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Oestrogen and progesterone concentration in Frieswal cows during superovulation

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Embryo transfer technology has been practised world wide for several years. The variable and unpredictable superovulatory response of donor cows remains the major limiting factor for successful embryo transfer in cattle (Adame *et al*. 1992), and the important factor that affects the superovulatory treatment (Kewon *et al*. 1987, Roberge *et al*. 1995). In view of this, the study was undertaken to know the circulatory levels of oestrogen and progesterone at different stages of superovulation in Frieswal animals.

Ten adult elite Frieswal cows were maintained under standard management conditions as per the Military Farm schedule. They were fed additional ration and mineral mixture @ 50 g/animal/day throughout the experimental period. Oestrus was regularly observed twice daily in the morning and evening followed by confirmation of oestrus by rectal palpation. These animals were divided into two groups of 5 cows each. In the first group of 5 cows, 280 mg of FSH-P (follotropin-V) was injected twice daily in descending doses schedule for 4 days. In the second group PMSG (folligon) was used in a single dose of 1500 IU as single i/m injection. All the ten animals were given injection of $PGF_2\alpha$ (illerin) @ 0.75 mg after 66 h of FSH/PMSG injection. The animals exhibited oestrus were inseminated with frozen-thawed semen after 12 hrs of estrus detection and were inseminated thrice up to 56 hrs. Samples of jugular venous blood were collected at the end of natural oestrus, prior to superovulatory treatment just before prostaglandin injection and at the end of induced oestrus from all the 10 cows for estimation of oestrogen (oestradial 17-β) and progesterone. Estimation of oestrogen and progesterone was done by enzyme linked immunosorbent assay (ELISA) technique using kit pathozyme. For analysis of data, RBD (randomized block design) and 't' test (paired/unpaired) were used as per standard statistical method of Snedecor and Cochran (1987). The minimum sensitivity of assay for oestradiol was pg/ml and cross reactivity was 100% for oestradiol whereas these values were 0.05 ng/ml and 100% for progesterone assay respectively.

It was observed that the mean concentration of hormone oestradiol 17-β in FSH superovulated donor animals (group 1) at the end of natural oestrus was 210 ± 136.64 (range 20–750) pg/ml. After day 10 of the cycle i.e. just before the start of superovulatory treatment the levels were 163 ± 37.20 (range 65–250) pg/ml and after 60 h of superovulatory treatment (i.e just before PG injection) were 109 ± 40.70 (range 10–290) pg/ml. These levels were 148 ± 54.44 pg/ml at the end of induced oestrus. In the second group (superovulated with eCG) the respective concentration of oestradiol 17β during the four different stages of sample collection were 93 ± 44.71 (range 30–270), 140 ± 47.04 (50–300); 90 ± 30.78 (25–200) and 85 ± 29.88 (5–190) pg/ml. Statistical analysis (RBD) revealed that concentration of oestradiol between different stages did not differ significantly similarly comparison (paired 't' test) of mean concentration of oestradiol 17β of different stages with two superovulatory groups of donors did not revealed any significant difference. Observations on oestradiol concentration of individual animal before PG injection, revealed a trend suggesting increased concentration at this stage probably giving an indication of number of developing estrogenized follicle, which could have turned out into a superovulatory response (with 175 pg/ml-14 CL and 200 pg/ml-10 CL), however, we observed a decline in the mean estradiol concentration (163 ± 37.20 to 109 ± 40.70 pg/ml and 140 ± 47.54 to 90 ± 30.78 pg/ml, in 1 and 2 groups, respectively, which does not support our aforesaid presumption, however, increase in concentration of estradiol before induction of oestrus with PG have been correlated with more number of follicle formation by other workers (Kweon *et al*. 1987, Agarwal *et al*. 1993).

The mean concentration of progesterone at the end of natural oestrus was 4.8 ± 2.12 and 4.9 ± 2.01 ng/ml in group 1 and 2 animals. These values were 4.95 ± 1.53 (range 1.25 – 9.5) and 4.45 ± 1.48 (1.5–10.0) (0.45–6.25) ng/ml in 2 groups just before the start of superovulatory treatment. The concentrations of progesterone after 60 h post FSH and PMSG treatment were 4.28 ± 1.19 (range 1–8) and 4.19 ± 0.99 ng/ml.

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Table 1. Serum concentration of oestradiol 17ß and progesterone of FSH and MSG superovulated doner animals at different stages of sample collection (mean±SE)

Hormone	Groups	End of normal oestrus	Before FSH/ PMSG injection	Before PG injection	End of superovulatory treatment
Oestradiol	1	210±136.64	163.0±37.20	109±40.70	148.0±54.44
	2	93.0±44.71	140.0±47.54	90.0±30.78	85.00±29.88
Progesterone	1	4.8±2.12	4.95±1.53	4.28±1.19	2.91±0.83
	2	4.9±2.01	4.45±1.48	4.19±0.99	3.65±1.58

However the values at the end of induced oestrus were 2.9 ± 0.83 (range 0.5 – 4.8) and 3.65 ± 1.58 (0.5–8.75) ng/ml in groups 1 and 2 respectively. Perusal of overall mean concentration (irrespective of stage of sample collection) of progesterone in 2 groups broadly indicates that there was a low superovulatory response with higher concentration of progesterone. Statistical analysis (RBD) did not reveal any significant difference in the concentration of progesterone between different stages of samples collection as well as between different animals. Comparison of mean concentration of progesterone of different stages with the 2 superovulated groups did not reveal any significant difference.

Observations on the progesterone concentration of individual cows in our experiment revealed no definite trend, the mean concentrations of progesterone at the end of natural oestrus were 4.8 ± 2.12 and 4.9 ± 2.01 for 2 groups whereas others have reported lower concentrations (Kharche *et al*. 1997, Prasad *et al*. 2003). The higher values of progesterone at the end of induced oestrus (2.91 ± 0.83 and 3.65 ± 1.58 ng/ml) suggests some unovulated follicle undergoing lutenization. This could also be a reason for lower recovery of embryos despite good superovulation response

SUMMARY

Ten normal adult healthy cyclic Frieswal cows were utilized for this study. These animals were divided into two groups of 5 animals each. Superovulation was induced in the first group by utilizing 280 mg of NIH-FSH P_1 in 8 divided doses given in 4 days. In the second group PMSG was utilized @ 1500 IU/animal as a single i/m injection. All the 10 animals were given injection of $PGF_2\alpha$ (PG) @ 0.7 mg after 66 hrs of FSH/PMSG injection for induction of oestrus and were subsequently inseminated with frozen thawed semen. The blood samples were collected for the estimation of oestradial 17β and progesterone at the end of natural oestrus, prior to superovulatory treatment, just before PG injection and at the end of induced oestrus.

The mean oestradiol 17β concentration estimated just before the start of superovulation treatment were 163 ± 37.20 and 149 ± 47.54 pg/ml whereas the concentrations of progesterone were 4.95 ± 1.53 and 4.45 ± 1.48 ng/ml in 1 and 2 superovulatory treatment groups respectively. It was observed that after 60 h of start of superovulatory treatment the levels of estradiol 17β were 109 ± 40.70 and 90 ± 30.78 pg/ml in group 1 and 2, these values at the end of induced oestrus were 148.0 ± 54.4 and 85.0 ± 29.88 pg/ml in group 1 and 2. Similarly, mean progesterone concentrations after 60 h of start of superovulatory treatment were 4.28 ± 1.9 and 4.19 ± 0.99 ng/ml in groups 1 and 2, which were 2.91 ± 0.83 and 3.65 ± 1.58 ng/ml at the end of induced oestrus. Here also levels were showing lower trend on compared to mean values obtained just before the start of experiment (i.e. 4.8 ± 2.12 and 4.9 ± 2.01 ng/ml).

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