Effects of $PGF_2\alpha$ and GnRH during Different Ovarian Status at Onset of Puberty in Murrah Buffalo Heifers (*Bubalus bubalis*)

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ABSTRACT : The objective of the investigation was to study the effect of intramuscular $PGF_2\alpha$ and GnRH on estrus behavior and ovarian response in Murrah buffalo heifers. Twelve Murrah buffalo heifers at 32 months of age that had not exhibited behavioral estrus symptom were included in the experiment. Out of 12,4 heifers were in follicular phase (plasma estradiol 57.05±12.52 pg/ml), another 4 heifers were in luteal phase (Plasma progesterone 2.24±0.25 ng/ml) while the ovaries of remaining four heifers were inactive (estradiol 23.70±1.66 pg/ml and progesterone 0.32±0.06 ng/ml). $PGF_2\alpha$ (25 mg, Lutalyse, im) and GnRH (200 ug, Fertagyl, iv) was administered to each heifer at interval of 10 days. The plasma progesterone concentration decreased within 48 hrs after $PGF_2\alpha$ injection and followed thereafter with follicular growth, estrus and ovulation. GnRH administration induced follicular growth, elevation of plasma estradiol concentration with subsequent exhibition of behavioral estrus in 2 out of 4 heifers having inactive ovary. The observation reveals that Murrah buffalo heifers at 32 months of age have developed receptors for $PGF_2\alpha$ and GnRH on ovarian and pituitary tissue respectively and response the single injection of $PGF_2\alpha$ and GnRH similar to the mature cycling animals. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1059-1062*)

Key Words : $PGF_2\alpha$, GnRH, Esturs, Buffalo Heifers

INTRODUCTION

One of the major causes limiting fecundity in buffaloes is its long prepubertal Period as the age of puberty varies from 24 to 36 months (Madan, 1988). The information on sequence of hypophyseal and gonadal activity towards onset of puberty is not available. Moreover, it has been reported that the concentration of plasma progesterone increases towards advancing age in Murrah buffalo heifers (Singh and Madan, 1998, 1998a) and before onset of puberty in beef heifer (Glencross, 1984). However, the information on temporal relationship of estradiol and progesterone with folliculogenesis, exhibition of behavioral estrus sysmptom, ovulation and functional corpus luteum formation at the onset of puberty in Murrah buffalo heifers is not available. The present investigation was undertaken to study the estrus behavior and ovarian activity at onset of puberty and the effect of $PGF_2\alpha$ and GnRH administration on gonadal activity in Murrah buffalo heifers with respect to folliculogenesis, intensity of estrus, ovulation and ovarian hormone secretion where single injection of prostaglandin in buffalo (Kamonpotana et al., 1979) and GnRH in Murrah buffalo heifers (Singh and Madan, 1998a) induced follicular growth ovulation and corpus luteum formation.

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MATERIALS AND METHODS

12 Murrah buffalo heifers (*Bubalus bubalis*) were selected in July to September (ambient temperature was ranging from 22 to 36℃) from institutes herd when they attained the age of 30 months. All animals were observed for exhibition of behavioral estrus symptom between 30 and 32 months of age. Each heifer was injected intramuscularly with $PGF_2\alpha$ (Lutalyse, 25 mg) on the day they attained the age of 32 month (Disignated as day 0). While synthetic GnRH (Fertagyl 200 μg) was injected intravenous (IV) on day 10 after $PGF_2\alpha$ administration. Both $PGF_2\alpha$ and GnRH were administered on respective days between 7 am and 9 am. Genatalia were examined per rectum and a potent teasure bull with trained attendant was used 6 hourly daily to detect the estrus. Blood samples from each animal were collected through endwelling jugular canula on day -2, 0 (Just before $PGF_2\alpha$ injection) 2, 4, 8, 10, 11, 13, 15, 19, 23 and 28 of $PGF_2\alpha$ injection in chilled heparinized test tubes.

Plasma separated was divided in different aliquots and stored at 20℃ for later estimation of estradiol and progesterone by radioimmuno assay (Singh and Madan, 1998b). The sensitivity of estradiol and progesterone assay was 2.5 pg and 20 pg per tube while the intra-assay and inter-assay coefficient of variation for estradiol as well as progesterone was 9.2% and 8.8%, as well as 4.9% and 5.7% respectively. When 20 pg, 40 pg, 80 pg, 160 pg and 320 pg of estradiol; 160 pg, 320 pg, 640 pg and 1280 pg of progesterone were added to charcoal treated plasma, the recovery rate was respectively 103.40%, 101.33%, 105.68%, 96.94%

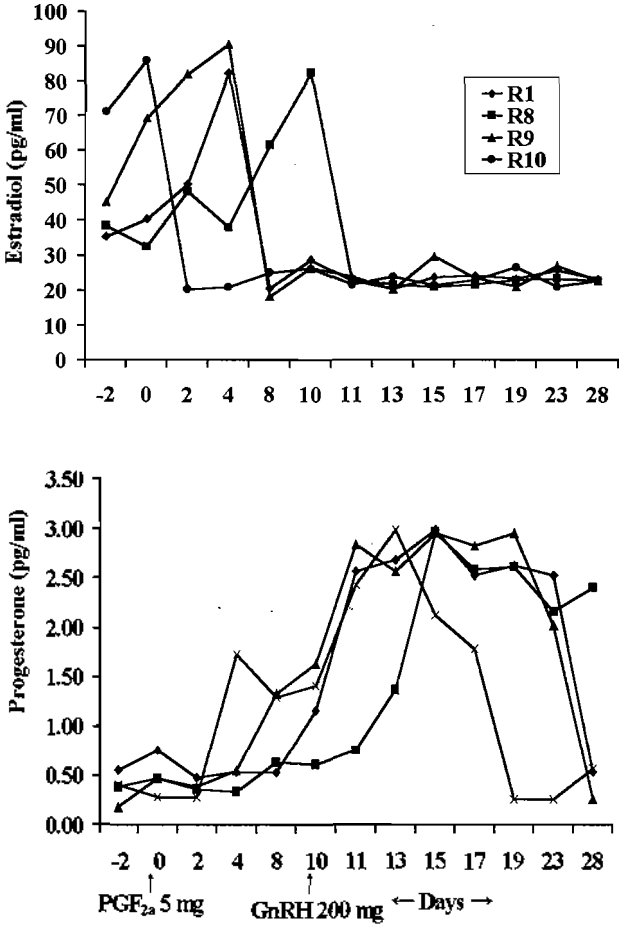

Figure 1. Estradiol and Progesterone concentration in Murrah buffalo heifers receiving $PGF_{2\alpha}$ (25 mg) intramuscularly and GnRH (200 μg) intravenously during follicular phase

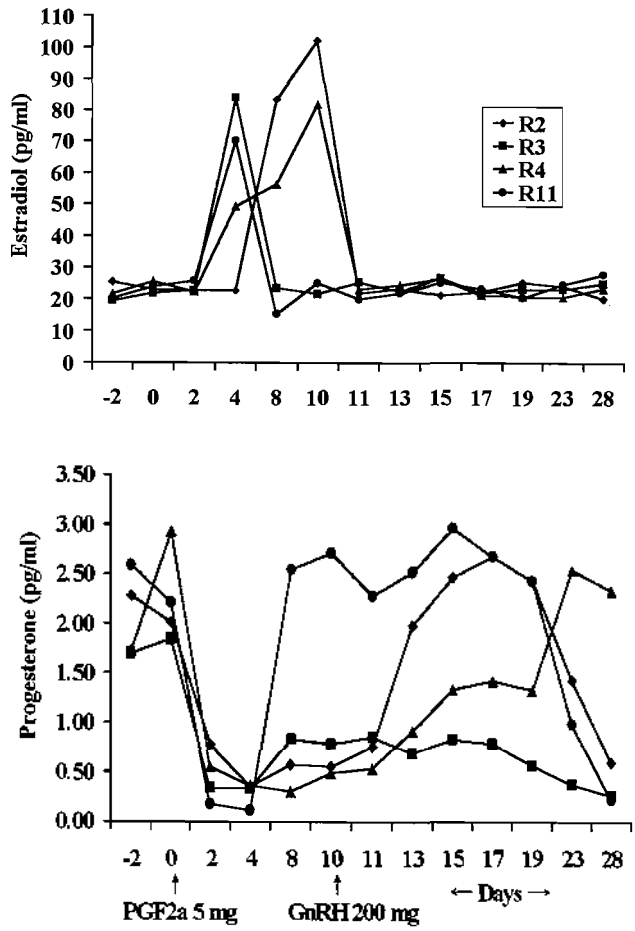

Figure 2. Estradiol and Progesterone concentration in Murrah buffalo heifers receiving $PGF_{2\alpha}$ (25 mg) intramuscularly and GnRH (200 μg) intravenously during Luteal phase

and 95.90% for estradiol, and 101.07%, 97.93%, 97.85% and 98.56% for progesterone. The pair 'T' test between the group and two way analysis of variance was done to compare the difference in hormone concentration between groups and between the samples by the method as described by Snedecor and Cochran (1967).

RESULTS

None of the heifers studied exhibited estrus behavior by 32 months of age. Moreover, 4 (R_1, R_8, R_9, and R_{10}) out of 12 heifers had already entered in follicular phase as their plasma estradiol (mean $\pm$ SE) concentration was significantly ($p<0.01$) higher than plasma estradiol concentration of remaining eight heifers on day-2. Individually their plasma estradiol concentration was 35.31 pg/ml (R_1), 38.41 pg/ml (R_8), 45.12 pg/ml (R_9) and 71.18 pg/ml (R_{10}) on day-2. After gradual increase in plasma estradiol concentration the highest value in each heifer was recorded when they detected in estrus either by teasure bull (R_1, R_8, R_9) or through behavioral estrus symptom (R_{10}). On rectal palpation distinct follicle on right ovary of all four heifers at estrus and ovulatory crypts within 12 to 24 hrs after estrus was recorded. The ovulation followed with increased concentration of progesterone by 48 to 72 hrs after detection of ovulatory crypts. The plasma progesterone concentration in these heifers between day-2 and day of estrus was maintained at less than 1.0 ng/ml (figure 1). In remaining eight heifers, neither follicle nor corpus luteum was detected through rectal palpation. However, four heifers (R_2, R_3, R_4, and R_{11}) were considered in luteal phase as their plasma progesterone concentration (mean $\pm$ SE) on day-2 and day 0 was significantly ($p<0.01$) higher than the plasma progesterone concentration of remaining eight heifers. The plasma progesterone concentration of individual heifers on day-2 and day 0 was 2.23 ng/ml and 2.01 ng/ml in R_2, 1.70 ng/ml and

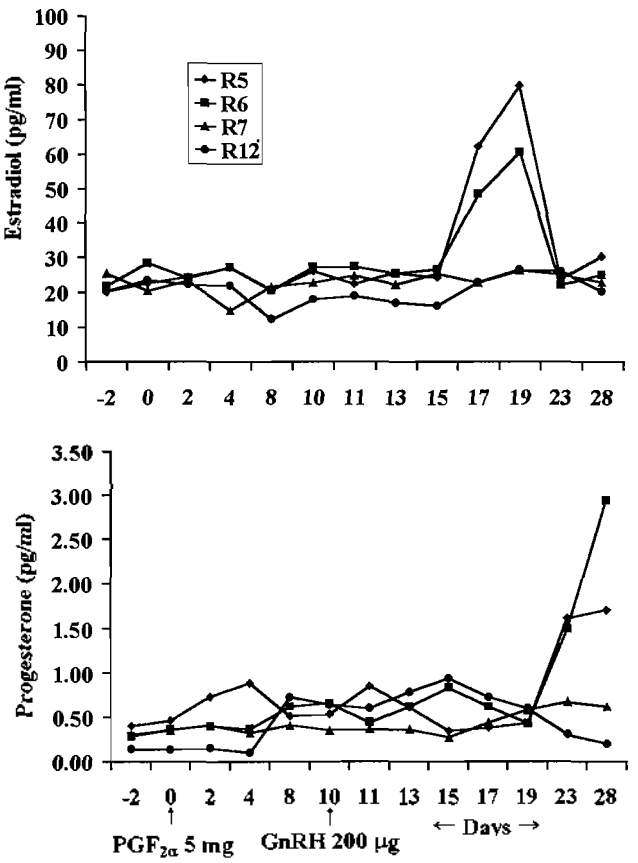

Figure 3. Estradiol and Progesterone concentration in Murrah buffalo heifers receiving $PGF_{2\alpha}$ (25 mg) intramuscularly and GnRH (200 μg) intravenously having inactive ovaries

1.84 ng/ml in R_3, 1.73 ng/ml and 2.93 ng/ml in R_4 and 2.59 ng/ml and 2.21 ng/ml in R_{11}. Their progesterone concentration decreased to less than 1.0 ng/ml by 48 hrs after $PGF_{2\alpha}$ injection with subsequent significant ($p<0.01$) elevation of estradiol between day 4 and day 10 (figure 2) over pretreatment value associated with follicular growth. Three heifers (R_2, R_3 and R_4) exhibited behavioral estrus symptom while one heifer (R_{11}) was detected in estrus by teasure bull. All four heifers ovulated within 12 to 36 h after detection of estrus. The ovulated follicles were transformed into palpable corpus luteum secreting elevated levels of progesterone on subsequent days (figure 2). Remaining four heifers were having neither follicle nor luteal tissue on their ovary as their plasma estradiol and prgesterone concentration were less than 30 pg/ml and 1.0 ng/ml respectively between day-2 to day 10 (day of GnRH) injection (figure 3).

At the time of GnRH injection three heifers R_2 R_4 and R_8 were having palpable follicle on their ovary secreting elevated level of estradiol with little uterine tone. Only two heifers (R_2 and R_8) exhibited behavioral estrus symptom 10 hrs after GnRH injection. By 24 hrs after GnRH injection ovulation was detected in all three heifers. Follicular growth with slight uterine tone and congested vaginal mucus memberane associated with elevated levels of estradiol (figure 3) was recorded in only 2 (R_5 and R_6) out of 4 noncycling heifers on 9th day after GnRH injection. However, none of these heifers exhibited behavioral estrus symptom although estrus was detected by teasure bull and ovulation was confirmed in one heifer (R_6) by presence of distinct corpus luteum and elevation of progesterone profile thereafter on subsequent days.

DISCUSSION

From the data recorded on physical examination of genitalia, estrus behavior and elevation of estradiol with palpable follicles in four heifers (R_1, R_8, R_9 and R_{10}) as well as increased concentration of progesterone in other four heifers (R_2, R_3, R_4 and R_{11}) and inactive ovary of remaining four heifer (R_5, R_6, R_7 and R_{12}) on day-2 and day 0 indicated that Murrah buffalo heifers attained different stages of maturity in respect of ovarian activity at 30 to 32 months of age. Most of them has achieved puberty at this age which was in line of support of previous report in relation to age of puberty in Murrah buffalo (Madan, 1988). The detection of plasma progesterone concentration more than 1 ng/ml on day-2 and day 0 followed with its decline to below 1 ng/ml by 48 hrs after $PGF_{2\alpha}$ injection and subsequent folliculogenesis indicated by the elevation of estradiol by day 4 to 10 (figure 2) suggest that these heifers were having corpus luteum on ovary secrete progesterone with developed feed back mechanism similar to the mature animals. Administration of $PGF_{2\alpha}$ is known would causes luteolysis within 24 hrs followed with folliculogenesis, estradiol secretion, resumption of estrous cyclicity and ovulation in cycling mature cow (Lebedev et al., 1980) and buffaloes (Kamonpotana et al., 1979). The secretion of progesterone more than 1 ng/ml in these four heifers without having palpable corpus luteum in two heifers (R_3 and R_4) was similar to the higher progestorone without distinct corpus luteum recorded before onset of puberty in beef heifers (Glencross, 1984).

The elevated levels ($p<0.01$) of estradiol associated with presence of palpable follicle on day-2 and subsequent increase in follicular size and estradiol concentration leading to ovulation with (R_{10}) and without (R_1, R_8 and R_8) exihibition of of behavioral estrus symptom in four heifers of follicular phase was due to less intense and shorter duration of cycle in three heifers which is common in buffaloes (Dobson and Kamonpotana, 1986). The record of ovulatory

crypt 24 to 36 hrs after detection of estrus and elevation of progesterone subsequently by 96 hrs after ovulation (figure 1) indicated that these four heifers were also have developed hypothalmo-hypophyseal and gonadal system at this age as they ovulated spontaneous and resumed luteal phase of sexual cycle for full cycle length. Similar temporal relationship of gonadal hormone with folliculogenesis ovulation and corpus luteum formation has been well documented in mature cattle, sheep and goat (Hafez, 1987), Swamp buffaloes (Kanai and Simizu, 1984) and Murrah buffaloes (Razdan et al., 1982). The presence of ovulatory crypt by 12 to 18 hrs with subsequent decrease in estradiol concentration by 24 hrs with subsequent elevation of progesterone by 72 hrs (R_2 and R_8) and 120 hrs (R_4) in the heifers having palpable follicle on ovary after GnRH injection indicated that GnRH injection though caused synchronised ovulation but the ovulated follicles took 72 to 120 hrs to secrete progesterone (figure 1, 2). Similar observations on the follicular growth, ovulation and ovarian hormone secretion in buffalo heifers of 24 months age receiving only GnRH (Singh and Madan, 1998a) and synchronized ovulation in superovulated buffalo heifers of 12 months (Singh and Madan, 1999) and 24 months (Singh and Madan, 1999a) age has already been reported. The follicular growth associated with elevation of estradiol on day 17 and 19, and detection of estrus on day 19 with subsequent ovulation followed with increased progesterone secretion by day 23 in two heifers (R_5 and R_6) having inactive ovaries on day 0 (figure 3) might be due to stimulation of ovarian growing follicles on day 10 by GnRH induced gonadotrophin release, as administration of GnRH induced increased release of FSH and LH which stimulated follicular growth and ovulation within 7 to 9 day of its administration in buffalo heifer at 24 months of age (Singh and Madan, 1998a).

Thus it can be concluded that by 30 to 32 months of age the hypothalmo-hypophyseal and gonadal systems of Murrah buffalo are fully mature to response the single injection of prostaglandin and GnRH in respect to luteolysis, folliculogenegsis, ovulation and corpus luteum formation similar to the mature animals.

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