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Superovulatory Response Following Transvaginal Follicle Ablation in Murrah Buffalo: Effect of FSH or PMSG+FSH

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Abstract

The current experiment was conducted to reduce the cost of superovulation by reducing the dose of FSH by partial replacement with PMSG. Five 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 groups. For FSH treated group, 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 group, 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 buffaloes treated with FSH or PMSG+FSH 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 FSH and PMSG+FSH treated buffaloes were 5.5 ± 2.5 and 2.6 ± 0.33, respectively. A significant increase in number of anovulatory follicles in FSH and PMSG+FSH (10 ± 0.0; 9.3 ± 0.3, respectively) was observed, which may be the reason for lower total embryo recovery and viable embryo recovery. In conclusion, superovulatory response of PMSG+FSH was similar to FSH treated group and can be used to reduce the cost of superovulation in MOET program.

Keywords: Murrah buffalo, 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

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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 have been established in buffaloes (Karaivanov, 1986; Patel *et al*., 2010) and in cattle (Lindsell *et al*., 1986; Goulding *et al*., 1990).

Superovulatory response in cattle is quite variable and differed with gonadotropins used (Elsden *et al*., 1978), batch of gonadotropin (Newcomb *et al*., 1979), duration of treatment (Garcia *et al*., 1982), total dose of gonadotropin (Bellows *et al*., 1969), additional hormones in the superovulatory scheme (Savage *et al*., 1987), ovarian status, season, age and the stage of the cycle at which administration of exogenous hormones is initiated, and similar results are reported in buffaloes (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). In this experiment, we aimed to reduce the dose of FSH by partial replacement with PMSG at initial stage of superovulation so as to take additional advantage of its long half-life. Hence, an experiment was designed to compare the response of Murrah buffaloes to superovulation with FSH or PMSG+FSH and the possibility of reducing the cost of hormone in MOET program in buffalo.

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 five 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).

Superovulation was induced in each animal with FSH or a combination of PMSG+FSH from the evening of day 10 of cycle. In brief, tapering dose of 600 mg FSH were given to donors for a period of 4.5 days in one group whereas other group 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. 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

Table 1: Regimen for superovulation using FSH and PMSG+FSH

		Treatment	
Day	**Time**	**FSH**	**PMSG+FSH**
0	AM	Heat	Heat
10	AM	Follicle Ablation	Follicle Ablation
	PM	**FSH 5 ml**	**PMSG 5 ml**
11	AM	FSH 5 ml	-
	PM	FSH 4 ml	FSH 4 ml
12	AM	FSH 4 ml	FSH 4 ml
	PM	FSH 3 ml	FSH 3 ml
13	AM	FSH 3 ml + **PG** 5 ml	FSH 3 ml + **PG** 5 ml
	PM	FSH 2 ml + **PG** 5 ml	FSH 2 ml + **PG** 5ml
14	AM	FSH 2 ml	FSH 2 ml
	PM	FSH 2 ml	FSH 2 ml
15	AM	A.I + **GnRH** 2.5 ml	A.I + **GnRH** 2.5 ml
	PM	A.I	A.I
16	AM	A.I	A.I
20	AM	Embryo recovery	Embryo recovery

with 0.1% BSA. Prostaglandin injection was again given on day 10 of superovulated cycle to cause lysis of the multiple corpora lutea. The schedule of treatment is shown in Table 1.

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). 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 t test was applied for statistical analysis.

Results and Discussion

The superovulatory responses using either FSH or PMSG+FSH in five Murrah buffaloes were 100 percent (2/2 ; 3/3 respectively).

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 (Fig. 1). Data pertaining to ovarian response are summarized in Table 2.

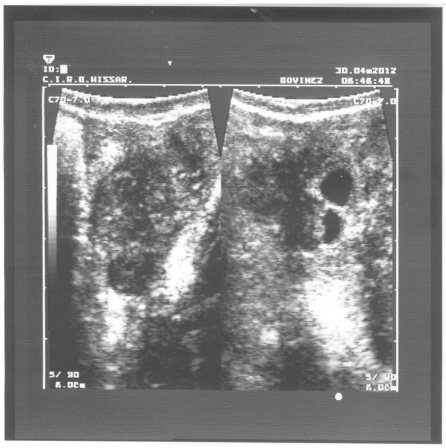

Fig. 1. Ultrasonogram of a superovulated Murrah Buffalo showing the corpora lutea on the ovaries on the day of embryo recovery. Two unovulated follicles are also visible on right ovary.

Table 2: Superovulatory response of FSH and PMSG+FSH treatments (No., Mean ±SE & %).

Characteristics	Treatments	
	PMSG+FSH	**FSH**
No. of buffaloes programmed	3	2
No. of buffaloes responded	3 (100 %)	2 (100 %)
Day of follicle ablation (OPU)		
Total No. of Follicles	10 (3.3±0.29)	11(5.5±0.5)
No. of small follicles (3-7 mm Ø)	7 (70%)	9 (81.8%)
No. of medium follicles (8-10 mm Ø)	0 (0%)	0 (0%)
No. of large follicles (>10 mm Ø)	3 (30%)	2 (18.2%)
First day after FSH or PMSG+FSH		
Total No. of Follicles	15 (5±0.0)	15 (7.5±1.5)
No. of small follicles (3-7 mm Ø)	15 (100%)	15 (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)	22 (11±1.0)
No. of small follicles (3-7 mm Ø)	26 (70%)	13 (59%)
No. of medium follicles (8-10 mm Ø)	8 (21%)	8 (36%)
No. of large follicles (>10 mm Ø)	3 (8%)	1 (4.5%)
Third day after FSH or PMSG+FSH		
Total No. of Follicles	42 (14±0.58)	31 (15.5±1.5)
No. of small follicles (3-7 mm Ø)	1 (2.3%)	2 (6.5%)
No. of medium follicles (8-10 mm Ø)	19 (45.2%)	13 (41.9%)
No. of large follicles (>10 mm Ø)	22 (52.3%)	16 (51.6%)
Day of Insemination		
No. of large follicles (>10 mm Ø)	40 (13.3±0.2)	22 (11±1.0)
No. of corpora lutea	1 (0.3±0.3)	1 (0.5±0.5)
First day after GnRH treatment		

No significant differences were detected among groups.

There were no differences between the mean numbers of follicles in buffaloes treated with FSH or PMSG+FSH 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 FSH and PMSG+FSH treated buffaloes were 5.5±2.5 and 2.6±0.33, respectively. A significant increase in number of anovulatory follicles in FSH and PMSG+FSH (10±0.0; 9.3±0.3,

respectively) was observed, which may be the reason for lower total embryo recovery and viable embryo recovery.

The optimal superovulatory response recorded in the present study could be due to prevention of atresia and enhanced selection of follicles. This is evidenced by the fact that number of small follicles decreased, whereas the medium and large sized follicles increased, following PMSG+FSH or FSH treatment. On the other hand, earlier studies in buffaloes (Taneja *et al*., 1995) and in cattle (Guilbault *et al*., 1991; Huhtinen *et al*., 1992) reported decreased superovulatory response when FSH treatment was initiated in the presence of dominant follicle which is suggested to have inhibitory effect on the other follicles of the wave.

The ovarian response of buffaloes to superstimulatory treatment has been less than one third of that reported in cattle. By using Folltropin or PMSG, only 50% to 55% of buffaloes respond and of those that respond, only two to four ovulations are induced, producing only one to two transferable embryos (Parnpai *et al*., 1985; Karaivanov, 1986; Vlakhov *et al*., 1986; Alexiev *et al*., 1988; Sharifuddin and Jainudeen, 1988; Singh *et al*., 1988; Misra *et al*., 1991; Kasiraj *et al*., 1992; Agarwal *et al*., 1996).

Endocrine studies have revealed that eCG-treated animals more frequently had abnormal LH and progesterone profiles than did the FSH treated cows (Mikel-Jenson *et al*., 1982; Greve *et al*., 1983). These were associated with reductions in both ovulation and embryo recovery rate (Callesen *et al*., 1986).

In conclusion, Superovulatory response in Murrah buffaloes treated with FSH or PMSG+FSH for superovulation were nonsignificantly different. These preliminary findings suggest that a small dose of PMSG (1000 IU) at the start of superovulatory treatment can be used as a replacement of FSH in reducing the cost of superovulation programe.

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