

Effect of transient suppression of LH surge on superovulatory responses in Ongole cows*

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ABSTRACT

The aim of the present study was to evaluate the effect of temporary suppression of LH surge during superovulation on ovarian response, total and viable embryo recovery and number of freezable (grade 1) embryos. Ongole cows (12) were randomly divided into 2 equal groups. Both groups 1 (control) and 2 (LH suppression) cows, received a silastic auricular implant containing 3 mg norgestomet (NOR) along with a 2 ml i/m injection containing 3 mg NOR and 5 mg estradiol valerate, 4 days before the start of gonadotrophin treatment. Superovulation treatment involved administration of 200 mg NIH-FSH-P1 in a twice daily descending dose schedule for 4 days and induction of luteolysis at 48 h (PGF₁) and 60 h (PGF₂) after initiating gonadotrophin treatment. In group 1 cows the implant was removed 12 h after PGF₁ whereas in group 2 it was removed at 60 h post PGF₁. At the time of implant removal the latter group was administered 25 mg pLH. Non surgical embryo collection was performed about 7–7.5 days after the onset of superestrus. The recovered embryos were evaluated and graded based on their morphology. The mean ovarian response (mean CL) was significantly higher in group 2 compared to group 1 cows. Similarly the total and viable embryo recovery and grade 1 embryos, respectively, were higher, albeit nonsignificantly, in group 2 than in group 1 cows. The fertilization rate in group 2 was comparable to that of group 1. The time of occurrence of LH surge (h post PGF₁) was significantly longer in group 2 donors compared to group 1. The study, therefore, revealed that temporary suppression of endogenous LH surge resulted in higher ovarian response, and it had no adverse impact on the fertilization rate, yield of viable as well as freezable embryos.

Key words: Embryo, LH surge, Norgestomet, Superovulation

Uncertainty as well as variability in superovulatory response (Mapletoft *et al.* 2002) within and between animals continues to be a frustrating task for extensive exploitation of superior females to realize more embryos and subsequently more progeny. Amongst various factors, the number of stimulated follicles that ovulate following superovulation contribute to variation in ovarian response (D'Occhio *et al.* 1997). Follicles smaller than 5 mm diameter at the start of gonadotrophin treatment may not ovulate (Vos *et al.* 1994) due to inadequate maturation or absence of sufficient LH receptors on these follicles at the time of preovulatory LH

surge (Xu *et al.* 1995). To overcome this problem attempts were made to increase the ovulation rate and embryo recovery by delaying the exposure of follicles to LH subsequent to super stimulation of follicular growth (Fricke *et al.* 1994, Vos *et al.* 1994). However, the yield of transferable (viable) embryos was compromised in these studies. The present study was therefore undertaken to evaluate the effect of delay in occurrence of LH surge on ovarian response and the yield of transferable embryos in superovulated Ongole cows.

MATERIALS AND METHODS

Parous, clinically normal, cyclic Ongole cows aged 6–14 years and weighing between 400 and 500 kg were used as donors. Based on the milk yield the daily ration of each animal consisted of 3–5 kg high protein feed, 20–30 kg chopped fodder (*Sorghum bicolor* or *Pennisetum purpureum*) and paddy straw *ad lib*. Water was available free choice. In general, animals were maintained under hygienic and optimum management conditions in loose housing system with a large, open paddock for free movement. They were also sent for grazing daily between 0900 and 1500 h to the

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adjoining pasture lands. Calves were allowed to suckle the dams and hand milking was practiced twice a day.

The donors (n=12) were equally divided into 2 groups. Four days before the start of superovulation treatment (day-4) the donor cows in both groups 1 (control) and 2 (LH suppression) received a silastic auricular implant containing 3 mg norgestomet (17 α -acetoxy-1 β -methyl-19-norpreg-4-en-3,20-dione) along with a 2 ml i/m injection containing an oily solution of 3 mg norgestomet and 5 mg oestradiol valerate. Superovulation treatment was initiated 4 days after application of norgestomet implant. In group 1 cows the implant was removed 12 h after PGF₁ injection whereas in group 2 cows the implant was removed 60 h after PGF₁ injection and simultaneously administered i/m 25 mg pLH.

The donors were superovulated by administering i/m 200 mg NIH-FSH-P1 (follitropin-V) in a twice daily descending dose schedule (40, 40, 30, 30, 20, 20, 10 and 10 mg) at 0600 and 1800 h for 4 days. Luteolysis was induced by injecting 50 mg dinoprost tromethamine (lutalyse) i/m at 48 h (PGF₁) and 60 h (PGF₂) after initiating gonadotrophin treatment. Animals in group 1 were inseminated at 48 and 60h post PGF₁ administration with frozen thawed semen in a 0.5 ml straw with at least 15 million live sperms post-thawing. Animals in group 2 were inseminated at 12 and 24 h after removal of the implant and administration of pLH. Non-surgical embryo collection was performed 9 days after PGF₁ treatment in group 1 whereas in group 2 it was performed 10 days after PGF₁ or 7.5 days after removal of implant and administration of exogenous LH. Collectively the embryo collections were conducted about 7–7.5 days after the onset of standing estrus (super-estrus). Prior to embryo collection the number of corpora lutea on each ovary were estimated per rectum. The embryo collection from donor cows was carried out using Rusch catheter, 18 gauge (IMV technologies) and Dulbecco's phosphate buffered saline with 0.1% BSA as flushing medium. The embryos were isolated, evaluated at 200 $\times$ magnification and classified as viable and non-viable embryos. Based on morphological features, the viable (transferable) embryos were further classified as grade 1 (excellent), grade 2 (good) and grade 3 (fair) embryos according to Mapletoft (1986).

The donors were subjected to blood collection for estimation of LH by enzyme immuno assay (EIA). Blood samples were collected beginning at 24 h post PGF₁ administration and at 4h interval thereafter up to 84 h. 10 ml of jugular venous blood was collected in sterile sodium heparin vacutainer tubes. Within 5 min after collection, the tubes were centrifuged for 15 min at 4000 rpm to separate plasma. The plasma was then pipetted into 5 ml screw capped plastic vials and stored at -20°C until assayed. Immunoenzymatic test kits were used to determine the precise time of preovulatory LH peak. An LH surge was defined as a more than 50% increase above the basal level

calculated as the mean of 3 sequential LH measurements. The greatest LH concentration observed during the sampling period was considered as the LH peak. Time of LH peak was the time at which maximum LH concentration occurred during the sampling period (Callesen *et al.* 1988). The intra- and inter-assay coefficients of variation were 5.6% and 7.1% respectively. The data collected was analyzed as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

An overall perusal of results in the present study revealed that all the donor cows responded to superovulation treatment (Table 1). The ovulatory response was estimated to be significantly higher ($P<0.05$) in group 2 compared to group 1 cows. The results are in keeping with earlier experiments that utilized PRID/eCG/GnRH protocol and recorded higher ovulation rate over control group (Van de Leemput *et al.* 2001). These studies collectively support the hypothesis that delaying the exposure of follicles to LH provides more time for the follicles to mature and then ovulate subsequent to superovulation treatment. Thus the positive effects of follicle coasting are clearly evident in our study too. Contrary to these observations, others studies revealed either no effect (Nogueira *et al.* 2002) or reduction in ovulation rate (Price *et al.* 1999) following delayed LH surge. Variations in breed and their management, hormonal treatments and above all individual response to hormonal stimuli may account for these observed differences in ovulation rate.

The present study did not observe any detrimental effect

Table 1. Effect of temporary LH suppression on ovulation rate and embryo recovery in superstimulated Ongole cows

Attributes	Group 1 (Control)	Group 2 (LH suppression)
No. of superovulation treatments	6	6
No. of responded treatments (%)	6 (100)	6 (100)
Mean No. of ovulations (CL)	10.50 $\pm$ 0.82 ^a	16.33 $\pm$ 1.99 ^b
Mean total ova/embryos	8.66 $\pm$ 0.88	12.83 $\pm$ 2.73
Mean transferable embryos	6.50 $\pm$ 0.82	9.17 $\pm$ 2.30
Fertilization rate (%)	92.75	91.56
Mean grade I embryos (%)	4.83 $\pm$ 0.76 (74.31)	7.50 $\pm$ 1.88 (81.78)
Mean basal LH (ng/ml)	2.59 $\pm$ 0.23	2.01 $\pm$ 0.15
Mean peak LH (ng/ml)	9.03 $\pm$ 1.21	4.98 $\pm$ 0.61
Time of LH peak (h after PGF ₁)	48.00 $\pm$ 1.95 ^c	72.00 $\pm$ 2.62 ^d
Duration of LH surge (h)	11.2 $\pm$ 0.8 ^c	6.0 $\pm$ 1.36 ^d
Onset of standing heat (h after PGF ₁)	43.0 $\pm$ 1.5 ^c	69.6 $\pm$ 3.12 ^d
Duration of estrus (h)	22.5 $\pm$ 2.87	25.2 $\pm$ 3.98

Means bearing different superscripts within a row differ significantly (a,b : $P<0.05$); (c,d: $P<0.01$).

of delaying the LH surge (group 2) on total ova/embryo recovery, viable embryo yield, fertilization rate and the number and percent freezable (grade I) embryos. Instead, it appears that group 2 cows were better placed in so far as all the above parameters are concerned. In agreement with our observation, others reported that by delaying the LH surge either with CIDR-B or GnRH agonist (Nogueira *et al.* 2002) or with GnRH agonist (D'Occhio *et al.* 1997) the viable embryo yield was either comparable or even higher than that of control cows. Contrary to the present finding Andreussi (2001) and Van de Leemput *et al.* (2001) reported that delaying of LH surge although increased the ovulation rate, the number of total ova/embryos and viable embryos were reduced and unfertilized ova increased. This decrease in fertilization rate and embryo recovery may be due to the detrimental effect of progesterone on oviduct micro environment (Nancarrow and Hill 1995).

Estimation of time of occurrence of LH peak enabled us to ascertain if presence of norgestomet implant suppressed the endogenous LH surge or not. As expected there was a significant ($P < 0.01$) delay in the occurrence of LH peak in group 2 cows. The LH peak occurred 48.00 ± 1.95 h post PGF₁ (36 h after implant removal) in control group as against 72.00 ± 2.62 h in LH suppression group. In other words, following administration of exogenous LH at 60h post PGF₁, cows in group 2 exhibited LH peak in 12.00 ± 1.4 h. However no significant differences were observed in the mean basal and peak LH levels between the 2 groups. In line with our observation in group 1 cows, Lafri *et al.* (2002) reported LH peak at 31.9 ± 0.7 h after implant removal in CIDR-B synchronised cycle. Our observation in group 2 cows is also in agreement with earlier report that LH surge was suppressed till the removal of PRID and the surge occurred in 2h after removal of PRID and simultaneous administration of GnRH which lasted for 6–8 h (Vos *et al.* 1994). Thus the delayed occurrence of LH peak in this study might have provided additional time for the follicular growth, which was evident by higher ovulation rate in group 2 donors.

The mean duration of LH surge was significantly shorter in LH suppression group (6.0 ± 1.36 h) compared to control group (11.2 ± 0.8 h). In agreement with our observation an earlier report (Van de Leemput *et al.* 2001) observed less duration of LH surge (6.0 ± 0.9 h) in NOR/eCG/GnRH group compared to control group (9.8 ± 0.9 h).

The onset of standing estrus (h post PGF₁) was significantly ($P < 0.01$) delayed in group 2 than in group 1 (69.6 ± 3.12 vs. 43.0 ± 1.5) donors. However, the duration of estrus was not different between groups ($P > 0.05$). The delayed onset of standing heat in treatment group 2 may also be related to the presence of NOR implant for an additional period of 60 h after PGF treatment. In line with our observation, Burns *et al.* (1993) reported suppression of estrus in cyclic cows till the norgestomet implant is removed.

It may be concluded that prolonged exposure to

progestational compounds suppress the LH surge; ensured more follicular growth as revealed by higher ovarian response and had no deleterious effect on viable as well as freezable embryo yield.

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