Effects of GnRH on the Plasma FSH, LH and Estradiol Levels at Estrus Induced with Injection of $PGF_{2\alpha}$ and eCG in Prepubertal Buffaloes (*Bubalus bubalis*)

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ABSTRACT : The experiment was conducted to study the effect of GnRH administration at induced estrus on pituitary and ovarian response in buffalo heifers. Eight Murrah river buffaloes of 12 to 13 months of age were treated with $PGF_{2\alpha}$ and eCG combination. GnRH (Fertagyl) 200 ug was injected (iv) at estrus in four heifers (treated group) while saline (2 ml, iv) was injected in remaining four heifers (control group). Blood was collected through jugular catheter to estimate plasma FSH, LH and estradiol level. The pretreatment plasma FSH, LH and estradiol values ranged from 8.46 ± 1.97 ng/ml to 12.31 ± 1.30 ng/ml, 0.87 ± 0.21 ng/ml to 1.19 ± 0.29 ng/ml and 19.09 ± 2.38 pg/ml to 20.24 ± 1.00 pg/ml respectively. The plasma estradiol concentration elevated significantly ($p<0.05$) within 24 hr after eCG administration and reached its peak levels of 154.09 ± 17.28 pg/ml and 181.95 ± 31.82 pg/ml at estrus in respectively treatment and control groups. The plasma FSH and LH concentrations did not increase during follicular development after eCG administration while initial significant ($p<0.05$) increases in both plasma FSH and LH concentrations occured within 5 and 10 min, reaching peak levels of respectively 110.06 ± 23.56 ng/ml and 13.15 ± 3.13 ng/ml within 90 min after GnRH injection was detected. A sharp and significant decline in plasma estradiol concentration (59.27 ± 8.78 pg/ml) associated with synchronized ovulation within 24 hours after GnRH injection was recorded. The observation suggest that the hypophysis of prepubertal buffaloes treated with eCG have gonadotrophins awaiting the releasing factor to evoke release of gonadotrophin during the follicular phase to induce synchronized ovulation. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 7 : 897-900*)

Key Words : Superovulation, GnRH, Gonadotrophins, Estradiol, Buffalo Heifers

INTRODUCTION

Despite consistent efforts during to augment the reproductive potential of buffaloes through embryo transfer technology, problems like weak ovarian response, high incidence of unovulated follicles and low number of embryo recovery remain unresolved. In spite of the availability of more large and medium sized follicles in the ovaries of buffaloes than cows before superovulation the total number of ovulations in buffaloes are less, and the number of large size follicles are more than in the cow at the time of flushing; this reveals an ovulation problem in buffaloes (Manik et al., 1998). The interval from onset of estrus to ovulation is spread over 100 hours in buffalo heifers receiving eCG as super- ovulating compound (Singh and Madan, 1998). The temporal relation of hypophyseal hormones with folliculogenesis and ovulation in superovulated buffaloes are not known well enough to relate the release patterns of hypophyseal and gondadal hormones coincident with folliculogenesis and ovulation. The present experiment was therefore undertaken in buffalo heifers receiving eCG as superovulating compound to study the pattern of circulating plasma FSH, LH and estradiol concentration during folliculogenesis and ovulation with and without GnRH administration at induced estrus, keeping in view that GnRH induces FSH and LH release (7 to 8 times) in untreated buffalo heifers (Singh and Madan, 1998a) and the preovulatory LH surge at estrus (Hafez, 1987) triggers the mechanisms of ovulation in farm animals.

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MATERIALS AND METHODS

Experimental animals and treatment

Eight, 12 to 13 month old Murrah buffaloes (*Bubalus bubalis*) were selected from the Institute's herd. All heifers were injected with 30 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Lutalyse, Upjohn, Sussex, U.K) intramuscularly. This was followed on day 14 by 2500 IU equine chorionic gonadotrophin (eCG, Folligon, Intervet) intramuscularly. A second dose (25 mg) of $PGF_{2\alpha}$ was injected intramuscularly on day 16. Genitalia were examined by rectal palpation and a vasectomized bull was paraded at 3 hourly intervals to detect estrus and ovulation. Four heifers BS, DS, FS and Hs (treated group) were injected intravenously with synthetic GnRH (200 ug, Fertagyl, Intervet, Holland) while another four heifers AS, CS, ES and GS (Control group) were injected intravenously with normal saline solution (2 ml) 12 hours after the onset

of estrus. All the sychronizing and superovulatory drugs were injected into the animal between 8 am and 9 am.

Blood sampling

Blood samples were collected from animals in chilled heparin treated test tubes through jugular catheter 2 days and 1 day before the 1st prostaglandin injection, and on the day of prostaglandin injection. Thereafter, samples were collected once on day 1, 3, 4, 5, 8, 12, 14 (day of PMSG administration) 15, 16, 17 and 18 (a day before exhibition of estrus symptoms). On the day of estrus the blood samples were collected 1 hr before and just before GnRH or saline injection, 5 min, 10 min, 20 min, 30 min, 40 min, 60 min, 90 min. 2 h, 4 h, 6 h, and 8 h after GnRH/saline injection. Thereafter, blood samples were also collected once on day 1, 3, 5, 7, 9, 13 and 18 of GnRH/saline injection. Plasma was separated by centrifuging at 3000 rpm for 10 min at 4℃ and stored at -20℃.

Hormone assay

The follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol were estimated by sensitive radioimmunoassay as described earlier (Singh and Madan, 1998b). The accuracies of determination of 1.25, 2.5, 5.0, 10.0 and 20.0 ng FSH, or 0.50, 1.00, 1.50, 2.00, 2.50 and 5.00 ng LH, added to 0.1 ml plasma, and of 20, 40, 80, 160 and 320 pg estradiol added to 1.0 ml charcoal treated plasma, were 105.26, 97.60, 104.40, 101.80 and 99.55% for FSH, 102.00, 107.00, 108.67, 107.00, 101.60 and 95.80% for LH, and 103.40, 101.33, 105.68, 96.94 and 95.90% for estradiol (n=10). The sensitivity of the assay was 0.08 ng/tube for FSH, 0.02 ng/tube for LH and 2.50 pg/ml for estradiol. The average intra-assay and inter-assay coeffient of variation was 4.5% and 4.9% for FSH, 5.1% and 6.1% for LH and 9.2% and 8.8% for estradiol. Paired t tests to compare the difference of mean of FSH and LH pre and post treatment, and two way analysis of variance to compare the difference of means of hormones, were calculated as described by Snedecor and Cochran (1967).

RESULTS

Follicle stimulating hormone (FSH)

The mean pretreatment plasma FSH level (basal value) in four heifers of the experimental group on day 2, day 1, and on day 0 of 1st $PGF_{2\alpha}$ injection was 9.89 ± 1.30, 9.35 ± 2.10 and 8.93 ± 1.93 ng/ml while in the control group i.e. was 8.46 ± 1.97, 12.31 ± 1.31 and 9.90 ± 1.61 ng/ml. The values of plasma FSH were maintained at similar levels in both experimental (9.74 ± 1.71 ng/ml to 11.47 ± 7.30 ng/ml) and control (7.47 ± 1.68 ng/ml to 12.26 ± 5.04 ng/ml) groups of heifers after eCG injection. The plasma FSH elevated significantly ($p<0.05$) at 5 min and reached peak level (110.06 ± 23.56 ng/ml) by 90 min; the value declined to pretreatment level by 6 h after GnRH injection. Thereafter, lower values ranged between 4.13 ± 1.03 to 9.12 ± 2.40 ng/ml throughout the experiment (figure 1). The mean peak value and area under peak of FSH in GnRH treated heifers were 116.25 ± 22.94 ng/ml and 6016.25 ± 1741.91 mm^2 respectively and were highly correlated (r=0.93). In two heifers the plasma FSH level started increasing by 40 min after GnRH injection and reached peak concentration of 79.34 ng/ml (BS) and 86.89 ng/ml (DS) by 90 min after GnRH injection. In the other two heifers the plasma FSH level elevated at 5 min and reached peak value of 118.55 ng/ml (FS) and 180.18 ng/ml (HS) by 60 min and 90 min respectively after GnRH injection.

Luteinizing hormone (LH)

The mean plasma LH concentration in the experimental group on day 2 and day 0 of 1st $PGF_{2\alpha}$ injection (figure 1) was 1.19 ± 0.29 and 1.03 ± 0.21 ng/ml while in the control group it was 0.87 ± 0.21 and 0.87 ± 0.19 ng/ml. The difference in pretreatment LH concentration of two groups was not significant ($p>0.05$). The plasma LH value maintained at similar level after PMSG injection. LH level elevated significantly ($p<0.05$) by 10 min, reaching peak level of 13.15 ± 3.13 ng/ml ($p<0.01$) by 90 min and declined to basal level by 4 h after GnRH injection. The mean peak value and area under peak were 16.99 ± 3.98 ng/ml and 7250.00 ± 2889.51 mm^2 respectively and were highly correlated. (r=0.82). Among control group heifers the basal value of FSH and LH was maintained throughout the experimental period.

Ovarian response

The $PGF_{2\alpha}$ injection did not have any influence on ovarian condition. The follicular development associated with significant ($p<0.05$) increase in plasma estradiol concentration was detected between 24 and 72 hours after eCG administration. After gradual increase the plasma estradiol concentration reached the peak value in both experimental (154.09 ± 7.28 pg/ml) and control 181.95 ± 31.82 pg/ml) groups on the day of estrus. A sharp and significant decline in estradiol concentration (59.27 ± 8.78 pg/ml) in the experimental group was detected by 24 h after GnRH injection while such a decline in estrogen in the same period after saline injection was not recorded in the control group (167.19 ± 31.25 pg/ml). The interval from onset of estrus to ovulation in the control group was long (30 to 100 hours) while in treated group the interval was

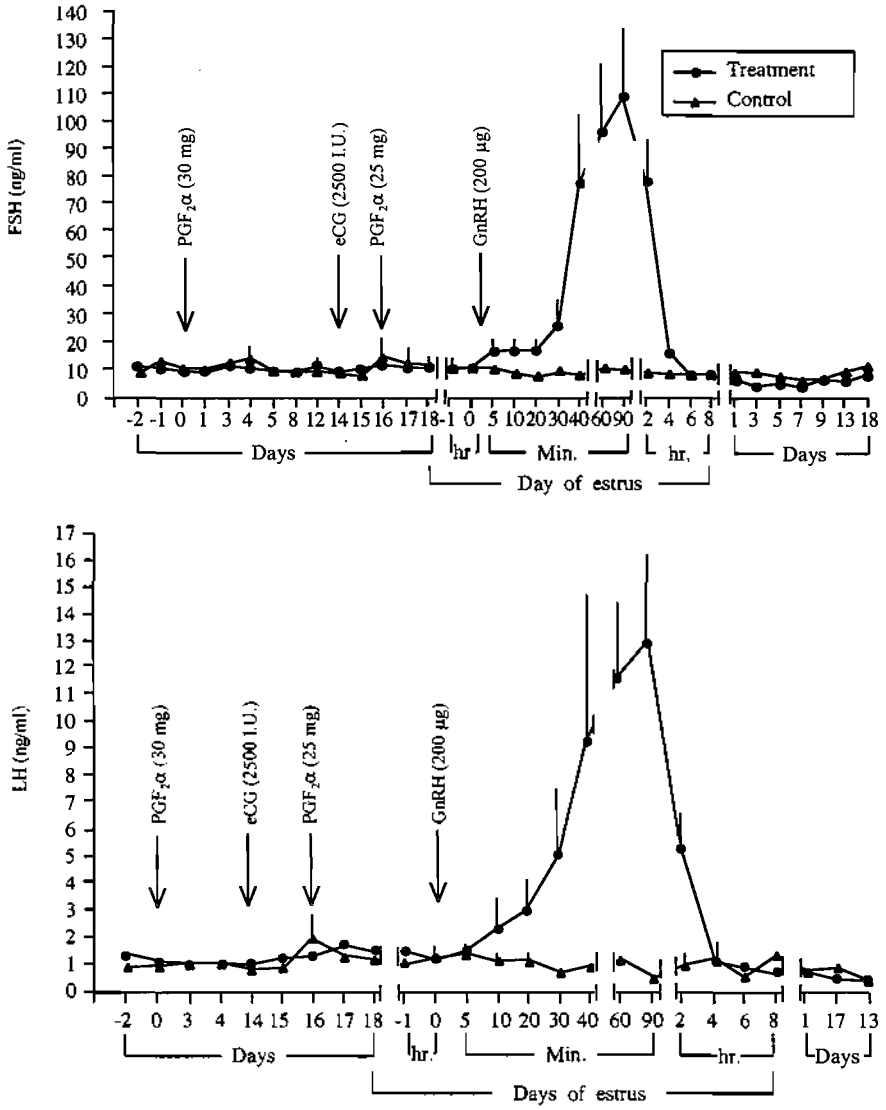

Figure 1. Changes in plasma levels of FSH and LH before and after GnRH injection at the induced estrus in prepubertal buffaloes treated with $PGF_2\alpha$ and eCG

short (26 to 36 hours) and ovulation was synchronized. However, the total numbers of ovulations in both treatment (5.5 ± 0.25) and control (4.75 ± 0.45) groups were similar ($p>0.05$).

DISCUSSION

The administration of $PGF_2\alpha$ did not have any effect on hypophyseal hormone release, folliculogenesis and gonadal hormone secretion However, the effect of eCG in respect to ovarian enlargement and progressive follicular development recorded in the present experiment was comparable to the observation recorded in cattle heifers (Takahashi, 1983) and cycling buffaloes (Schallenberger et al., 1990). The maintenance of a basal level of FSH during follicular development between administration of eCG and onset of estrus in present experiment was in contrast to the higher release of gonadotrophin during follicular development in eCG treated cows (Yadav et al., 1986). However, it was similar to the FSH concentration recorded during a corresponding period in eCG treated heifers (Moseley et al., 1984) and cycling buffaloes (Schallenberger et al., 1990). The non detectable preovulatory LH surge in the present experiment might be due to the fact that blood samples were not collected at frequent intervals around the onset of estrus; a preovulatory LH surge in buffaloes treated with eCG is expected to occur shortly before or just after the onset of estrus (Schallenberger et al., 1990). As another possibility, the LH surge might have not occurred during the follicular phase because the buffalo heifers of present experiment were prepubertal non-cycling. Their

hypophyseal-pituitary system needs pre-activation to respond to the elevated levels of estradiol for LH release because in the prepubertal animal receiving only PMSG as superovulating compound, LH always stayed at same basal level; the need to use HCG for optimising ovulation is well documented (Saumande, 1978). The interval from GnRH injection to initial significant elevation of plasma FSH and LH concentration, peak release of FSH and LH and area under peak in heifers of present experiment was higher than the buffalo heifers of similar age receiving only GnRH (Singh and Madan, 1998a). The higher peak release, and area under peak, of gonadotrophin (FSH and LH) after GnRH treatment in present experiment than the values reported earlier for FSH and LH (Singh and Madan, 1998a) could be due to the initial elevation of estradiol after PMSG administration before GnRH injection in present experiment which might have primed the pituitary gonadotrophs. This could have resulted in a progressive increase in pituitary responsiveness to GnRH by achieving an increase in GnRH recepter affinity that affected hypophyseal storage and a release pattern of gonadotrophin in buffalo heifers after stimulation from exogenous GnRH administration, as reported earlier in cattle heifers (Imakawa et al., 1986; Beck and Convey, 1977). Besides, a higher level of estradiol primes the pituitary tissue for increase in peak release of gonadotrophin but suppresses the pituitary tissues in eliciting a fast GnRH induced FSH and LH release (Padmanabhan et al., 1978). This might be the consequence of a longer interval (90 min) from GnRH injection to peak release of FSH and LH in the present experiment than the interval (10 min) from GnRH injection to peak FSH and LH release in buffalo heifers of the same age having lower basal level of estradiol (Singh and Madan, 1998a). During the present experiment too, there was a higher release of FSH and LH after administration of GnRH at induced estrus in two heifers (FS and HS) having higher plasma estradiol concentrations than in the remaining two heifers (BS and DS) with low circulating estradiol. This observation is supported by the evidence that the magnitude and duration of LH release after GnRH treatment in heifers is directly proportional to the circulatory levels of estradiol at the time of GnRH administration (Lucy and Stevenson, 1986). The induced release of FSH in the treatment group might have stimulated fast development of growing follicles while induced release of LH could have triggered the mechnisms responsible for ovulation, resulting into synchronized ovulation within 12 to 20 h after GnRH administration similarly to normal cycling animals (Hafez, 1987) and eCG treated buffalo heifers 24 months old (Singh and Madan, 1999). The results show that the gonads of prepubertal buffaloes have developed receptors for eCG, and the hypophysis has gonadotrophin which awaits the releasing factor GnRH from the hypothalamus to evoke its release during the follicular phase for synchronized ovulation in eCG treated buffalo heifers.

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REFERENCES

Beck, T. W. and E. M. Convey. 1974. LH after estradiol and GnRH in spayed heifers. J. Anim. Sci. 39:198 (Abstr.).

Hafez, E. S. E. 1987. Reproduction in Farm animals. 5th Ed. Lea and Fibigar, Philadalphia.

Imakawa, K., M. L. Day, D. D. Zalesky, M. Garcia-winder, R. J. Kittock and J. E. Kinder. 1986. Regulation of Pulsatile LH Secretion by ovarian steroids in heifers. J. Anim. Sci 63:162-168.

Lucy, M. C. and J. S. Stevenson. 1986. Gonadotropin-releasing hormone at estrus: Luteiruzing hormone, estradiol and progesterone during the Periestrual and Post - insemination in dairy cattle. Biol. Reprod. 35:300-311.

Manik, R. S., S. K. Singla and M. L. Madan. 1998. Comparative Follicular Dynamics in Superovulated Crossbred Cows and Water Buffaloes. Asian-Aust. J. Anim. Sci. 11:65-70.

Saumande, J. 1978. Relationship between ovarian stimulation by PMSG and Steroid secretion. In: Control of Reproduction in the Cow (Ed. J. M. Sreenan). The Hague, M. Mijhoff. pp. 169-194.

Schallenberger, E., H. G. Wagner, R. Papa, P. Hast and H. Tenhumberg. 1990. Endocrinological evaluation of the induction of Superovulation with PMSG in water buffalo (*Bubalus bubalis*). Theriogenology 34:379-392.

Singh, C. and M. L. Madan. 1998. Superovulation in buffalo heifers. Ind. J. Anim. Sci. 68:62-63.

Singh, C. and M. L. Madan. 1998a. Pituitary and gonadal response to GnRH in Prepuberal buffalo heifer (*Bubalus bubalis*). Asian-Aus. J. Anim. Sci. 11:78-83.

Singh, C. and M. L. Madan. 1998b. Hypophyseal and Gonadal response to GnRH in buffalo heifers (*Bubalus bubalis*). Asian-Aus. J. Anim. Sci. 11:416-421.

Singh, C. and M. L. Madan. 1999. Ovarian response to equine chorionic gonadotrophin (eCG) and GnRH in buffalo heifers (*Bubalus bubalis*). Ind. J. Anim. Sci. 69:490-493.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. 6th ed. Oxford and IBH Publishing Company, Calcutta, New Delhi.

Takahashi, Y. 1983. Superovulation with PMSG-PGF_2 alpha and non-surgical embryo recovery in Holstein Friesian heifers. Japanese J. Anim. Reprod. 29:74-79.

Yadav, M. C., J. S. Walton and K. E. Leslie. 1986. Plasma concentration of Luteinizing hormone and progesterone during superovulation using Follicle stimulating hormone and pregnant mare serum gonadotrophin. Thereogenology. 26:523-540.