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Effect of Presuperovulatory Treatment of Buserelin on Superovulatory Response and Embryo Recovery in Cattle under Subtropical Conditions of Meghalaya

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ABSTRACT

An experiment was conducted with 24 healthy cyclic cows between 2nd to 5th lactation by under intensive system of rearing. The animals were randomly divided into Control (C) and Experiment (E) groups with 12 animals in each The control and experiment groups were again further subdivided in groups C_1 and C_2 & E_1 and E_2 comprising six in each. Onset of oestrus was considered as day 0 for superovulatory treatment. The animals of groups C_1 and C_2 & E_1 and E_2 were injected with 5 ml NSS (i/ml) & 21µg GnRH (i/m) (Buserelin acetate) on day 8 of the oestrous cycle, respectively. Each of the experimental animals of groups $C_1$and E_1 was superovulated with 400 mg FSH (i/m) in divided and equal doses at 12 h intervals for four days on day 10 to 13 of the oestrous cycle and consequently, each of the experimental animals of groups C_2 and E_2 was also superovulated with 2000 I.U.PMSG injection (i/m) as single dose on day 10 of the oestrous cycle. $PGF_2\alpha$ 2ml was injected (i/m) at morning time on day 12 of oestrous cycle. At the superovulatory oestrus, all the animals were bred three times at 12 h intervals using frozen semen. The animals were examined per rectally on day 6 of the induced oestrus after first insemination to detect the superovulatory response. Flushing was performed on day 7 of induced oestrus to retrieve embryos from the superovulated cows. In the present experiment, superovulatory response in GnRH treated groups of cows (groups E_1 and E_2) were found to be better as compared to that of the animals of control groups but the duration of induced oestrus (h) recorded in groups C_2 (49.02 ± 0.07 h) and E_2 (50.40 ± 0.27 h) were found to be significantly higher ($P<0.05$) than the value recorded in groups C_1 and E_1.

Keywords: Follicle Stimulating Hormone, GnRH, PMSG

INTRODUCTION

Superovulation is a key step in the embryo transfer technology in cattle and requires administration of a gonadotropin preparation that mimics the effect of follicle stimulating hormone (FSH). It is an important and integral part of the fast developing multiple ovulation and embryo transfer (MOET) programmes. Superovulation is done by application of gonadotropin like porcine pituitary FSH (Barman et al. 2012) with or without luteinizing hormone in mithun cows or pregnant mare serum gonadotropin (Saumande and Chupin 1981) with or without luteinizing hormone in cattle. The success of embryo transfer technique depends largely on the reliable method for the induction of superovulation to harvest maximum number of normal fertile ova or embryos. Although various advances have been achieved in superovulation technique, it still remains a weak link in the chain of events influencing the final outcome of embryo transfer in cattle. Beneficial effects of prostaglandins as well as GnRH have been well documented and are now being used for improvement of reproductive efficiency in large ruminants (Kumar et al. 2010). Therefore, the present study was conducted to determine the effect of presuperovulatory treatment of buserelin on

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superovulatory response and embryo recovery in cattle under subtropical conditions of Meghalaya in order to understand their reproductive physiology.

MATERIALS AND METHODS

The present experiment was conducted at dairy unit, ICAR Research Complex for NEH Region, Umiam, Meghalaya. Twenty four healthy cyclic cows between 2nd and 5th lactation, free from genital abnormalities on per rectal examination, having no previous history of infectious diseases were selected for the present experiment. Experimental animals were maintained under intensive system of rearing and they were examined regularly to ascertain their health condition and strict hygienic measures were taken in the shed. The experimental animals were divided in two groups *viz*. Control (C) and Experiment (E) comprising twelve in each. The control group was again further subdivided in groups C_1 and C_2 comprising six in each and similarly experiment group (E) was also further subdivided into E_1 and E_2 comprising six in each. Onset of oestrus was considered as day 0 for superovulatory treatment. The animals of groups C_1 and C_2 & E_1 and E_2 were injected with 5 ml NSS (i/ml) & 21µg GnRH (i/m) (Buserelin acetate) on day 8 of the oestrous cycle, respectively. Each of the experimental animals of groups $C_1$and E_1 was superovulated with 400 mg FSH (i/m) (Folltropin, Veterpharma Inc, Canada) in divided and equal doses at 12 h intervals for four days on day 10 to 13 of the oestrous cycle. The treatment schedule for superovulation with FSH 400 mg was as 50/50, 50/50, 50/50 and 50/50 mg respectively and consequently, each of the experimental animals of group C_2 and E_2 was also superovulated with 2000 I.U.PMSG injection (i/m) (Folligon, Intervet International Gmbh, Germany) as single dose on day 10 of the oestrous cycle. PGF_2á 2ml (Cloprostenol, Intervet International Gmbh, Germany) was injected (i/m) at morning time on day 12 of oestrous cycle. At the superovulatory oestrus, all the animals were bred three times at 12 h intervals using frozen semen. The animals were examined per rectally on day 6 of the induced oestrus after first insemination to detect the superovulatory response. Flushing of uterine horns for non-surgical embryo collection using modified Dulbecco's phosphate buffer saline (mDPBS) with 1% heat treated oestrus cow serum was performed on day 7 of induced oestrus to retrieve embryos from the superovulated cows according to the method described by Neto et al. (2005). Data obtained from the experiment were statistically analyzed as per the method described by Snedecor and Cochran (2007).

RESULTS AND DISCUSSION

The results of the present experiment are being presented in Table 1 and 2. Animals responding to superovulatory treatment in groups C_1, C_2, E_1 and E_2 were 100%, 83.33%, 100% and 83.33%,

Table 1: Effect of presuperovulatory treatment of buserelin on superovulatory response and embryo recovery in cattle

Treatment groups	No. of animals	Oestrus response (%)	Mean ovulation rate (Mean ± SE)	Total embryos recovery (Mean ± SE)	Viable embryos (Mean ± SE)	Anovulatory follicles (Mean ± SE)	Degenerated embryos (Mean ± SE)	Unfertilized ova (Mean ± SE)
C_1 (5 ml NSS+FSH: 400 mg)	6	100.00	6.83^{NS}±1.01	4.17^{NS}±0.91	2.5^{NS}±0.80	0.67^{NS}±0.49	0.67^{NS}±0.49	1.0^{NS}±0.44
C_2 (5 ml NSS+PMSG 2000 I.U.)	6	83.33	5.33^{NS}±1.11	1.83^{NS}±0.79	1.67^{NS}±0.65	1.83^{NS}±0.79	0.17^{NS}±0.16	0.5^{NS}±0.49
E_1 (21 µg Buserelin acetate + FSH: 400 mg)	6	100.00	8.17^{NS}±1.30	4.83^{NS}±1.22	2.33^{NS}±0.20	0.67^{NS}±0.20	1.67^{NS}±0.40	0.15^{NS}±0.16
E_2 (21 µg Buserelin acetate +PMSG 2000 I.U.)	6	83.33	5.5^{NS}±1.06	2.83^{NS}±1.64	1.83^{NS}±1.16	0.83^{NS}±0.30	0.83^{NS}±0.54	0.17^{NS}±0.16

^{NS}: Non-significant

Table 2: Effect of presuperovulatory treatment of buserelin on occurrence of induced and natural oestrus in cattle

Treatment groups	No. of animals	Oestrus response (%)	Duration of induced oestrus (hrs.) (Mean±SE)	Interval from induced oestrus to the onset of subsequent natural oestrus (days) (Mean±SE)	Duration of natural oestrus (hrs.) (Mean±SE)
C_1 (5 ml NSS+FSH: 400 mg)	6	100.00	43.05^{a}±1.15	20.02^{NS}±0.68	24.85^{NS}±0.03
C_2 (5 ml NSS+PMSG 2000 I.U.)	6	83.33	49.02^{b}± 0.07	20.15^{NS}±0.15	26.06^{NS}±0.22
E_1 (21 µg Buserelin acetate + FSH: 400 mg)	6	100.00	44.25^{ac}±0.03	22.5^{NS}±1.38	25.50^{NS}±0.25
E_2 (21 µg Buserelin acetate +PMSG 2000 I.U.)	6	83.33	50.40^{b}± 0.27	21.08^{NS}±0.27	27.50^{NS}±0.10

Means with different superscripts in a column differ significantly (P<0.05), NS-non significant

respectively. 100 percent oestrus response was observed in animals of groups C_1 and E_1.These findings were in close agreement with the earlier report of Barman et al. (2012). However, the report of Mathur et al. (2006) on oestrus response following superovulation in Frieswal cows was 75 per cent only.

In the present experiment, superovulatory response in GnRH treated groups of cows were found to be better as evidenced by apparently lower number of palpable follicles as well as apparently higher numbers of corpora lutea as compared to that of the animals of control groups. This might be due to increased growth and maturation of follicles by combined effect of GnRH and FSH/ PMSG (Nilchuen et al. 2011) or due to follicle luteinisation and possible ovulation followed by the emergence of new follicular wave (Schmidt et al. 1996) or also due to the involvement of some factors including progesterone profile of donor at the time of superovulation, increased number of follicular population and endogenous hormonal milieu (Arora et al. 1996).

The numbers of unovulated follicles obtained in animals of group C_2 were apparently higher as compared to groups C_1, E_1 and E_2 but analysis of variance revealed non-significant differences in respect of anovulatory follicles between GnRH (buserelin acetate) treated and control groups. This might be due to insufficient endogenous LH surge (Taneja et al. 1988).

The number of embryos recovered were found to be apparently higher in animals of group E_1 (4.83 ± 1.22) which might be due to increased growth and maturation of follicles caused by combined effect of GnRH (Buserelin acetate) and FSH and also due to genetic variation (Nilchuen et al. 2011) but statistically found to be non-significant (P>0.05) between the treated and control groups.

The duration of induced oestrus (h) recorded in the present experiment were as 43.05 ± 1.15, 49.02 ± 0.07, 44.25 ± 0.03 and 50.40 ± 0.27, respectively for groups C_1, C_2, E_1 and E_2. Although apparently higher value was observed in group E_1 as compared to the value recorded in group C_1 but analysis of variance revealed no significant difference (P>0.05) between groups C_1 and E_1. Similar findings have been also recorded by Bhuyan et al. (2006). The value recorded in group E_2 were found to be apparently higher than the value recorded in group C_2 but no significant differences (P>0.05) were found between the two respective groups in respect of duration of induced oestrus. The present findings were in close agreement with the earlier report of Abdoon et al. (2000). The duration of induced oestrus (h) recorded in groups C_2 and E_2 were found to be significantly higher (P<0.05) than the value recorded in groups C_1 and E_1. This might be due to 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) or could be attributed to

increased follicular growth caused by hormone pregnant mare serum gonadotropin (Abdoon et al. 2000).

The mean time intervals from induced oestrus to the onset of subsequent natural oestrus in the present experiment were 20.02 ± 0.68, 20.15 ± 0.15, 22.5 ± 1.38 and 21.08 ± 0.27 days, respectively for groups C_1, C_2, E_1 and E_2. Although analysis of variance revealed no significant differences for time intervals recorded from induced oestrus to the onset of subsequent natural oestrus among the groups C_1, C_2, E_1 and E_2 but apparently longer time interval was recorded in group E_1 (22.5 ± 1.38 days) followed by groups E_2, C_2 and C_1. This may be attributed to elevated concentrations of circulating P4 due to more numbers of CL. The high concentration of P4 exerts negative feedback effect on LH secretion; thereby causing the attenuation of follicular growth (Barman et al. 2012).

The mean duration of subsequent natural oestrus (h) recorded in the present experiment were 24.85 ± 0.03, 26.06 ± 0.22, 25.50 ± 0.25 and 27.50 ± 0.10 h, respectively for groups C_1, C_2, E_1 and E_2. Although apparently higher values were observed in group E_2 followed by groups C_2, E_1 and C_1 but analysis of variance revealed no significant differences for duration of subsequent natural oestrus among different groups. The present findings recorded in respect of duration of subsequent natural oestrus were in close agreement with the earlier report of Bhuyan et al. (2006).

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