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Effect of superovulatory treatment on serum estradiol -17ß and progesterone concentration and its correlation with embryo recovery in mithun cows

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ABSTRACT

The present experiment was conducted to observe the effects of superovulatory treatments on serum estradiol-17ß and progesterone concentration and its correlation with embryo recovery in mithun cows (*Bos frontalis*). Healthy cyclic mithun cows (20) between second and fifth lactation were randomly divided into 4 groups (n=5) viz. A, B, C and D. Animals were maintained under intensive system of management at NRC on Mithun, Nagaland. Each animal of groups A, B, C and D was treated, respectively, with 400 mg FSH, 300 mg FSH, 400 mg FSH + 1500 IU hCG and 300 mg FSH + 1500 IU hCG in divided and reducing doses during 4 days starting on day 11 of estrous cycle. Prostaglandin $F_2\alpha$ was injected intramuscularly on day 13 of estrous cycle after sixth injection of FSH. hCG was injected intramuscularly 12 h post oestrus. Results revealed that serum oestradiol-17ß was higher in cows treated with 400 mg FSH compared to 300 mg that became significant on day 4 of treatment in group C (10.20±0.34 pg/ml) than group B (9.25±0.35 pg/ml). Significantly higher serum oestradiol was recorded at induced oestrus in all the treatment groups.

Serum progesterone level decreased to a significant level on the day of induced oestrus in all the 4 groups irrespective of treatment. Serum oestradiol-17ß at induced oestrus had positive correlations with the number of total embryos recovered. It may be concluded that the serum estradiol and progesterone concentration are good indicator of embryo recovery in superovulated mithun cows.

Key words: Exogenous hormone, Mithun cows, Oestrous cycle, Superovulation

Mithun (*Bos frontalis*), a semi-domesticated rare bovine species, is basically used for beef purpose besides its use in barter trade, marriage and gift etc. There is immense scope to increase milk as well as meat production by exploiting the rate of reproductive potential through application of embryo biotechnology. The multiple ovulation and embryo transfer (MOET) can be used for facilitating the genetic improvement of the animal (Nilchuen *et al.* 2011). However, uncertainty as well as variability in superovulatory response within and between donors continued to be a frustrating task for extensive exploitation of superior females to realize more number of embryos (Rao *et al*. 2010). It is important to estimate oestrogen, mainly oestradiol-17β concentration in the blood samples of superovulated cows to detect the response of the ovary to hormonal treatment for production of embryos. An increase in the concentration of oestradiol -17ß at the end of hormonal stimulation reflected the number and the quality of the developing follicles and the ovulation rate could be predicted by plasma concentration of oestradiol -17β (Prasad *et al*. 2003). The quantitative estimation of progesterone also provides a reliable means to monitor the functional state of ovary or reproduction (Navotny *et al.* 2005). The progesterone level is used as a tool to predict the ovarian response and the number of embryos in superovulated cows**.** However, very meager amount of work has been done on superovulation in mithun and no written evidence so far has been available in this regard. Therefore, the present work was undertaken to study the effect of superovulatory treatments on serum estradiol-17ß and progesterone concentration in mithun cows to get better embryo recovery.

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MATERIALS AND METHODS

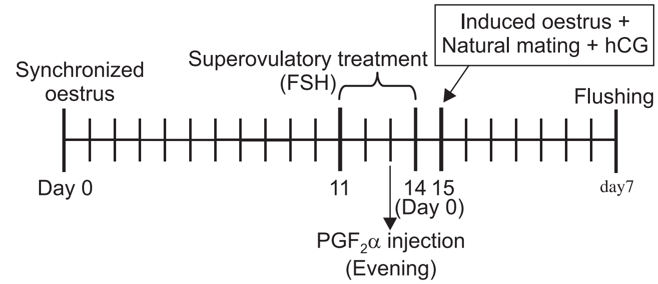
Experimental animals: The present experiment was carried out at National Research Centre on Mithun, Nagaland in association with Department of Veterinary Physiology at College of Veterinary Science, Guwahati, Asom. Healthy cyclic mithun cows (20) of second to fifth lactation were selected. All the animals were having normal genitalia on per rectal examination with no history of infectious or reproductive diseases. Experimental animals were maintained under intensive managemental system and were offered concentrate feed @ 3.5 kg/day/animal along with 10–12 kg green grasses, adequate straw, tree leaves and drinking water *ad lib*.

Hormonal treatment: The experimental animals were randomly divided into 4 groups, viz. A, B, C and D consisting of 5 mithun cows in each group. Each of the experimental animals was treated with intravaginal progesterone device + $PGF_2\alpha$ (cloprostenol) @ 0.5 mg/animal for synchronized oestrus followed by superovulatory treatment and collection of embryo as per the following experimental design.

Animals of group A, B, C and D were treated respectively with 400 mg FSH (folltropin-V), 300 mg FSH, 400 mg FSH + 1500 IU hCG (chorulon) and 300 mg FSH + 1500 IU hCG in divided and reducing doses at 12 h interval for 4 days.

Treatment was started on day 11 of oestrous cycle. The treatment schedule for 400 and 300 mg FSH from day 11 to day 14 was 60/60, 55/55, 45/45, 40/40 and 50/50, 40/40, 35/35 and 25/25 mg respectively. Prostaglandin $F_2\alpha$, 2 ml, corresponding to 0.5 mg prostaglandin $F_2\ \alpha$ per animal, was injected intramuscularly after sixth injection of FSH. hCG was injected @ 1500 IU intramuscularly 12 h post oestrus in each animal of groups C and D.

Detection of oestrus and mating: Occurrence of oestrus in treated animals was observed at 4 h interval following $PGF2\alpha$ treatment. A vasectomized mithun bull was used to detect oestrus along with visual observations for external signs. Cows in oestrus were allowed to mate naturally under observation 12–18 h of oestrus with mithun bull of proven fertility allowing 3 times of breeding a day by the bull during the oestrus period.



Serum Oestradiol-17β and progesterone assay: Blood (5–7 ml) was collected from each experimental animal by jugular venipuncture in a clean sterilized test tube on day 3 before treatment and on the days of treatment. Subsequently, collections were made on the day of induced oestrus following treatment and at 3 days interval till subsequent natural oestrus. Serum was separated within 8 h of blood collection, centrifuged at 3000 rpm for 15 min and stored at –20°C until radio immuno assay (RIA) was performed for estimation of serum estradiol-17β and progesterone concentration. Serum oestradiol-17β and progesterone was assayed following COAT-A-COUNT method of radio immuno assay (RIA) using commercial kits. The intra- and inter-assay coefficient of variations were 5.4 and 5%, and 9.6 and 9.2% for serum oestradiol and serum progesterone respectively.

Statistical analysis: Data obtained from the experiment was analysed statistically using two way analysis of variance (ANOVA) as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Serum oestradiol-17β concentration in superovulated mithun cows: The mean oestradiol concentration prior to treatment ranged between 4.82±0.27 and 5.20±0.31 pg/ml (Table 1). This was in close agreement to the earlier reports (Dhali *et al.* 2006).

Table 1. Serum estradiol-17ß concentration (pg/ml) in mithun cows following 4 different superovulatory treatments

Groups	3 day before treatment	Days of treatment				Day of induced estrus	Days post-estrus			Subsequent natural estrus
		Day 1	Day 2	Day 3	Day 4		Day 4	Day 8	Day 12	
Gr. A	4.90_{G} ±0.22	5.20_{G} ±0.17	6.30_{F} ±0.19	7.40_{E} ±0.28	9.75^{ab}_{D} ±0.34	28.90_{A} ±0.54	13.00_{C} ±0.49	4.20_{H} ±0.17	9.00^{b}_{D} ±0.17	19.20^{b}_{B} ±0.35
Gr. B	4.85_{GH} ±0.12	5.30_{G} ±0.37	6.42_{F} ±0.58	7.50_{E} ±0.30	9.25^{b}_{D} ±0.35	28.70_{A} ±0.42	12.35_{C} ±0.39	4.50_{H} ±0.30	9.50^{ab}_{D} ±0.35	20.00^{b}_{B} ±1.45
Gr. C	5.20_{GH} ±0.31	5.72_{G} ±0.41	6.80_{F} ±0.56	7.59_{E} ±0.36	10.20^{a}_{D} ±0.34	29.50_{A} ±0.48	13.50_{C} ±0.38	4.80_{H} ±0.18	10.20^{ab}_{D} ±0.38	21.50^{a}_{B} ±0.93
Gr. D	4.82_{H} ±0.27	5.47_{G} ±0.21	6.85_{F} ±0.16	7.48_{F} ±0.22	9.50^{ab}_{E} ±0.32	28.95_{A} ±0.47	13.00_{C} ±0.56	4.40_{H} ±0.27	11.63^{a}_{D} ±0.36	20.35^{ab}_{B} ±1.06

[a, b] Figures with different superscripts in a column differ significantly (P<0.01).[A, B, C, D, E, F, G, H] Figures with different subscript in a row differ significantly (P<0.01).

Table 2. Serum progesterone concentration (ng/ml) in mithun cows following 4 different superovulatory treatments

Groups	3 day before treatment	Days of treatment				Day of induced estrus	Days post-estrus			Subsequent natural estrus
		Day 1	Day 2	Day 3	Day 4		Day 4	Day 8	Day 12	
Gr. A	2.53_A ±0.27	2.78_A ±0.21	2.75_A ±0.16	2.70_A ±0.16	1.35_B ±0.12	0.49_D ±0.03	2.80_A ±0.38	1.05_C ±0.17	0.65_D ±0.04	0.45_D ±0.03
Gr. B	2.42_A ±0.26	2.73_A ±0.25	2.70_A ±0.24	2.62_A ±0.18	1.32_B ±0.12	0.52_C ±0.05	2.75_A ±0.40	0.95_C ±0.05	0.69_C ±0.06	0.42_C ±0.04
Gr. C	2.69_B ±0.28	2.92_B ±0.09	2.87_B ±0.21	2.81_B ±0.18	1.25_C ±0.07	0.45_D ±0.07	3.67_A ±0.48	1.20_C ±0.17	0.62_D ±0.08	0.49_D ±0.08
Gr. D	2.62_B ±0.22	2.82_B ±0.19	2.78_B ±0.20	2.70_B ±0.23	1.28_C ±0.09	0.48_E ±0.07	3.20_A ±0.46	1.10_C ±0.17	0.77_D ±0.05	0.47_E ±0.03

$^{A, B, C, D, E}$ Figures with different subscript in a row differ significantly (P<0.01).

Table 3. Correlation coefficient of serum estradiol-17ß concentration (at induced estrus) and progesterone concentration (on day 4 of post-induced estrus) with the number of palpable CL, palpable follicles and total embryo recovery

Particulars	Correlation co-efficient				
	Estrogen	Progesterone	Total corpora lutea	Total embryo recovered	Total palpable follicle
Estrogen	1	–0.121	0.511*	0.202	0.429
Progesterone	–0.121	1	0.107	0.093	0.016
Total corpora lutea	0.511*	0.107	1	0.609**	–0.340
Total palpable follicles	0.429	0.016	–0.340	–0.079	1
Total embryo recovered	0.202	0.093	0.609**	1	–0.079

*Correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level.

The serum estradiol level increased significantly (P<0.01) during the period of treatment and reached a peak value on the day of induced estrus within the treatment groups. On the contrary, Chakravarty *et al.* (2005) reported a decreasing trend of serum estradiol-17ß concentration following superovulatory treatment with FSH in both local non-descript and crossbred cows of Asom. Peak concentration of serum estradiol-17ß recorded on the day of estrus might be due to development, growth and maturation of more numbers of follicles (Bonia and Goswami 2011, Kharche *et al*. 2008). However, significantly higher level (P<0.01) of estradiol-17 ß was found in group C than group B on day 4 of treatment which might be due to synergistic effect of higher doses of FSH and hCG.

The estradiol level reduced significantly (P<0.01) on day 8 post induced estrus in all the groups, which might be due to high progesterone level resulting from negative feedback suppression of estradiol-17ß (Sirohi *et al.* 2005). The serum estradiol-17ß concentration on the day of induced oestrus was significantly higher (P<0.01) as compared to value on the day of subsequent natural oestrus, which might be due to ovarian follicular influence following exogenous hormone administration (Nath *et al*. 2003). The serum estradiol-17ß concentrations recorded on the day of subsequent natural oestrus were in close agreement with the earlier reports (Dhali *et al*. 2005) in female mithun cows. Following induction of oestrus on day 12 of the cycle, oestradiol concentration was recorded significantly (P<0.01) higher in group D animals (11.63±0.36 pg/ml) than that of the animals of group A (9.00±0.17 pg/ml). However, the oestradiol concentration did not differ significantly among A, B and C, and B, C, and D groups. This was reflected in higher recovery of embryo in group D than in group A. At subsequent natural oestrus, the serum oestradiol-17ß concentration rose to a significantly higher level (P<0.01) in group C compared to groups A and B. However, the values between groups C and D did not differ significantly. This might be due to synergistic effect of FSH and hCG on ovarian follicular growth and maturation.

A nonsignificant positive correlation existed between estradiol-17ß concentrations; total numbers of palpable follicles and embryos recovered in all experimental mithun cows and were in close agreement with the earlier report (Mishra *et al.* 2000). However, significantly positive (P<0.01, r = 0.511) correlation between serum oestradiol-17ß concentration at induced oestrus and the number of total corpora lutea (CL) palpated on day 7 after oestrus in mithun cows were observed which was in agreement with the reports of Arosh *et al.* (2001). In the present experiment, the correlation studies suggested that the concentration of oestradiol at induced oestrus in all the 4 different groups of mithun cows can be used as marker to predict the ovulation rate, number of follicles present and responded for superovulatory treatment (Arosh *et al.* 2001, Chakravarty *et al.* 2005).

Serum progesterone concentration in superovulated mithun cows: The mean concentrations of serum progesterone in mithun cows following different superovulatory treatment are depicted in Table 2. The serum progesterone concentration ranged between 2.42±0.26 and 2.69±0.28 ng/ml in mithun cows under different experimental groups prior to treatment. It is in close agreement with reports of Bonia and Goswami (2011). The level remained apparently unchanged up to day 3 of treatment. However, it declined significantly (P<0.01) on day 4 following treatment in animals of all the groups. This might be due to luteolytic effect of circulating oestrogen secreted from increased follicular growth caused by exogenous administration of superovulatory hormone (Kharche *et al.* 2001). The level further decreased to a significant (P<0.01) level on the day of induced oestrus in all the groups. The reason might be due to lysis of existent CL (Chakravarty *et al.* 2005; Sirohi *et al.* 2005) or negative feedback suppression of progesterone by higher circulating estrogen (Singh *et al.* 1998). The serum progesterone level increased significantly (P<0.01) on day 4 post induced oestrus in all the groups and again declined significantly on day 8 i.e. 1 day after flushing. The values of serum progesterone recorded on day 8 of post induced oestrus were slightly higher than the reported values of Chakravarty *et al.* (2005). A further decreased level was recorded on day 12 of post-induced oestrus and maintained serum profile without any significant variation while observed at subsequent natural oestrus in all the experimental groups which is in agreement with Kharche *et al.* (2008). The level also did not vary significantly between induced oestrus and subsequent natural oestrus in any of the groups.

On the other hand, it was observed that the serum progesterone concentration did not vary significantly among different treatment groups in any days under observation. The serum progesterone concentrations recorded on the day of subsequent natural oestrus were in close agreement with Mondal and Prakash (2003).

The serum progesterone concentration on day 4 post induced oestrus correlated positively with ovulation rates, number of palpable follicles and embryos recovered but the values were nonsignificant. This was in close agreement with the results of Arosh *et al.* (2001).

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