

Pituitary and Gonadal Response to GnRH in Prepubertal Buffaloes (*Bubalus bubalis*)

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ABSTRACT: The objective of this study was to investigate the responsiveness of hypophysis and gonads to synthetic GnRH among prepubertal buffalo heifers at 12 months of age. Peripheral plasma FSH, LH, estradiol and progesterone level were measured in blood samples collected at 1 hr before and up to 18 days subsequent to the administration of 200 μ g GnRH (n=6) or saline (n=6) in Murrah buffalo heifers. The pretreatment peripheral plasma FSH, LH, estradiol and progesterone among GnRH treated heifers were 7.35 ± 0.45 ng/ml, 1.08 ± 0.3 ng/ml, 22.93 ± 1.06 pg/ml and 0.27 ± 0.04 ng/ml respectively. A quick elevation ($p < 0.01$) of FSH and LH within five min of GnRH administration was observed in

all heifers. Although the peak FSH (89.57 ± 23.43 ng/ml) and LH (7.52 ± 3.08 ng/ml) reached by 10 min of GnRH administration, yet the animals differed both in terms of their amplitude response of FSH and LH release as well as in terms of time which animals took to exhibit maximum response to GnRH administration. The GnRH administration did not cause alteration in plasma estradiol and progesterone level. The present study suggests that the pituitary of 12 month buffalo heifers has capacity to synthesize and store of gonadotropin and have developed receptors for GnRH for a spike of gonadotropin release.

(Key Words: GnRH, Response, Prepubertal Buffaloes)

INTRODUCTION

Buffaloes being predominant dairy animals contribute to a great extent in the economy of Asian countries where about 96% of world buffalo population are found. These animals suffer from certain acute limitation in reproductive phenomenon in terms of long prepubertal period of 2.5 to 3.5 years and is considered one of the causes limiting fecundity in buffaloes (Madan et al., 1983). Hypothalamus plays principal role for augmentation of pituitary function through its releasing hormone. The increased release of LH (Schams et al., 1981; Sysoev and Bogacleva, 1980; Gonzalez-Padilla et al., 1975b) and progesterone (Gonzalez-Padilla et al., 1975a) towards advancing age during prepubertal period considered to act as primer sensitizer to modulate hypothalamic hypophyseal system and intrinsic rhythm to bring about onset of puberty in animals. The organisation of micromolecular components of the pituitary gonadotrophs and subsequent gonadotropin secretion is continuously modified by changes in the feed back sensitivity of the hypothalamic hypophyseal system. The spontaneous

release of gonadotropin hormone from prepubertal to pubertal age determine the release pattern of hypothalamic hormone (GnRH) and sensitivity of hypophyseal gonadotrophs to GnRH. However, the extent of maturation and coordination of hypothalamo-hypophyseal system is obtained by the knowledge of both hypothalamic and pituitary activity in terms of hypothalamic and hypophyseal hormone release (Knobil 1980). The information on release pattern of pituitary and gonadal hormone is not enough to provide sufficient knowledge regarding the responsiveness of hypophysis and gonads to hypothalamic hormone during prepubertal period in buffaloes. To obtain this basic information is essential where the efforts are being made to augment the reproduction in buffaloes.

MATERIALS AND METHODS

Animals and treatments:

Prepubertal Murrah buffalo heifers (n=12) at age group of 11 to 13 months were used in this study. The animals were maintained under general herd managemental condition at animal farm of NDRI, Kamal, Two hundred μ g synthetic GnRH (Fertagyl) was

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Received May 6, 1997; Accepted August 18, 1997

administered intravenously through the catheterized jugular vein of 6 Murrah buffalo heifers (Experimental group) between 7 am to 9 am. Another six Murrah buffalo heifers received sterile saline (2 ml, intravenous) at an identical time schedule and were considered control group. Blood samples were collected in chilled heparinized tubes from the catheterized jugular vein at 1 hr before treatment, immediately before treatment and at 5, 10, 20, 30, 40, 60, 90, 120, 240, 360 and 480 min subsequent to GnRH and saline injections. Blood samples were also collected on 1st, 5th, 7th, 9th, 13th and 18th day of GnRH/saline injections. The plasma was separated and divided in different aliquots and stored at -20°C for subsequent analyses of FSH, LH, estradiol and progesterone.

A double antibody RIA standardized in our lab (Palta and Madan 1995) was used for estimation of FSH and LH levels in the plasma sample. Highly purified bovine FSH (B FSH subunit, USD-FSH-BP₃) and BLH (USDA bovine LH-I-1) was used for iodination (Niswender et al., 1969) and reference standard for FSH and LH. The anti FSH and anti LH serum developed in this laboratory (Palta and Madan, 1995) was used at final dilution of 1:15,000 and 1:100,000 respectively. The sensitivity of FSH and LH assay was 0.08 ng/tube and 0.02 ng/tube respectively. The intra and interassay coefficient of variation for FSH were 4.5% and 4.9% and for LH were 5.1% and 6.1% respectively. The progesterone in blood plasma was estimated by the method described earlier (Prakash and Madan, 1986). The sensitivity of the assay was 20 pg/tube. the intra-and interassay coefficient of variation were 4.9% and 5.7% respectively.

RIA based on the method of Echterncamp et al. (1976) was used for estimation of estradiol in blood plasma. Estradiol purchased from Sigma chemical company, St-Louis, USA and anti-serum (Estradiol DJB 186-2) was gifted by Dr. D. J. Bolt, Animal science institute, Beltsville, Maryland, USA and labeled estradiol (2, 4, 6, 7-³H Estradiol 17B) received from Amersham International PLC Amersham, U.K., were used for estradiol estimation. The estradiol was extracted from 1,000 μl plasma for one minute with 5 ml diethyl ether twice. The ether was evaporated to dryness. The residue was dissolved in 500 μl 0.1 M sodium phosphate buffer (pH 7.0) and 100 μl labeled estradiol (10,000 CPM) was added, vortexed and incubated at 4°C for 10 hours. 100 μl of antiserum (1:36,000) was then added to all tubes. The tubes were vortexed and incubated in water bath at 40°C for 30 minutes. The tubes were then transferred to ice water and incubated for 90 minutes. Five hundred μl of cold charcoal mixture (0.625% activated charcoal and 0.0625%

dextran suspension) under constant stirring was added to each tube. Tubes were then centrifuged at 3,000 rpm at 4°C for 10 minutes. The supernatant containing the antisera bound estradiol was decanted into glass scintillation vials. Ten ml of scintillation fluid (0.1 g popop and 4.0 g ppo in 1,000 ml toluene) was poured in all the vials and vials were tightly capped. The mixture was left for 12 hours at a room temperature. The hormone concentration in each vial was determined by liquid scintillation counter (LKB) programmed for microquantitation of hormones. The sensitivity of the assay was 2.5 pg/tube. The intra and inter-assay coefficient of variation were 9.2% and 8.8% respectively.

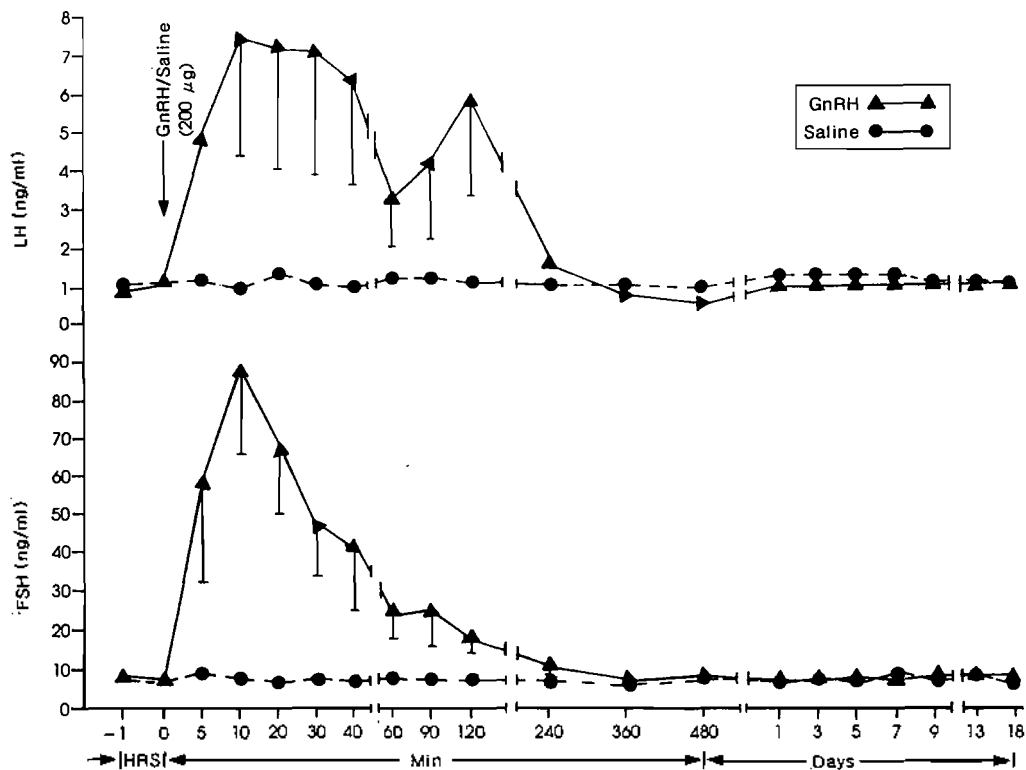
The pair 't' test to compare the difference of mean for FSH and LH pre and post GnRH treatment and the two way analysis of variance to compare the differences of means of hormones were obtained according to the methods described by Snedecor and Cochran (1967).

RESULTS

The mean pretreatment FSH and LH values in the experimental group heifers were 7.35 ± 0.45 ng/ml, 1.08 ± 0.08 ng/ml, respectively where as in control groups the values were 7.93 ± 0.43 and 1.05 ± 0.15 ng/ml, respectively. The difference between the pretreatment blood values of FSH and LH detected in both treatment and control group was statistically non-significant. All heifers receiving GnRH responded to the treatment uniformly and a quick elevation of FSH (52.71 ± 20.02 ng/ml) as well as LH (4.80 ± 1.86 ng/ml) within 5 min of GnRH administration was detected in each heifer of experimental groups. The peak release of FSH was uniform and reached at 10 min of GnRH administration (figure 1, table 1). However, the animals differed in terms of their amplitude of LH release as well as in terms of the time which the animals took to exhibit the maximum LH response. The peak levels of LH in four heifers were detected at 10 min while in remaining two heifers at 20 min of GnRH administration. In addition to the single fast elevation of LH peak, a phasic elevation of LH level first at 10 to 20 and 2nd at 120 min of GnRH injection was detected in 3 heifers. The values of FSH and LH declined to pretreatment levels by 240 to 360 min of GnRH administration. (figure 1, table 1). The peak release of FSH ($r=0.88$) as well as LH ($r=0.98$) and area under peaks of FSH and LH were highly correlated. The injection of saline in control group did not cause any alteration in FSH and LH levels.

Table 1. Plasma (Mean \pm SE) FSH and LH concentration following GnRH administration (200 ug, 0 hr) in 12 Month buffalo heifers

Time	FSH (ng/ml)		LH (ng/ml)	
	Treatment group (GnRH)	Control group (Saline)	Treatment group (GnRH)	Control group (Saline)
-1 hr	7.99 \pm 0.57	7.64 \pm 0.36	0.94 \pm 0.09	1.02 \pm 0.06
0 hr	7.35 \pm 0.45	7.93 \pm 0.43	1.08 \pm 0.08	1.05 \pm 0.15
5 Min	52.71 \pm 20.02	8.50 \pm 0.34	4.80 \pm 1.86	1.21 \pm 0.13
10 Min	89.57 \pm 23.43	7.94 \pm 0.33	7.52 \pm 3.08	1.05 \pm 0.15
20 Min	67.05 \pm 17.34	7.24 \pm 0.68	7.22 \pm 3.31	1.36 \pm 0.18
30 Min	46.35 \pm 12.36	7.69 \pm 0.41	7.09 \pm 3.41	1.12 \pm 0.22
40 Min	40.84 \pm 15.05	7.32 \pm 0.26	6.32 \pm 2.89	1.00 \pm 0.11
60 Min	25.05 \pm 7.52	8.18 \pm 0.35	3.17 \pm 1.02	1.19 \pm 0.11
90 Min	25.12 \pm 9.53	8.19 \pm 0.21	4.14 \pm 1.89	1.19 \pm 0.11
2 hr	18.00 \pm 3.42	7.71 \pm 0.49	5.92 \pm 2.66	1.01 \pm 0.14
4 hr	10.71 \pm 1.37	7.49 \pm 0.25	1.47 \pm 0.23	0.99 \pm 0.13
6 hr	7.59 \pm 0.54	7.43 \pm 0.50	0.79 \pm 0.08	1.00 \pm 0.09
8 hr	7.51 \pm 0.55	6.77 \pm 0.28	0.60 \pm 0.07	0.86 \pm 0.06
1st day	6.80 \pm 0.44	6.91 \pm 0.15	1.04 \pm 0.11	1.31 \pm 0.14
3rd day	7.39 \pm 0.56	7.32 \pm 0.65	0.92 \pm 0.07	1.01 \pm 0.19
5th day	7.61 \pm 0.43	7.86 \pm 0.67	1.06 \pm 0.10	1.18 \pm 0.22
7th day	7.39 \pm 0.38	8.16 \pm 0.26	0.97 \pm 0.10	1.20 \pm 0.08
9th day	7.73 \pm 0.52	7.38 \pm 0.35	0.95 \pm 0.15	1.06 \pm 0.08
13th day	7.88 \pm 0.81	7.89 \pm 0.33	1.07 \pm 0.14	1.07 \pm 0.13
18th day	7.58 \pm 0.51	6.83 \pm 0.56	1.03 \pm 0.10	1.00 \pm 0.08

**Figure 1.** Circulating plasma FSH and LH concentration in response to GnRH in prepubertal buffaloes.

The mean pretreatment value of plasma estradiol in treatment and control group was 22.93 ± 1.06 pg/ml and 21.20 ± 0.54 pg/ml respectively and the value of progesterone in same group was 0.27 ± 0.04 ng/ml and

0.26 ± 0.03 ng/ml respectively. The injection of GnRH and saline did not have any influence on estradiol and progesterone level (figure 2, table 2).

Table 2. Plasma (Mean \pm SE) estradiol and progesterone concentration following GnRH administration ($200 \mu\text{g}$, 0 day) in 12 month buffalo heifers

Time	Estradiol (pg/ml)		Progesterone (ng/ml)	
	Treatment (GnRH)	Control (Saline)	Treatment (GnRH)	Control (Saline)
0 day	22.93 ± 1.06	21.20 ± 0.54	0.27 ± 0.04	0.26 ± 0.03
1st day	22.68 ± 0.89	21.79 ± 0.65	0.20 ± 0.01	0.25 ± 0.03
3rd day	22.92 ± 0.76	23.18 ± 0.82	0.24 ± 0.03	0.22 ± 0.02
5th day	23.71 ± 0.46	22.47 ± 0.96	0.27 ± 0.04	0.29 ± 0.04
7th day	23.64 ± 1.38	21.92 ± 0.60	0.22 ± 0.02	0.23 ± 0.02
9th day	22.19 ± 0.64	23.25 ± 0.74	0.26 ± 0.04	0.24 ± 0.04
13th day	22.41 ± 0.42	22.18 ± 0.58	0.23 ± 0.03	0.22 ± 0.01
18th day	22.28 ± 0.75	21.53 ± 0.39	0.21 ± 0.02	0.18 ± 0.02

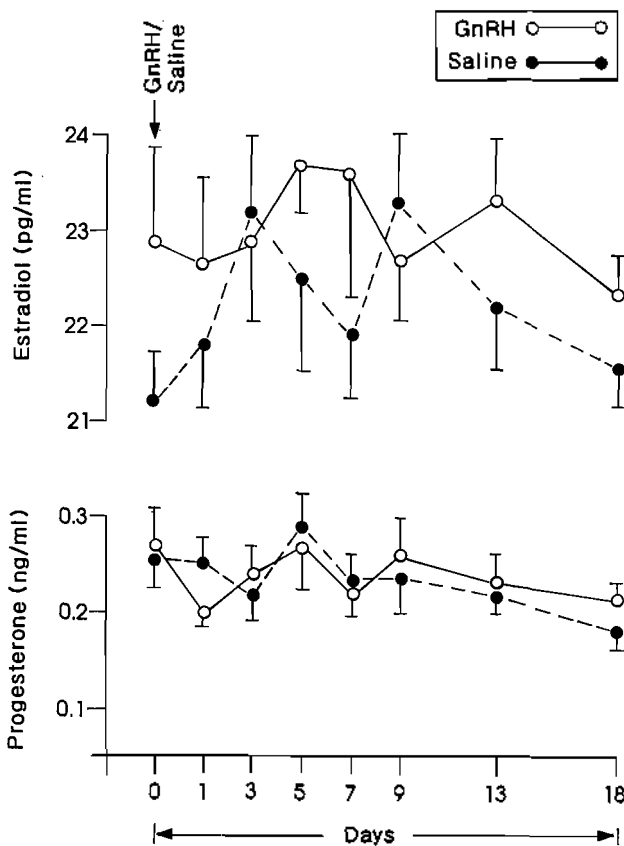


Figure 2. Plasma estradiol and progesterone concentration in response to GnRH in prepubertal buffaloes.

DISCUSSION

The plasma FSH concentration detected in 12 month-old buffalo heifers was lower than the value reported earlier in parous buffaloes (Razdan et al., 1985; Palta and Madan, 1995). The gradual increase in circulatory level of FSH from prepubertal period to approaching puberty has been observed in cattle heifers (Desjardin and Hafis, 1968). Detection of lower levels of FSH in 12 month buffalo heifers agree with the above value as FSH release in buffalo heifer remains at lower level during prepubertal period which increase to a higher level towards the onset of puberty (Razdan et al., 1985).

In fact GnRH even at lower doses of 5 to 80 μg acts directly on the pituitary to cause gonadotropin release in heifers (Zolman, 1973; William et al., 1975). Similarly when exogenous GnRH was administered in 12 month buffalo heifers a 12 fold increase in the peak release of FSH and a 7 fold increase in that of LH within 10 to 20 min of GnRH administration was detected. The pattern of FSH and LH release, the shape and slope of FSH and LH curve following GnRH administration recorded in our experiment was similar to the pattern of FSH and LH release after GnRH administration in heifers (Barnes et al., 1980; McLeod et al., 1984. and Zolman, 1973). However, the peak release of FSH and LH and time to attain peaks of FSH and LH release in 12 month buffalo heifers in our study was lower than the peak FSH and LH release and

time to attain peak release of FSH and LH during post partum and estrus buffaloes (Palta and Madan 1995) and buffalo heifers of 24 months and 36 months of age (Singh, 1988). This may be due to either lower concentration of receptors and readily releasable pool of gonadotropin at this age as the receptor concentration in cattle are dynamically changing with physiological stage and LHRH receptor levels are known to be depressed during the period of noncyclicality in heifers (Schoenemann et al., 1985). Besides phasic elevation of LH in 3 GnRH treated heifers suggests that the GnRH influence has an overriding effect on the phasic intrinsic pulsatile fluctuation in the LH release in buffalo heifers. Similar biphasic response having two LH peaks at the interval of 30 min (Madej et al., 1980) and at the interval of 60 min (Pichova et al., 1983) in GnRH treated heifers have been recorded.

The estradiol and progesterone concentration of 12 month-old buffalo heifers maintained at similar base level after 200 μ g GnRH administration may be due to the fact that single injection of GnRH was not sufficient to induce ovarian activity in 12 months buffalo heifers as observed in cattle heifers (Barnes et al., 1980). However, the estradiol values obtained in this study was higher than the values recorded in cattle heifers (Booth et al., 1975; Hill et al., 1972) while it is similar to the value of estradiol 17B recorded in 9 to 12 month old Murrah buffalo heifers (Jain and Pandey, 1985). The plasma progesterone value obtained in 12 month buffalo heifers is similar to the values reported in prepubertal cattle heifers (Gonzalez-Padilla et al., 1975b) and prepubertal Murrah buffalo heifers (Jain and Pandey 1985). There was no effect of GnRH administration on estradiol and progesterone release in these heifers.

ACKNOWLEDGEMENTS

We are grateful to Dr. D. J. Bolt, Anim. Sci. Inst., USDA, Beltsville, MD, USA for supplying us purified preparations of LH, FSH, and estradiol and to Dr. G. P. Talwar, Director, NII, New Delhi, for generous gift of progesterone monoclonal antibody.

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