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Periovulatory steroid hormone profile in relation to superovulatory responses in native Ongole cows

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ABSTRACT

Plasma progesterone (P4) and 17-β estradiol (E2) concentrations were estimated and correlated with ovulatory response, total and viable embryo recovery rates in six Ongole cows (*Bos indicus)* superovulated during mid luteal phase (day 10-11) of the estrus cycle using FSH. The mean P4 and E2 levels on the day of superovulation were found to be 1.45 ng/ml and 37.83 pg/ml respectively. No correlations were observed between plasma P4 levels on day-4 and ovulatory response or between plasma E2 on day `0' and CL response. Positive correlations were observed between plasma P4 levels starting from day 3 to 7 and superovulatory responses (number of palpated CL, mean total and viable embryos). However, a negative correlation was observed between E2 on day-4 and ovulatory resonse. Determination of plasma P4 and E2 levels before, during and after ovarian super stimulation may help in predicting ovulatory response, total and viable embryo recovery in Ongole cows.

Key words : Progesterone, 17-β estradiol, Ongole, embryo

Wide variation in response to superovulation is the single most important limiting factor for production of high quality embryos in commercial embryo transfer programs (Walton and Stubbings, 1986; Elsden and Seidel, 1982; Neumann *et al.*, 1994) and very little is known about the endocrine factors that effect embryo production especially in *bos indicus* cattle (Gradela *et al.*, 1996). Apart from several other factors (Monniaux *et al.*, 1983; Armstrong, 1993; Lopes da Costa *et al.*, 2001), the circulating concentrations of endogenous hormones and their temporal relationship may also account for the variability in ovulatory response (Haupat, 1979; Sreenan *et al.*, 1980; Betteridge, 1993). Determination of periovulatory steroid hormone profiles (Booth *et al.*, 1975; Mehmood *et al.*, 1991) during superovulation has helped to predict the superovulatory response (Lemon and Saumande, 1972; Saumande and Batra, 1985) in cattle. Scanning of reports on steroild hormone profiles and their relationship with ovarian responses indicate variable results. The present study was conducted to analyze plasma P4 and E2 levels in superovulated Ongole cows and to establish correlations between steroid hormone profiles and superovulatory response and embryo recovery.

MATERIALS AND METHODS

Six parous, cyclic Ongole cows aged 8-10 years having normal genitalia were used as donors. They were fed a ration of 1.5 - 2.5 kg concentrate, 10 - 20 kg green fodder and paddy straw *ad lib*. They were sent for grazing between 9 AM to 3 PM to the adjoining fields and water was available free choice. Calves were allowed to suckle the dams and hand milking was carried out twice daily. Superovulation was initiated during mid luteal phase (day 10-11) of the cycle (estrus-0) by administrating 200 mg of NIH-FSH-PI (Folltropin-V, Vetrepharm Inc., London, Ontario, Canada) intramuscularly, in a twice daily descending dose schedule (40/40, 30/30, 20/20 and 10/10 mg) for 4 cosecutive days. Luteolysis was induced by intramuscular administration of 50 mg dinoprost tromethamine (Lutalyse, Upjohn, USA) in 2 equally divided doses at 48 and 60 hrs after initiating superovulatory treatment. The animals were inseminated 3 times from the start of standing estrus and at 12 hrs interval thereafter using pedigreed frozen semen. Embryos were recovered by non surgical method on day 7 (Newcomb *et al.*, 1978). They were evaluated (Lindner

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and Wright, 1983) and graded on the basis of their morphology (Boland *et al.*, 1978).

Blood samples were collected daily at 6 AM by jugular venipuncture on day-4 (day of initiation of treatment), day-1 (24 hours post PG3), day `0' (day of super estrus) and days 1 to 7 after super estrus. The samples were immediately centrifuged at 2500 rpm for 20 min and the separated plasma was stored at -20°C until assayed for steroid hormones.

Pathozyme® progesterone and Pathozyme® estradiol test kits (Omega diagnostics Limited, Scotland, UK) were used for Enzyme Immuno Assay (EIA) of P4 and E2. The instructions included with the kit were followed scrupulously. The test kits contained P4 standards of 0, 0.5, 3.0, 10, 25 and 50 ng/ml and E2 standards of 0, 10, 30, 100, 300 and 1000 pg/ml. The sensitivity of the assay was 0.05 ng/ml for P4 and 1.0 pg/ml for E2. Based on the mean absorbance values of samples the P4 and E2 concentrations were calculated using Ledicare dgn. software provided with Eliskan, ELISA reader (Ranbaxy, India).

The data are expressed either as mean±standard error (SE). Correlations were established (Steel and Torrie, 1960) between circulating steroid hormone levels and superovulatory responses and only statistically significant correlations were presented.

RESULTS AND DISCUSSION

The plasma steroid hormone profiles (P4 and E2) before, during and after superovulation in native Ongole donors were presented in Table 1.

All treated (n=6) cows responded to superovulation. An average of 11.30±1.4 ovulations were palpated per rectum and 9.5±1.88 total ova/embryos were recovered of which 5.8±1.66 were viable. The total embryo recovery was 79.2 per cent of total ovulations and viable (transferable) embryos were found to be 61.4 per cent of recovered embryos (Table 2). The number of anovulatory follicles (2.6±0.56) was positively correlated with E2 concentrations on day 3 ($r=0.87$, $P < 0.05$).

The mean plasma P4 levels at the beginning of superovulation treatment (day-4) were observed to be 1.48±0.38 ng/ml (range 0.7 - 3.3 ng/ml). There was a precipitous drop in P4 levels to 0.47±0.09 ng/ml by day-1 (24 hrs post PG3) confirming the luteolytic activity of $PGF_2\alpha$ (Kamonpatana *et al.*, 1987) followed by gradual increase up to day 2. However, a sharp increase in P4 levels to 7.23±0.69 ng/ml was noticed after day 3 and reached maximum concentrations by day 7 (day of embryo recovery). The P4 profiles in the present study are in keeping with previous repots in cattle (Allen and Foote, 1987; Sarvaiya *et al.*, 2003).

The elevated P4 values from day 3 onwards were indicative of higher ovulation rate as also reported by Booth *et al.* (1975) and Boland *et al.* (1985) in cattle and Misra *et al.* (2000) in buffalo. In the present study no correlation was observed between P4 concentration on day-4 and the ovulation rate which is in agreement with the reports of Tamboura *et al.* (1985), Lindsell *et al.* (1986) and Gradela *et al.* (1996). Contrary to the present finding Yadav *et al.* (1986) and Goto *et al.* (1988) reported positive correlation between suprovulatory response and plasma P4 levels at the beginning of treatment.

The mean total and viable embryos were positively correlated with P4 concentrations on day 5 ($r = 0.84$ and 0.97, $P < 0.05$) day 6 ($r = 0.63$ and 0.71) and day

Table 1. Mean(±SE) plasma P4 and E2 concentrations in superovulated Ongole cows

Hours post PG3	Day of cycle	Progesterone ng/ml	Estradiol 17β pg/ml
-48	-4	1.48±0.38	37.83±4.08
24	-1	0.47±0.09	88.28±3.71
48	0	0.62±0.27	149.48±35.40
72	1	0.98±0.42	51.48±15.82
96	2	1.98±0.43	47.48±14.29
120	3	7.23±0.69	63.60±21.84
144	4	9.30±1.08	33.55±7.43
168	5	11.70±1.36	33.78±14.87
192	6	15.07±1.48	34.90±12.34
216	7	16.25±1.48	35.98±9.74

Table 2. Superovulatory response and embryo recovery in Ongole cows

Attributes	Mean ± SEM
No. of ovulations	11.3±1.4
No. of anovulatory follicles > 10 mm diameter	2.6±0.56
Total no. of ova/embryos recovered (%)	9.5±1.88 (79.2)
Viable embryos recovered (%)	5.8±1.66 (61.4)

7 (r = 0.53 and 0.54) after super estrus. Collectively estimation of plasma P4 from day 3 onwards may be a useful index of superovulatory response, total and viable embryo recovery rates.

The E2 concentration at the beginning of FSH injection was found to be 37.83±4.08 pg/ml (range 22.4-49.8 pg/ml) which increased steadily to reach maximum levels of 149.48±35.40 pg/ml on day `0' (day of super estrus) indicating high degree of folliculogenesis. The levels plummeted sharply by day 1 and gradually reached basal concentrations by day 4. The study failed to find a relationship between CL response and E2 on the day of superestrus as also reported relationship between CL response and E2 on day of superestrus as also reported by Gradela *et al.* (1996) in Nelore cows. However, a negative correlation was observed between E2 on day-4 and ovulatory response (r = -0.66). The mean as well as per cent total and viable embryos were positively correlated with plasma E2 on day `0' but negatively correlated on day 6 of super estrus. The positive correlation of E2 on day day `0' with embryo quality (r = 0.71) indicate that estrogens are important steroidal signal during initial critical phase of oocyte maturation (Osborn and Moor, 1983). High estrogen levels in follicular fluid are correlated with successful fertilization (Laufer *et al.*, 1984) and only those oocytes originating from E2 rich follicles (Yoshimura and Wallach, 1987) would result in higher fertilization and subsequent pregnancy rates.

In conclusion these results suggest that periovulatory steroid hormone profile on the day of FSH injection is not a good predictor of ovulatory response in native (*Bos indicus*) Ongole cows. Plasma P4 concentrations from day 3 onwards and E2 levels on the day of superestrus were directly related to quality of recovered embryos.

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