Changes in Plasma Levels of Inhibin and Follicle Stimulating Hormone in Buffaloes Superovulated with eCG[a]

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ABSTRACT : The present study was undertaken to investigate the effect of stimulation of follicular development with eCG on the peripheral levels of inhibin and FSH in Murrah buffaloes. Estrus was synchronized in five normally cycling females by insertion of Crestar (Intervet, Boxmeer, Holland) implants for nine days. Estradiol valerate was administered i.m. on the day of implant insertion. On the 10th day of the induced estrous cycle a single dose of 3000 IU eCG (Folligon, Intervet, Boxmeer, Holland) was given, followed by treatment with 25 mg of PGF_2 alpha (Lutalyse, Upjohn, Belgium) 48 h later. Blood samples were obtained during the induced estrus, on cycle day 10 (luteal phase), at the superovulatory estrus (43 h after PGF) and during the periovulatory period (64 h after PGF). Ultrasonography was done daily to monitor follicular development. Plasma concentrations of inhibin and FSH were determined by specific radioimmunoassays. Differences between mean±SEM values of different phases of the cycle were compared by ANOVA. The mean number of small (2-5 mm), medium (6-9 mm) and large (>10 mm) follicles observed two days after eCG treatment and on the day of superovulatory estrus was 2.8±0.31, 5.2±0.30 and 1.4±0.09 and 1.9±0.21, 2.8±0.40 and 5.0±0.83, respectively. The mean number of ovulations was 3.6±0.37 and the mean number of unovulated follicles was 6.1±0.47. Most of the follicles >10 mm in diameter had ovulated (72%). The mean ±SEM of plasma inhibin concentration (2584.15±17.92 pg/ml) during the superovulatory estrus was significantly higher ($p \leq 0.05$) than during the induced estrus (749.87±17.29 pg/ml), the luteal phase (1099.54±24.98 pg/ml) and periovulatory period (1682.71±29.88 pg/ml), respectively. Mean±SEM plasma FSH concentration during the induced estrus (10.35±0.41 ng/ml) was not different from that during the superovulatory estrus (8.52±0.39 ng/ml), but was significantly higher ($p \leq 0.05$) than during the luteal phase (2.81±0.42 ng/ml) and periovulatory period (5.7±0.28 ng/ml). These data indicate that treatment with eCG in buffaloes for inducing superovulation results in a significant elevation in plasma inhibin levels and a decrease in plasma FSH levels during the superovulatory estrus.Thus, we suggest that the elevated plasma inhibin coming from fully developed follicles continued for a long time which results in inhibition of FSH leading to poor ovulation in the remaining follicles, which may be the cause of suboptimal superovulatory response. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 9 : 1205-1209*)

Key Words : Buffalo, Superovulation, Ultrasonography, Inhibin, FSH

INTRODUCTION

There cannot be two opinions about the fact that in livestock with relatively long generation intervals the most proven and viable technique to increase animal crop productivity is to go for superovulation, ova/embryo harvesting and transfer to a number of foster mothers for attaining several offsprings at a time. Various attempts to induce superovulation in buffaloes have met with little success, so much so Madan et al. (1988) considered even the presence of two or more corpora lutea after superovulatory treatment as a positive sign of superovulation unlike only three or more corpora lutea in the case of cows (Seidel, 1981; Kaneko et al., 1995) and investigations are being conducted to find out the reasons for this poor response at several centres including our laboratory. The differential feed back regulation of FSH had not been searched because GnRH was regarded the sole regulator of this hormone. Inadequacy of estradiol-17β as the only negative feedback signal for regulation of serum FSH levels has been established by controlled experiemnts (Butcher, 1977; Welschen et al., 1978). Lately, inhibin a glycoprotein produced by ovarian granulosa cells has been found to be implicated in the feed back regulation in the species studied so far. The radioimmunoassay for inhibin has been developed and circulating inhibin levels in various species of animals reported (Hamada et al., 1989; Taya et al., 1991; Kaneko et al., 1992, 1995). Kaneko et al. (1990) demonstrated that inhibin in peripheral blood rises drastically in cows induced to superovulate with porcine FSH and equine gonadotrophins (Kaneko et al., 1992). Sreenan et al. (1987) reported that immunization against inhibin in ewes resulted in enhanced ovulation rates. The present investigation was, therefore, undertaken to investigate the effect of stimulation of follicular development with eCG on

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peripheral levels of inhibin and FSH in buffaloes.

MATERIALS AND METHODS

Animals

The experiment was conducted on five (5-7 years old) non-lactating cyclic female Murrah buffaloes (*Bubalus bubalis*) at the regimental dairy of the Army Equine Breeding Stud at Hisar. The animals were fed twice daily with *ad libitum* green fodder. The concentrate feed of crushed green oats, barley and wheat bran containing recommended amount of mineral mixture and salt was given as supplement and the animals also had free access to water. The animals were induced into a synchronous estrus by implantation of Crestar (Intervet, Holland) containing Norgestomet with simultaneous 2 ml i.m. administration of oestradiol valerate. All animals exhibited signs of estrus within 34-44 h after implant removal on 9th day. Estrus was confirmed by using visible signs, teaser bull parading and ultrasound scanning for the presence of follicles on the ovary (Ultrasound Scanner Vet 200, Pie Medical, The Netherlands).

Superovulatory regime

A single i.m. injection of eCG (Folligon : Intervet : Holland) comprising a total dose of 3000 IU per animal was given during mid-luteal phase (day 10 of induced estrous cycle) followed by administration of 25 mg of PGF2α (Lutalyse:Upjohn:Belgium), 48 h after the eCG injection.

Ultrasonography

Follicle recruitment and development were monitored ultrasonographically daily after administering superovulatory treatment on day 10 of the Norgestomet induced estrous cycle (day of estrus = day 0). The follicles were categorised into three groups, small (2-5 mm), medium (6-9 mm) and large ($\geq$10 mm). One day after the superovulatory estrus i.e., around 72 h post-PG administration the ovarian response was monitored for the occurrence of ovulations and the follicles which were not ovulated are termed as unovulated follicles (>6 mm).

Blood sampling

The animals were fitted with indwelling jugular vein catheters (Silastic^R : Dow Corning Corporation, Midland Michigan, USA) as and when required at least 24 h before sampling commenced. Blood sampling at 1 h interval was done for five hours as per following schedule (a) on initiation of estrus after removal of ear implants. (b) on day 10 of induced estrous cycle (luteal phase) (c) on the day of superovulatory estrus after expression of heat symptoms by animals starting 43 h after PG administration (d) one day after the superovulatory estrus starting 64 h after PG injection. These samples were termed as samples around 'superovulation'. Blood was collected in heparinized tubes, plasma separated and stored frozen at -20°C pending hormonal analysis.

Assays

Plasma i.r. inhibin levels were measured by a sensitive heterologous double antibody RIA (Hamada et al., 1989) using purified bovine 32 kDa inhibin for iodination, rabbit antiinhibin antiserum (TNDH-1) and partially purified bovine inhibin as reference preparation. The cross reactivity of various inhibin related peptides and other peptide hormones in the assay system have been previously reported (Hamada et al., 1989). The sensitivity of the inhibin RIA was 3.9 pg/tube based on 95 per cent confidence limit of the zero standard. The intra and inter assay coefficient of variations were 7.2 and 14.6 per cent, respectively.

Plasma FSH levels were estimated using a modified heterologous double antibody RIA as described by Razdan et al. (1982) in this laboratory. Plasma FSH levels were determined using antiovine FSH serum (NIDDK anti oFSH1 (AFP 528113), NIDDK oFSH-1 AFP 75714 for radioiodination and NIDDK oFSH RP-1 as reference standard. The sensitivity of RIA for FSH was 0.125 ng/tube and the intra and interassay coefficient of 5.2 per cent and 10.2 per cent for FSH.

Calculations and statistics

The data obtained for particular hormones in all four phases of superovulation studied in particular animal was tabulated and was expressed in mean±SEM. The data obtained were statistically analysed through an ANOVA for split plot design treating the animals as plots and each period of sampling as sub-plots. The hypothesis was tested by mean sum of squares of animals X phase interaction as the error term. And the differences between means were tested by Duncan's multiple range test.

RESULTS

Onset of induced and superovulatory estrus

All animals implanted with Crestar exhibited in estrus within 34-44 h after the implant removal. Superovulatory treatment was administered on day 10 of induced estrous cycle. Within 43 h after PG administration all animals were observed to be in superovulatory estrus i.e. estrus after eCG treatment. The data on ovarian response is summarised in table 1. Results contained in this table shows that there are relatively a large number of follicles which do not ovulate after superovulatory treatment.

Table 1. Ovarian response to exogenous hormone during a superovulatory regime in buffaloes

Animal No.	Ovary	50 h post superovulatory treatment			During superovulatory estrus			72 h post PG administration	
		Small 2-5 mm	Medium 6-9 mm	Large >10 mm	Small 2-5 mm	Medium 6-9 mm	Large >10 mm	Ovulated	Unovulated
56	Right	2	4	1	2	2	5	5	5
	Left	2	3	1	1	1	3	2	3
105	Right	2	2	0	1	2	2	2	2
	Left	1	2	1	1	1	1	1	3
245	Right	1	2	1	1	1	2	2	3
	Left	2	2	1	0	1	2	2	3
388	Right	1	2	1	1	2	2	1	2
	Left	1	4	0	1	1	3	3	2
465	Right	1	2	0	1	1	2	1	3
	Left	1	3	1	0	0	3	2	3
Mean ± SEM		2.8±0.31	5.2±0.30	1.4±0.09	1.9±0.21	2.8±0.40	5.0±0.83	3.6±0.37	6.1±0.47

Circulating inhibin

Plasma inhibin levels ranged between 593.68 to 3257.46 pg/ml during various phases of experimentation. Mean±SEM plasma inhibin concentration during superovulatory estrous period were found to be significantly higher ($p<0.05$) than induced estrous period, luteal phase and periovulatory periods, respectively. Mean±SEM and ranges during different periods are presented in table 2.

Circulating FSH

Plasma FSH levels ranged from 1.67 to 18.39 ng/ml during various phases of experimentation. Mean±SEM and range during four periods are presented in table 2.

DISCUSSION

The onset of estrus after $PGF_{2\alpha}$ administration was between 34-44 h amongst the animals studied. Similar findings have been reported in buffaloes by other workers (Madan et al., 1988; Singh et al., 1988; Taneja et al., 1995). Ultrasonic imaging of ovaries 72 h after $PGF_{2\alpha}$ injection confirmed the ovulations reported in table 1. Mean number of ovulations 3.6±0.37 observed in this study are comparable to those reported earlier (Vlahov et al., 1986; Madan et al., 1988; Sodhi et al., 1997). However, others (Jain et al., 1988; Matharoo et al., 1996) reported lower number of ovulatory follicles during superovulation with eCG.

The mean FSH levels reported in this study during induced estrous period were lower than the ones reported earlier (Razdan et al., 1982) during hotter and cooler months. Possibly the process of artificial induction of estrus in buffaloes does not stimulate the hypophyseal gonadal axis to the same extent as at the onset of a normal estrus. However, the levels are comparable to the report (Madan et al., 1988) during induced estrous period. Mean FSH levels around superovulation were significantly lower ($p<0.05$) than superovulatory estrous and induced estrous periods.

Peripheral inhibin concentrations during induced estrus and luteal period were of the same order as reported earlier (Palta et al., 1996). Treatment with eCG 48 h before the administration of PG for luteolysis caused a marked increase in peripheral inhibin concentration during superovulatory estrous period compared to induced and luteal periods. This outcome is similar to earlier finding in which peripheral inhibin concentration were reported to be significantly higher in cows treated for superovulation

Table 2. Mean±SEM plasma concentrations of inhibin (pg/ml) and FSH (ng/ml) during different phases of superovulation in buffaloes

Hormone		Induced estrus (Day 0)	Luteal phase (Day 10)	Superovulatory estrus (43 h after PG)	Around superovulation (64 h after PG)
Inhibin	Range	593.68-911.32	647.45-1443.59	1677.24-3257.46	1137.19-2214.10
	Mean	749.87 ± 17.29^{D}	1099.54 ± 24.98^{C}	2584.15 ± 17.92^{A}	1682.71 ± 29.88^{B}
FSH	Range	7.42-18.39	1.67-6.43	5.37-16.42	3.94-9.77
	Mean	10.35 ± 0.41^{A}	2.81 ± 0.42^{C}	8.52 ± 0.39^{A}	5.71 ± 0.28^{B}

Note: Mean bearing dissimilar superscripts differ from each other ($p\leq0.05$).

with FSH (Kaneko et al., 1990) or eCG (Kaneko et al., 1992) and with eCG and neutra eCG in buffaloes (Palta et al., 1997). This elevation in inhibin levels is a consequence of a eCG induced increase in the number of antral follicles, since antral follicles have been reported to be the primary source of peripheral inhibin in sheep, cattle and buffalo (Mann et al., 1989; Rodgers et al., 1989; Campbell et al., 1991; Palta et al., 1992).

A significant increase in inhibin concentraton during superovulatory estrous period results in the suppression of plasma FSH concentration during this period. The source of inhibin is fully developed follicles. Formation of follicles after eCG treatment continued for a prolonged period, which results in increased levels of inhibition. This in turn suppresses ovulation of remaining follicles. Kaneko et al. (1992) observed an increase in inhibin levels concomitantly along with estradiol-17β levels in cows superovulated with eCG and suggested that both inhibin and estradiol-17β act in combination to cause suppression of FSH. Takedomi et al. (1995) in cows reported that after superovulation with single injection of pFSH dissolved in polyvinylpyrolidone (PVP), the inhibin levels were markedly increased which subsequently suppressed the release of bFSH from pituitary into circulation.

From the present study it could be concluded that treatment with eCG in buffalo for inducing superovulation results in a significant elevation in plasma inhibin levels which has resulted in inhibition of FSH levels resulting in large number of unovulated follicles, which may be the cause of suboptimal superovulatory response in buffaloes.

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