory response of Murrah buffaloes subjected to repeated therapeutic doses of FSH and PMSG+FSH in two successive periods. Three elite, multiparous, Murrah buffaloes, 80 to 120 days postpartum, were included in multiple-ovulation embryo transfer (MOET) schedule. The estrus was synchronized with one injection of prostaglandin. Superovulatory treatment was started from day 10 of induced estrus after ablation of dominant follicle of over 10 mm diameter, using 5.0 MHz convex-array intravaginal transducer using a B-mode scanner with aspiration assembly. Study was undertaken in two consecutive periods so each animal was treated with PMSG+FSH in first superovulation period and FSH on the Second period after short interval period (14 day cycle). For FSH treated, Folltropin was administered in a twice-daily descending dose schedule (0–5, 5–4, 4–3, 3–2, 2–2 ml; 20 mg/ml, total dose 600 mg of FSH. For PMSG+FSH treated, an injection of PMSG (1000 IU) was given in the evening of day 10 followed by dose 400 mg FSH and schedule as of FSH treated group from the evening of day 11. All donors received prostaglandin injection on day 13 in morning and evening. Donors were inseminated with frozen thawed semen of proven bull on Day 15 a.m. and p.m. and Day 16 a.m. Nonsurgical embryo collection was carried out on Day 5 post-insemination. There were no differences between the mean numbers of follicles in the same buffaloes treated with PMSG+FSH or FSH at any time throughout

the experimental period. At AI, similar numbers of corpora lutea were observed for PMSG+FSH and FSH. On the day of flushing, almost similar mean numbers of corpora lutea counted in PMSG+FSH and FSH treated buffaloes In conclusion, ovarian response in Murrah buffaloes treated with PMSG+FSH or FSH for two superovulatory periods were nonsignificantly different.

***Keywords**:* Murrah buffalo, Repeated superovulation, FSH, PMSG

Introduction

Superovulation is required in embryo transfer program to expedite the propagation of animals with high genetic merit for desirable trait. However, large variations in the number of ovulations and/or embryos that result from this procedure and high cost of the superovulatory treatment remain major obstacles in such program. A considerable improvement has been reported in MOET (multiple ovulation and embryo transfer) technology in cattle but in buffaloes, superovulatory response is still poor (Misra, 1991).

Two different types of gonadotrophins have been used to induce superovulation in the buffalo; gonadotrophins (FSH) extracted from the pituitaries of porcine or other domestic species, or pregnant mare serum gonadotrophin (PMSG) collected from the sera of pregnant mare (Murphy *et al*., 1984; Alkemade *et al*., 1993).

Protocols for the superovulation using either PMSG or FSH or PMSG+FSH have been established in buffaloes (Karaivanov, 1986; Patel *et al*., 2010; Abd-Allah *et al*., 2013 a).

Superovulatory response in buffaloes is quite variable and differed with gonadotropins used, batch of gonadotropin, duration of treatment, total dose of gonadotropin, additional hormones in the superovulatory scheme, ovarian status, season, age and the stage of the cycle at which administration of exogenous hormones is initiated (Rahil *et al*., 1989; Taneja *et al*., 1995).

Conventional regimens for ovarian superovulation involve gonadotropin treatment between Day 8 and 12 after estrus, roughly coincident with the emergence of the second follicular wave (Lindsell *et al*., 1986). FSH is widely used for superovulation in buffaloes. Initial studies with PMSG resulted in a poor response of donor while FSH from porcine was the most commonly used hormone for superovulation in buffaloes (Misra *et al.,* 1993). However, FSH has the disadvantage of higher cost, short half-life and need for multiple injections, though yields better superovulatory response (Monniaux *et al*., 1983). In contrast, PMSG is cheaper with long half life, require single dose but gives poor superovulatory response (Moor *et al*., 1984; Murphy and Martinuk, 1991).

Abd-Allah *et al.* (2013 a) reported that superovulatory response in Murrah buffaloes treated with FSH or PMSG+FSH was nonsignificant different.

Repeated superovulation with FSH or PMSG has been found to cause reduction on ovualtory response in sheep (Paulsson, 1962) and Cows (Turman and Watman, 1978), In contrast, other studies have not shown a reduction on ovualtory response in Cows (Jauinudeen *et al*., 1966) and sheep (Gordon, 1975). However, such effect in buffaloes has not been recognized during previous studies. Moreover, there is no information available on repeated superovulation in buffaloes so our study was conduct to determine the superovulatory response of Murrah buffaloes to repeated doses of FSH and PMSG+FSH in two consecutive times.

Materials and Methods

Location, The experimental animals and management

The study was carried out at the Central Institute for Research on Buffaloes, Hisar, Haryana, India during May 2012 in the Murrah herd, managed under semi-intensive system of management. During day time, animals were allowed to graze on natural pastures of the farm land and fed concentrate ration as per the milk yield.

Superovulation

The estrum of three multiparous elite Murrah buffaloes were synchronized with one injection of prostaglandin, (Lutalyse - 5 ml i/m; Hoechst, India). Animals found in heat (day 0) were not inseminated and programmed for superovulation from day 10 of cycle. On day 10, largest follicle (>10 mm) present on ovary was ablated with 7.5-MHz micro convex array transvaginal transducer equipped ultrasound (Esaote, Aquila Vet) guided aspiration needle connected to a regulated vacuum pump (K-MAR-5100, Cook IVF Co. Australia).

Study was undertaken in two consecutive periods with short interval period (14 day cycle) so each animal was treated with PMSG+FSH in first superovulation period (1st SOV) and FSH on the Second period (2nd SOV) after short interval period (14 day cycle).

For 1st SOV, the superovulatoion treatment started on the day of 12 of consecutive estrous cycle (estrous cycle = 0 day), the same animals was given PMSG 1000 I.U. on day 10 followed by 400 mg FSH in tapering manner from evening of day 11 for a period of 3.5 days.

For 2nd SOV, each animal was given FSH from the evening of day 10 of cycle. In brief, tapering dose of 600 mg FSH was given to donors for a period of 4.5 days.

In both periods (1st and 2nd SOV), Luteolysis was induced with prostaglandin given in the morning and evening of day 13 of cycle. Donors were inseminated with frozen thawed semen at 48, 60 and 72 hrs after the first prostaglandin injection. An injection of GnRH (10 µg Buserelin acetate, Receptal) was given at the time of 1st insemination in all buffaloes. Non-surgical embryo collection was attempted on day 5 post-breeding using D-PBS supplemented with 0.1% BSA. Prostaglandin injection was again given on day 10 of superovulated cycle to cause lysis of the multiple corpora lutea.

Ultrasound examinations of the ovaries were done with a B mode ultrasound scanner (Toshiba, SSA 220, Just Vision) equipped with an intraoperative 7.0 MHz microconvex transducer to record the ovarian response during the superovulation programe (Abd-Allah *et al*., 2013 b). Ovarian follicular response was analyzed by comparing different sized follicles, i.e. small (4–6 mm), medium (6–9 mm) and large (10 mm and above) on Day 10, day of superovulatory induced estrus and the day of embryo recovery. Superovulatory response was confirmed by determining number of corpora lutea on the day of embryo recovery with transrectal ultrasonography. The study was conducted during the low-breeding season for buffalo, in the months of peak summer season in semi-arid region of north India.

Statistical Analysis

The means and standard errors for all variables were calculated and are presented. All data was statistically analyzed by chi-square and Student "t" test.

Results and Discussion

For the two superovualtory periods (1st and 2nd SOV), the superovulatory responses using PMSG+FSH or FSH in three Murrah buffaloes were 100 percent (3/3). These results confirms the previous findings of the same author (Abd-Allah *et al.* 2013 a) who found 100% of superovulatory response in murrah buffaloes treated either PMSG+FSH or FSH but the differences between these two studies were the present study used the same animal in two successive periods and the previous study used different animals.

The superovulatory response in these buffaloes was evaluated by number of graafian follicles formed on day of estrous (after FSH or PMSG+FSH) and number of corpora lutea formed on Day 7 after the oestrus (Day of Flushing),

using real time, transrectal ultrasonography. Data pertaining to ovarian response are summarized in Table 1.

Table 1. Ovarian response of murrah buffaloes repeatedly superovulated for two consecutive periods with PMSG+FSH and FSH (No., Mean±SE & %).

	1st SOV	2nd SOV
No. of buffaloes programmed	3	3
No. of buffaloes responded	3 (100%)	3 (100%)
Day of Follicle Ablation		
Total No. of Follicles	10 (3.3±0.29)	9 (3±0.50)
No. of small follicles (3-7 mm Ø)	7 (70%)	6 (66.7%)
No. of medium follicles (8-10 mm Ø)	0 (0.0%)	0 (0.0%)
No. of large follicles (>10 mm Ø)	3 (30%)	3 (33.3%)
First day after FSH or PMSG+FSH		
Total No. of Follicles	15 (5.0±0.0)	13 (4.3±0.3)
No. of small follicles (3-7 mm Ø)	15 (100%)	13 (100%)
No. of medium follicles (8-10 mm Ø)	0 (0.0%)	0 (0.0%)
No. of large follicles (>10 mm Ø)	0 (0.0%)	0 (0.0%)
Second day after FSH or PMSG+FSH		
Total No. of Follicles	37 (12.3±0.3)	34 (11.3±0.3)
No. of small follicles (3-7 mm Ø)	26 (70.3%)	25 (73.5%)
No. of medium follicles (8-10 mm Ø)	8 (21.6%)	7 (20.6%)
No. of large follicles (>10 mm Ø)	3 (8.1%)	2 (5.9%)
Third day after FSH or PMSG+FSH		
Total No. of Follicles	42 (14±0.58)	39 (13±0.50)
No. of small follicles (3-7 mm Ø)	1 (2.4%)	1 (2.5%)
No. of medium follicles (8-10 mm Ø)	19 (45.2%)	18 (46.2%)
No. of large follicles (>10 mm Ø)	22 (52.4%)	20 (51.3%)
Day of Insemination		
No. of large follicles (>10 mm Ø)	40 (13.3±0.2)	38 (12.6±0.2)
No. of corpora lutea	1 (0.3±0.3)	1 (0.3±0.3)
First day after GnRH treatment		
No. of large follicles (>10 mm Ø)	34 (11.3±0.3)	31 (10.3±0.3)
No. of corpora lutea	1 (0.3±0.3)	1(0.3±0.3)

Day of Flushing		
No. of follicles (>10 mm Ø)	28 (9.3±0.3)	25 (8.3±0.3)
No. of Corpora Lutea	8 (2.6±0.3)	7 (2.3±0.3)

No significant differences were detected among two SOV.

There were no differences between the mean numbers of follicles in the same buffaloes treated with FSH or PMSG+FSH in 1st and 2nd SOV at any time throughout the experimental period. At AI, similar numbers of corpora lutea were observed for PMSG and FSH, respectively. On the day of embryo recovery, almost similar mean numbers of corpora lutea counted in 1st and 2nd SOV were 2.6±0.3 and 2.3±0.3, respectively.

In the present study, there was no decrease in the repeated superovulation in the two successive periods. This is agreement with Juainuudeen *et al.* (1966) who repeatedly superovulated cows for four successive periods and found that the ovarian response did not decrease on the second period.

This may be attributed to no immunological refractoriness developed and also short time interval between two successive superovulation periods.

In conclusion, ovarian response in Murrah buffaloes treated with FSH and PMSG+FSH for superovulation were nonsignificantly different. These results indicate that repeated superovulation with FSH and PMSG+FSH did not lead to decreased response throughout the two superovulatory periods. Further Investigations should include multiple superovulation for long periods.

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