CIRCULATING OVARIAN STEROID PROFILES IN SURTI BUFFALOES SUPEROVULATED WITH FSH AND DIFFERENT DOSES OF PMSG

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ABSTRACT

Eighteen Surti buffaloes of 2 to 6 lactations were superovulated during breeding season (August to February) using FSH and 2 doses of PMSG. Animals were divided into three groups. Group I (n=6) and Group II (n=6) donors were superovulated with single i/m dose of 2500 and 3000 IU PMSG, respectively, on day 10 of the estrous cycle, whereas Group III (n=6) donors were treated intramuscularly with 400 mg FSH per donor in 8 equal doses 12 hours apart for four days. Jugular samples were collected from all donors at five different stages of MOET, i.e, Day 0 (Heat), Day 10 (PMSG treatment), Day 12 (2^{nd} $PGF_{2\alpha}$ inj.), Day 14 (Superovulatory estrus) and Day 20 (Flushing) for serum separation. No significant variation was found in serum concentrations of progesterone and estradiol-17ß among three groups of donors during various stages of MOET. However, the serum concentrations of progesterone and estradiol-17ß at various stages of MOET within the group varied significantly ($p<0.05$). The number of CLs had significant positive correlations with the serum progesterone ($r = 0.125$) and estradiol-17ß ($r = 0.482$) profile on pooled basis. The mean serum concentrations of progesterone were found to be highest on the day of embryo collection (1.83 ± 0.63; 3.01 ± 0.23 and 3.35 ±0.68 ng/ml) in 3 groups (Group I, II and III, respectively) of donors, whereas the mean serum concentrations of estradiol-17ß were found to be the highest on the day of superovulatory estrus (42.67 ± 5.44; 46.17 ± 6.27 and 50.17 ± 7.12 pg/ml, respectively).

Key words: Buffalo, MOET, Serum progesterone, Estradiol-17ß, Superovulatory response.

INTRODUCTION

The most well-known effect of eCG-induced superovulation is the increase in plasma progesterone and estradiol-17ß concentrations during superovulation (Alcivar *et al.*, 1992). Progesterone concentrations increase during the luteal phase of the cycle and remain higher in eCG-stimulated animals even after prostaglandin-induced luteolysis. Progesterone concentrations are not significantly affected by superovulation with FSH preparations containing little LH contamination (Folltropin, for example). Superovulation also increases plasma estradiol-17ß concentrations, and generally the stimulation is greater with eCG than with FSH. As the ruminant CL does not secrete estradiol-17ß, this steroid is clearly of follicular origin. Superovulation increases the number of growing follicles, thus the increase in plasma estradiol-17ß concentrations could be the result of an increase in healthy estrogen-secreting follicles, or it could be due to a direct stimulation of steroidogenesis in the follicles present. The increase in plasma estradiol-17ß observed in FSH-stimulated animals compared to non-stimulated animals is likely due to an increased number of follicles (Goff *et al.*, 1986). Owing to the long half-life of eCG, follicles are still being stimulated after ovulation, and estradiol-17ß levels remain high. High early luteal-phase concentrations of estradiol-17ß have deleterious effects on the oviduct, decreasing fertility. The very high estradiol-17ß concentrations seen in FSH stimulated buffalo may also be involved in the poor embryo recovery rates observed in this species. It is believed that high estradiol-17ß levels set off the prostaglandin-induced luteolytic cascade (Armstrong, 1993). Hence, the study

was carried out to know the steroid hormone profile (progesterone and estradiol-17ß) at different stages of MOET in Surti buffaloes.

MATERIALS AND METHODS

Eighteen donor buffaloes with age group of 6 to 14 years and of 2 to 6 lactations were used in this experiment. Donor animals were subdivided into group-I, II & III, having six buffaloes in each group. Group-I and II buffaloes (n=6 each) were superovulated with PMSG (Folligon®, Intervet India Pvt. Ltd.) @ 2500 and 3000 IU/animal in single i/m dose, respectively. Group-III buffaloes (n=6) were superovulated with FSH (Folltropin®-V, Bioniche, Canada) @ 400 mg/animal given i/m at 12 hours intervals in 8 equal divided doses for four days. The superovulatory estrus in donor animals was induced at 48 hours of superovulatory treatment by i/m administration of 25 mg Tiaprost tromethamine (Iliren®, Intervet India Pvt. Ltd.) in all the groups. At superovulatory estrus, donor buffaloes were bred three times at 12 hours intervals by AI using fresh liquid semen. All the buffaloes of group I, II and III were injected with hCG (Chorulon®, Intervet India Pvt. Ltd., India) @ 1500 IU on the day of superovulatory estrus to improve ovulation rate and transport of embryos into the uterus. Flushing was performed in all the donors on day 6 of superestrus.

Jugular blood samples were collected from all the donors over 5 stages, viz. on day 0 (heat), day 10 (PMSG treatment), day 12 (2nd PG injection), day 14 (superovulatory estrus) and day 20 (flushing). The serum separated out was stored at -20°C till analysis.

The progesterone and estradiol were estimated by radioimmunoassay (RIA) employing standard technique of Kubasik *et al.* (1984) and Robertson *et al.* (1979) respectively. Labelled antigen (with I^{125}), antibody coated tubes and standards were procured from M/S immunotech (A Beckman Counter Company) France. These tubes were read in gamma counter (IC 4702, I125 gamma counter ECIL, INDIA). The values of progesterone were calculated against a standard curve of 0.1 to 40 ng/ml on the logit log paper and represented as ng/ml. Whereas the values of estradiol were calculated against a standard curve of 8 to 5000 pg/ml on the logit log paper and represented as pg/ml.

Differences between means of hormone profile between groups and between stages were compared using simple and two factors factorial completely randomized design (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The mean values of the serum progesterone and estradiol-17ß in all 3 groups of donors are given in Table 1. No significant variation was found in serum concentrations of progesterone and estradiol-17ß among three groups of donors under study during various stages of MOET. However, the serum concentrations of progesterone and estradiol-17ß at various stages of MOET within the group varied significantly ($P<0.05$).

In Group I buffalo donors treated with 2500 IU PMSG (Table 1), the serum concentrations of progesterone in stage II (initiation of multiple ovulations), III ($PGF_{2\alpha}$) and V (flushing) were significantly higher ($P<0.05$) than in stage I (synchronous estrus) and IV (first AI), whereas the serum concentration of estradiol-17ß in stage IV was significantly higher ($P<0.05$) than in other stages (Stage I, II, III and V). The mean numbers of CLs and unovulated follicles palpated in this group were 2.67 and 1.67. No embryo was recovered from this group.

In Group II donors treated with 3000 IU PMSG (Table 1), the serum concentrations of progesterone in stage II (initiation of multiple ovulations), III (PG2 inj.) and V (flushing) were significantly higher ($P<0.05$) than in stage I (synchronous estrus) and IV (first AI), whereas the serum concentration of estradiol-17ß in stage IV was significantly higher ($P<0.05$) than in stage II and stage III. The mean numbers of CLs and unovulated follicles palpated in this group were 3.50 and 2.17. Only one good quality embryo was recovered from this group.

In Group III donors treated with FSH-P, serum progesterone concentrations was significantly higher ($P<0.05$) in stage V (flushing), III ($PGF_{2\alpha}$) and stage II (initiation of multiple ovulations), than in stage I (synchronous estrus) and IV (first AI), whereas the

serum concentration of estradiol-17ß in stage IV(first AI) and V (flushing) was significantly higher (P<0.05) than in the stage I and the stage II (Table 1). The mean numbers of CLs and unovulated follicles palpated in this group were 4.67 and 1.00. Only one degenerated embryo was recovered from this group.

Out of total five stages of blood collection, only one stage (Stage V) that is on the day of flushing was subjected to group-wise correlation analysis to estimate whether the levels of serum progesterone and estradiol-17ß can explain the variation in the number of ovulations. The number of CLs had significant positive correlations with the serum progesterone (r= 0.125) and estradiol-17ß (r= 0.482) profile on pooled basis, irrespective of three groups.

Under the present study, the mean serum concentrations of progesterone were found to be highest on the day of embryo collection (1.83 ± 0.63; 3.01 ± 0.23 and 3.35 ±0.68 ng/ml) in all the 3 groups (Group I, II and III, respectively) of donors, whereas the mean serum concentrations of estradiol-17ß were found to be the highest on the day of superovulatory estrus (42.67 ± 5.44; 46.17 ± 6.27 and 50.17 ± 7.12 pg/ml, respectively). Hammam and Salama (1997) reported the highest level of plasma progesterone on the day of embryo collection, whereas Raghava *et al.* (1997) reported highest level of plasma estradiol-17ß on the day of superovulatory estrus. Their findings are in collaboration with the results of the present study.

The unovulated follicles observed in present study occur both with FSH as well as PMSG superovulation treatment. The Group III donors treated with FSH-P had lowest unovulated follicles followed by Group I & II buffalo donors, treated with 2500 & 3000 IU PMSG. The most probable cause seems to be the hormonal asynchrony particularly the LH surge. The changes prerequisite to a successful ovulation do not follow and unovulated follicles and cystic ovaries are manifested. Missing LH surge has been described by Bevers and Dieleman, 1987 in PMSG superovulated animals. Petr *et al.*(1991) observed that preovulatory LH surge fail to occur in 4 PMSG superovulated cows despite GnRH treatment. This suggests possibilities of gonadotrophin surge inhibitory factors from preovulatory follicles. Unovulated follicles probably fail to acquire LH receptors in granulosa cells and this might happen due to number of reason as for example, atresia of follicles, gonadotrophic status of animal and local secretions in the ovaries affecting gonadotrophic uptake of growing follicles.

Despite satisfactorily accomplished flushing in the present study, overall embryo recovery rate was poor. Many workers confronted with situation like this, where a proportion of donors did not yield embryos (Bielanski and Yadav, 1990; Reinhard and Rohn, 1992). As observed by Maurer and Echtemkamp (1992) donor which did not yield embryo might have a delayed onset of LH peak, low magnitude of LH surge and low peripheral plasma progesterone concentration. This hormonal asynchrony might produce an undesirable uterine environment for male and female gametes or embryo which leads to fertilization failure or embryonic death. Besides that high estrogen: progesterone ratio affect the embryo transport within fallopian tube (Misra *et al.*, 1998), low number of primodial follicles (Danell, 1987) and poor superovulatory response in buffaloes(Madan *et al.*, 1996) are responsible for poor embryo recovery.

In conclusion the results suggest that there is possible underlying role of sound profile of ovarian circulating steroids contributing towards better results of MOET with FSH than both the doses of PMSG (2500 IU and 3000 IU). The numbers of unovulated follicles on the ovary were more after treatment with PMSG than FSH. The effects on the reproductive system of various endocrine changes should not be ignored, as they may contribute to the great variation encountered in standard superovulatory protocols.

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