



Superovulatory treatment response and embryo recovery in mithun cows

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Mithun (*Bos frontalis*), a semi-domesticated rare bovine species considered to be descendents of wild gaur (*Bos gaurus*), is found mainly in the North Eastern Hill Regions of India. Lots of work is reported in cattle and buffaloes regarding superovulation and embryo recovery from India and abroad (Zbylut and Jaskowski 1999, Mishra and Dhanda 2003). However, very meagre amount of work have been taken up on superovulation in mithun. Since, the animal is free ranging and the area under forest as well as grazing land is gradually decreasing which is threatening to the existence of this rare species. Therefore, there is an urgent need of scientific intervention for proper management as well as conservation of this beautiful hilly animal. Hence, the present work was undertaken to study the superovulatory treatment response and embryo recovery rate in mithun cows.

Experimental animals: Healthy cyclic mithun cows (20) between second and fifth lactation were maintained under intensive managerial system. They were offered concentrate mixture @ 3.5 kg/day/animal along with 10–12 kg green grasses, adequate straw, tree leaves and drinking water *ad lib*. The experimental animals were randomly divided into groups A, B, C and D consisting of 5 mithun cows in each.

Superovulatory treatment: All the animals were treated with intra-vaginal progesterone device + PGF₂α analogue for synchronization of oestrus before superovulatory treatment. Animals of groups A, B, C and D were treated with 400 mg FSH (folotropin-V) 300 mg FSH, 400 mg FSH + 1500 IU hCG (chorulon) and 300 mg FSH + 1500 IU hCG, respectively. FSH was given in divided and reducing doses at 12 h intervals for four days starting from day 11 to day 14

of estrous cycle. Cloprostenol, a PGF₂α analogue was injected intramuscularly @ 0.5 mg/animal on day 13 of estrous cycle. hCG was injected @ 1500 IU intramuscularly 12 h post oestrus in each of the animals of groups C and D.

Detection of oestrus and mating: Estrus was observed at 4 h interval following PGF₂α treatment. A healthy mithun bull was used to detect oestrus along with visual observations for external signs. Acceptance of the bull by the cow was considered as onset of oestrus, while rejection of male by the female and/or disappearance of external signs were considered to be the cessation of oestrus. Cows in oestrus were allowed to mate naturally with healthy mithun bull having good libido for 3 occasions during oestrus period.

Flushing and collection of embryo: On day 7 of induced oestrus, all animals were examined per rectally for presence of corpora lutea on ovarian surface and were subjected to “flushing” for retrieval of embryos. The animals were kept on fasting for 24 h and water was withdrawn 12 h prior to flushing. Embryos were collected non-surgically following the procedure described by Karihaloo (1988). Embryos were searched out in 5–10 ml of flushed out media in 65 mm plastic petridishes with grids using a stereozoom microscope. Each

Table 1. Response and duration of oestrus in mithun cows following four different superovulatory treatments

Superovulatory treatment group	Oestrus response (%)	Interval from superovulatory treatment to onset of induced oestrus (h)			Duration of induced oestrus (h) (mean±SE)
		From PGF ₂ α (mean±SE)	From last folltropin-V injection (mean±SE)		
Gr. A	100.00	48.60±0.80	24.60±0.80		25.00±0.71
Gr. B	100.00	47.60±0.40	23.60±0.40		24.00±0.71
Gr. C	100.00	49.40±0.58	25.40±0.58		26.00±0.71
Gr. D	100.00	47.90±0.86	23.90±0.86		24.50±0.74
Overall	100.00	48.37±0.35	24.37±0.35		24.87±0.37

*Mean of 5 observations.

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Table 2. Number of palpable follicles and corpora lutea (CL) on day 7 after oestrus in mithun cows following 4 different superovulatory treatments

Superovulatory treatment group	Number of palpable follicle (mean $\pm$ SE)			Number of corpora lutea (mean $\pm$ SE)			Ovulation percentage		
	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total	Right ovary	Left ovary	Overall
Gr. A	1.00 $\pm$ 0.32 (0–2)	0.80 $\pm$ 0.20 (0–1)	1.80 ^{NS} $\pm$ 0.20	1.60 ^b $\pm$ 0.24 (1–2)	1.80 ^{ab} $\pm$ 0.20 (1–2)	3.40 ^b $\pm$ 0.24	61.54	69.23	65.39
Gr. B	0.60 $\pm$ 0.24 (0–1)	1.00 $\pm$ 0.32 (0–2)	1.60 ^{NS} $\pm$ 0.24	1.80 ^b $\pm$ 0.20 (1–2)	1.40 ^b $\pm$ 0.24 (1–2)	3.20 ^b $\pm$ 0.20	75.00	58.33	66.67
Gr. C	0.80 $\pm$ 0.20 (0–1)	0.40 $\pm$ 0.24 (0–1)	1.20 ^{NS} $\pm$ 0.37	2.80 ^a $\pm$ 0.37 (2–4)	2.40 ^a $\pm$ 0.24 (2–3)	5.20 ^a $\pm$ 0.20	77.77	85.71	81.74
Gr. D	0.60 $\pm$ 0.24 (0–1)	0.80 $\pm$ 0.37 (0–2)	1.40 ^{NS} $\pm$ 0.24	1.80 ^b $\pm$ 0.37 (1–3)	2.00 ^{ab} $\pm$ 0.32 (1–3)	3.80 ^b $\pm$ 0.49	75.00	71.43	73.22

*Mean of 5 observations; ^{NS} nonsignificant; ^{a, b} figures with different superscripts in a column differ significantly ($P < 0.05$, $P < 0.01$); figures in parentheses indicate range.

chamber of the petridish was thoroughly examined, and upon locating the embryo, zoomed to high power to view the embryo closely. Embryos were collected using a 10 μ l unopette fitted in one ml disposable syringe with an adopter.

Evaluation of embryo: The embryos were then washed 2 to 3 times using holding media in 35 mm plastic petridishes and transferred finally into another 35 mm plastic petridish containing 2 ml holding media and marked the dish with animal number and respective horn. The embryos in dishes were examined using inverted phase contrast microscope under $10 \times 10 - 20$ magnifications to determine their stages and grades, and marked the status of embryos on the lid of dish. Data obtained from the experiment was analysed statistically (Snedecor and Cochran 1989).

All the data related to superovulatory response and embryo recovery are given in Tables 1–3. All the experimental mithun cows (100%) under 4 different groups, viz. groups A, B, C and D exhibited oestrus behaviour. Our results are in agreement with Bhuyan *et al.* (2006) in cows following treatment with FSH. However, Mathur *et al.* (2006) reported that only 75% Frieswal cows were responded to superovulatory treatment.

The time interval from PGF₂ α injection to the onset of induced oestrus had an overall mean of 48.37 ± 0.35 h irrespective of groups (Table 1). Although, apparently higher value was recorded in group C followed by groups A, D and B. But the values did not differ significantly among different treatment groups.

An overall mean value of 24.37 ± 0.35 h of the time interval from last FSH injection to the onset of induced oestrus was recorded in the present experiment. Analysis of variance revealed no significant variation in respect of time interval from last treatment to onset of induced oestrus among different superovulatory treatment groups.

The intervals from PGF₂ α injection to onset of oestrus were 48.60 ± 0.80 , 47.60 ± 0.40 , 49.40 ± 0.58 and 47.90 ± 0.86 h respectively in cows of groups A, B, C and D. The values recorded among different treatment groups were statistically not significant. The present findings were in close conformity with the reports of Dutt and Kharche (2002) in cows. The

Table 3. Correlation co-efficient with the number of palpable corpora lutea (CL), embryo recovery and palpable follicles in mithun cows following four different superovulatory treatments

	Correlation co-efficient		
	Total corpora lutea	Total embryo recovered	Total palpable follicle
Total corpora lutea	1	0.609*	–0.340
	.	0.004	0.143
	20	20	20
Total embryo recovered	0.609**	1	–0.079
	0.004	.	0.741
	20	20	20
Total palpable follicle	–0.340	–0.079	1
	0.143	0.741	.
	20	20	20

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

intervals recorded in the present study were longer than reported by Mustapha *et al.* (2008) in cattle but shorter than the reports of Bhuyan *et al.* (2006). The variation of time interval might depends on the presence of large follicle at the time of prostaglandin injection (Mughal *et al.* 1998).

The overall mean value of duration of induced oestrus was 24.87 ± 0.37 h. The duration of oestrus in mithun cows did not differ significantly among different superovulatory treatment groups. The duration of induced oestrus was apparently higher in group C followed by groups A, D and B. The present findings are in agreement with the earlier reports of Munnich and Busch (1992). Variations in duration of oestrus might be influenced by the method of treatment, dose, age, breed, season, cyclicity and stages of reproduction (Mughal *et al.* 1998).

Analysis of variance revealed no significant variation in respect of the numbers of palpable follicles between the right and left ovaries and also among different superovulatory treatment groups. The total mean numbers of palpable follicles both on right and left ovaries were recorded as

1.80±0.20, 1.60±0.24, 1.20±0.37 and 1.40±0.24, respectively for groups A, B, C and D in superovulated mithun cows (Table 2). No significant differences were obtained among different treated groups of mithun cows in respect of total numbers of palpable follicles.

The number of corpus luteum was significantly ($P<0.05$, $P<0.01$) higher on right ovary and on total (right and left) basis in mithun cows under group C compared to that of other treated groups. However, on the left ovary, the value recorded significantly ($P<0.05$) higher in group C compared to group B treated animals but did not differ significantly between groups A and D (Table 2).

The total mean numbers of corpora lutea palpated on day 7 post oestrus was 3.40±0.24, 3.20±0.20, 5.20±0.20 and 3.80±0.49, respectively, in mithun cows of groups A, B, C and D. Number of corpus luteum was significantly ($P<0.05$) higher in mithun cows under group C compared to that of other treatment groups. This might be due to increased growth and maturation of follicles by higher doses of FSH along with potent LH activity of hCG (Schmitt *et al.* 1996). Significantly higher numbers of corpora lutea in group C might be due to involvement of some factors including progesterone profile of the donor animal at the time of superovulation, influence of time on the onset of oestrus, increased number of follicular population and additional numbers of ovulation, drugs and doses used, age and body weight of animals and endogenous hormonal profile.

The overall ovulation percentages for both right and left ovaries were 65.39, 66.67, 81.74 and 73.22, respectively for groups A, B, C and D in treated mithun cows. Statistical analysis revealed no significant differences among different treatment groups in respect of ovulation percentage. The ovulation rate in treated mithun cows was higher than the earlier reports of Agarwal *et al.* (1992) in lactating cows following superovulatory treatment with FSH. However, it was lower than reported by Mustapha *et al.* (2008).

In the present experiment, a significantly ($P<0.01$) positive correlation ($r = 0.609$) between the number of corpora lutea (CL) and embryo recovery in all the 4 groups of mithun cows was observed. No significant correlations existed between the number of CL, palpable follicles and embryo recovery in the present experiment (Table 3). The overall embryo recovery percentages were as 17.65, 12.50, 23.08 and 21.05, respectively, for groups A, B, C and D.

The mean number of embryo recovered and the embryo recovery percentages in this experiment did not differ significantly among different groups and between the right and left horn following superovulatory treatment with 2 different doses of FSH with or without hCG in mithun cows. In the present experiment, a significantly ($P<0.01$) positive correlation ($r = 0.609$) between the number of corpora lutea (CL) and embryo recovery in mithun cows was observed which corroborated the earlier reports of Dutt and Kharche (2000). The overall embryo recovery percentages were

recorded as 17.65, 12.50, 23.08 and 21.05, respectively in groups A, B, C and D. The present findings are in agreement with the reports of Mishra *et al.* (2002) in Sahiwal cows following superovulatory treatment with FSH. Lower embryo recovery rate might be influenced by factors like lesser ability of fimbriae to trap ova from enlarged superovulated ovary, premature entry of ova/embryos into the uterus and subsequent degeneration, low recovery of fluid, faster movement of ova, low progesterone profile etc. (Sarvaiya *et al.* 2003). Advanced age of the donor cows could also be a cause of the lower embryo recovery rate (Valencia *et al.* 2005). The lower embryo recovery rate might also be due to environmental factors since heat stress during summer season reduced the rate of embryo recovery (Peixoto *et al.* 2006).

In the present experiment, 15 embryos could be recovered, being the number 3, 2, 6 and 4, respectively, from groups A, B, C and D in superovulated mithun cows. All the recovered embryos were of morula stage except one (16.67%) blastocyst in group C. While grading the embryos following the methods of Reddy (1994), grade A embryos were highest (80.00%) followed by grade B (20.00%). None of the embryo was categorized as grade C or D. The present findings recorded in four different treatment groups of mithun cows in respect of stages and grading of embryos were in close conformity to the reports of Bhuyan (2004).

SUMMARY

Cyclic mithun (*Bos frontalis*) cows (20) between second and fifth lactation, reared under intensive managerial system were randomly divided into groups A, B, C and D comprising 5 cows in each group. Animals of groups A, B, C and D were treated with 400 mg FSH, 300 mg FSH, 400 mg FSH + 1500 IU hCG and 300 mg FSH + 1500 IU hCG, respectively. Hundred per cent oestrus response was recorded following treatment in all the groups. Time interval from PGF₂α injection to onset of oestrus and duration of induced oestrus were 48.60±0.80, 47.60±0.40, 49.40±0.58, 47.90±0.86 h and 25.00±0.71, 24.00±0.71, 26.00±0.71, 24.50±0.74 h in animals under groups A, B, C and D respectively. Mithun cows treated with 400 mg FSH plus 1500 IU hCG showed better superovulatory response in terms of average number of palpable corpus luteum (5.20±0.20) and embryo recovery percentage (23.08%) compared to other treatment groups.

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