African Journal of Biotechnology Vol. 9(14), pp. 2169-2172, 5 April, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Hormonal responses to GnRH injection given at different stages of the estrous cycle in water buffaloes

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Accepted 8 February, 2010

The objective of the present study was to evaluate the hormonal responses of buffaloes to GnRH injections given at different moments of the estrous cycle. The estrous cycles of 15 buffaloes were synchronized with 2 im injections of prostaglandin F2α given 11 days apart. The buffalos were randomly assigned to 1 of 3 groups. Buffaloes in the control group received no treatment, whereas GnRH6 buffaloes received a GnRH injection between days 5 and 7 and GnRH16 buffaloes received a GnRH injection between days 15 and 17 of the estrous cycle (estrus = day 0). Up to day 6, the plasma progesterone levels in the three groups were low (1 ± 0.16 ng/ml) but gradually increased until day 12 (4.62 ± 0.41 ng/ml). The amount of plasma progesterone concentrations decreased from day 15 of the estrous cycle in all groups of buffaloes. No significant differences were observed in the plasma level of progesterone and estradiol concentrations of the three groups during the estrous cycle. The peak levels of plasma estradiol were observed on day 20 and the differences were not significant (11.34 ± 1.77, 11.74 ± 1.91 and 12.72 ± 2.49 pg/ml in the control, GnRH6 and GnRH16 groups, respectively (P> 0.05). In conclusion, GnRH given at the beginning or at the end of the estrous cycle did not alter the profile of progesterone and estradiol concentration in buffaloes.

Key words: Hormonal response, GnRH, estrous cycle, buffalo, progesterone.

INTRODUCTION

Today, it is well accepted that injection of a GnRH agonist at any stage of the estrous cycle in cattle 1) increases the number of medium-sized follicles within 3 days of treatment, 2) eliminates the large follicles by ovulation or atresia and 3) induces the emergence of a new follicular wave within 2 to 3 days of treatment (De Rennis and Peters, 1999; Kohram et al., 1998a, b).

The administration of GnRH and GnRH agonists induces an acute release of gonadotropins in cattle (Campanile et al., 2008) and buffaloes (Rastegarnia et al., 2004). High concentrations of plasma LH and FSH following treatment with GnRH are typically greater than concentrations associated with the pre-ovulatory gonadotropin surge but the release of gonadotropins, however, is of shorter duration (Campanile et al., 2008).

In buffaloes, treatment with GnRH (gonadoreline) has been shown to induce ovulation that is accompanied by the appearance of luteal tissue on the ovary within 14 h and increased circulating concentrations of progesterone within 96 h (Rastegarnia et al., 2004). In cattle, treatment with hCG or GnRH induces ovulation and the formation of an accessory CL (Kohram et al., 1998b; Rastegarnia et al., 2004). In buffalo, hCG treatment also induces ovulation and the formation of an accessory CL (Rastegarnia et al., 2004). GnRH and GnRH analogues have been used in river buffaloes to reduce post-partum anestrus period, induce ovulation at the end of superovulation programs and synchronize follicular emergence and ovulation in estrus synchronization programs and fixed time insemination protocols (Rastegarnia et al., 2004).

Therapeutic approaches based on the use of steroid

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Abbreviations: GnRH, Gonadoreline; **LH,** luteinizing hormone; **FH,** follicle stimulating hormone.

hormones (Bo et al., 1995; Burns et al., 2005) or of GnRH (Beal, 2003; Twagiramungu et al., 1994) have also been shown to alter hormonal profile and follicular development. Kohram et al. (1998a, b) showed that hormonal changes did not occure during estrous cycle in heifers and cattle using GnRH injection at different stages of the estrous cycle. Therefore, the objective of the present study was to evaluate the hormonal response of water buffaloes to GnRH injections given at different moments of the estrous cycle.

MATERIALS AND METHODS

Animals and treatment

The estrous cycles of 15 buffaloes were synchronized with 2 intramuscular (im) injections of prostaglandin F2α (Synchromate®, 500µg cloprostenol sodium, Bremer pharma GmbH, Germany) given 11 days apart. The buffaloes were randomly assigned to 1 of 3 groups. In control group of animals no injection of GnRH was performed. GnRH administered (Cystorelin, 2 ml, im) between days 5 and 7 (GnRH6 group) or between days 15 and 17 (GnRH16 group) of the estrous cycle (estrus = day 0).

Blood sampling

Blood samples were collected once daily from the jugular vein into heparinized tubes beginning at day that second PGF2α inject until the day of next estrous. Plasma was harvested within 1 h of collection and stored at -20°C until progesterone or estradiol assay. Progesterone concentrations were measured using progesterone kit (Progesterone RIA, Kavoshyar, Iran). Sensitivity of the assay was 3pg/tube, and intra-assay coefficients of variation were 7.4 and 7.0% for reference samples containing 7.0 and 26.0 pg/tube, respectively. Estradiol concentrations were measured as previously described with using an Estradiol MAIA kit (Specteria Estradiol RIA; Orion corporation, Orion Diagnostica, Espoo, Finland). Sensitivity was 0.031pg/tube. Intra-assay coefficients of variation were 11.1, 8.4 and 22.9% for samples containing 0.14, 0.68 and 5.16pg/tube, respectively. Inter-assay (n = 2) coefficients of variation for the same samples were, 4.9, 12.4 and 7.4%, respectively.

Statistical analyses

Profiles of the mean progesterone and estradiol concentrations were compared by least squares analysis of variance and using the general linear model (GLM) procedure of SAS. The multivariate analysis included sources of variation due to groups, days (repeated measures) and their interactions (MANOVA SAS).

RESULTS

The plasma progesterone and estradiol concentrations Up to day 6, the plasma progesterone levels in the three groups were low (1 ± 0.16 ng/ml) then they gradually increased until day 12 (4.62 ± 0.41 ng/ml; Figure 1). They maintained high levels (4.99 ± 0.49 ng/ml) between days 12 and 15. The amount of plasma progesterone concentrations decreased from day 15 of the estrous cycle and reached less than 1 ± 0.11 ng/ml on day 20 in all groups of buffaloes. No significant differences were observed in the plasma level of progesterone and estradiol concentrations of the three groups during the estrous cycle. The peak levels of plasma estradiol were observed on day 20 and the differences were not significant (11.34 ± 1.77, 11.74 ± 1.91 and 12.72 ± 2.49pg/ml in the control, GnRH6 and GnRH16 groups, respectively; $P > 0.05$; Figure 1).

DISCUSSION

The pattern of reproductive hormones during the estrous cycle in buffaloes are generally similar to cattle (Jainudeen and Hafez, 2000), although other authors have reported lower concentrations of these hormones in buffalo than in cattle (Srivastava et al., 1999). In the present study, there was a typical pattern of P4 decline and E2 surge near the time of ovulation.

Progestins are a group of hormones with similar physiological activity, the most important being progesterone. This latter has a dominant role in regulating the oestrus cycle. In buffaloes, during the normal cycles, plasma P4 levels drop sharply 2 or 3 days before the luteinizing hormone (LH) peak, start to rise 2 to 4 days after the LH surge reaching the highest characteristic mid-luteal phase (dioestrus) levels (ranging between 5 and 12ng/ml in Mediterranean buffalo cows or between 4 to 6ng/ml in Murrah buffalo cows). P4 blood concentration can change during the seasons. During the fresh season, the highest progesterone levels of the luteal phase was reported in comparison to that recorded during the hot dry or hot wet seasons (Srivastava et al., 1999).

Basal concentrations of progesterone for several days around the time of estrus are a common feature of most farm animals. A transient rise in progesterone concentration just before estrus reported for cattle (Ayalon and Shemesh, 1974), was not evident in water buffaloes of the present study. The concentration of blood progesterone during estrus in our study was 0.49 ng/ml and remains close to 1 ng/ml for the next 3 to 4 days which is in agreement with Arora et al. (1982a) that reported blood concentration of progesterone of 0.1 to 0.3 ng/ml. The first significant increase in progesterone concentration in our study occured about 5 days after estrus which was in contrast with the studies of Ahmed et al. (1977). In their study the first significant increase in progesterone concentration was on day 7. The high level progesterone values in our study were 4 to 6 ng/ml which is in agreement with Arora et al. (1982b) who the reported peak progesterone concentration (4.0 to 5.1 ng/ml).

Estrogens are hormones produced by the ovary and are transported in the body by binding proteins. Estrogens act on the central nervous system in order to induce behavioural estrus in females and the most important of these hormones is estradiol. In buffalo the general pattern

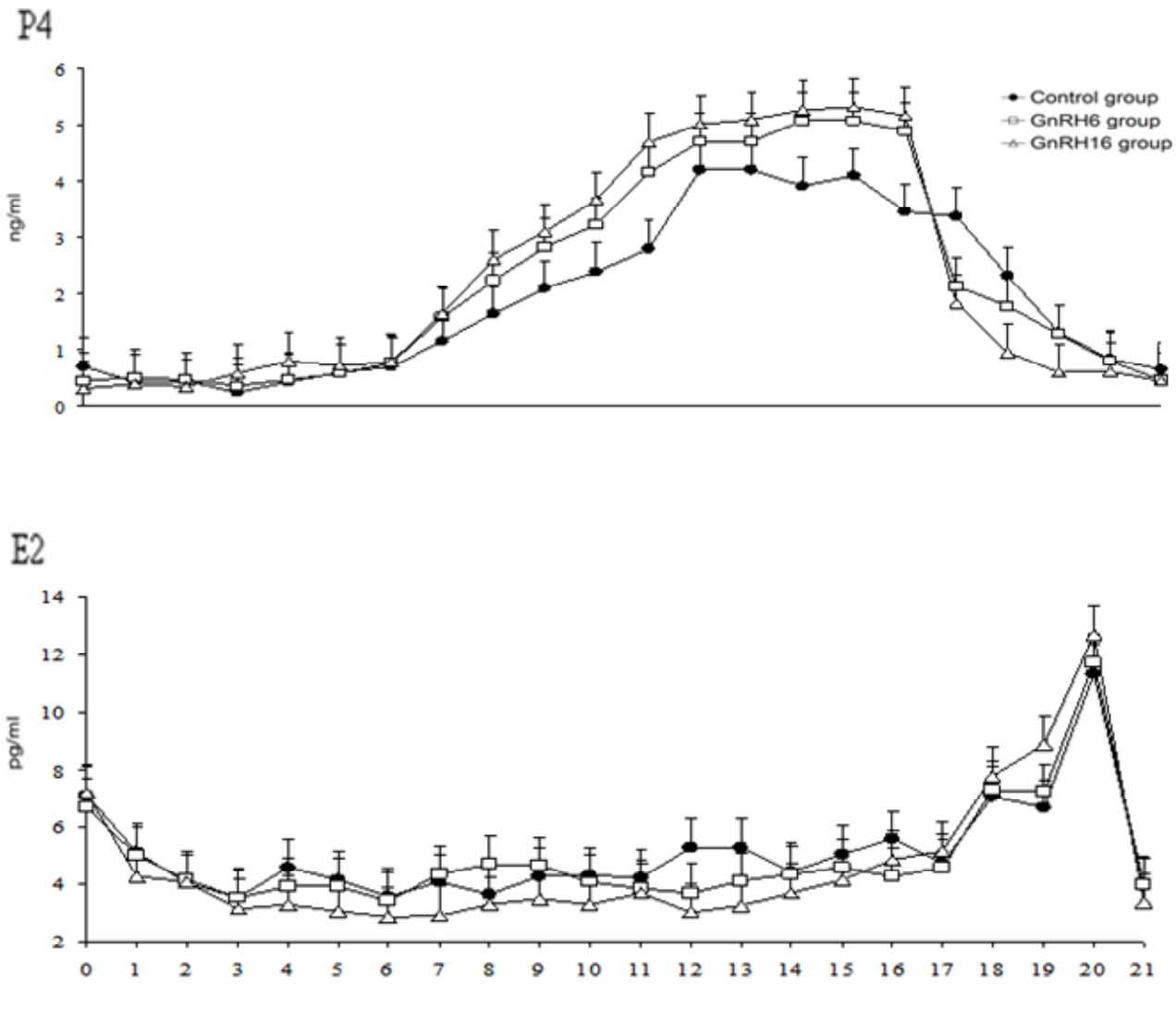

Figure 1. Progesterone and estradiol concentrations (mean ± SEM) during the estrous cycle in buffaloes.

of secretion of the estradiol-17ß indicates a surge which takes place on the d preceding the LH peak (Malfatti, 2003; Singh et al., 2001) or frequently very close to the LH peak with blood levels ranging between 9 and 13 pg/ml (Seren et al., 1994), respectively, in Murrah and Mediterranean buffaloes. Following the LH surge, there is a rapid decline in estradiol secretion and this results in the cessation of estrus after a relatively constant time interval (about 12 h). The basal estradiol levels during the luteal phase of the cycle range between 3 and 8 pg/ml, but some minor elevations can be observed in the early luteal phase, up to 1.3pg/ml (Malfatti, 2003; Singh et al., 2001). Circulating estradiol concentrations in our study remains low during the luteal phase with minor fluctuations (3 to 6 pg/ml) around days 4 and 16 which is in agreement with the previously reports (Batra and Pandey, 1982). The mean estradiol peak concentrations were 11.93pg/ml on day 20 of the estrous cycle, followed by a decline to 3 to 5 pg/ml within one day. Other studies report the mean of estradiol peak concentrations of 30 to 35 pg/ml on the day of estrus (Batra and Pandey, 1982). This pattern is indicative of enhanced estradiol production by the preovulatory follicles during the proestrus phases. Overall, estradiol-17ß concentrations were similar to or slightly lower than those previously recorded for buffalo (Batra et al., 1980; Knopf et al., 1989) and cattle (Ayalon and Shemesh, 1974; Dobson, 1978; Lemon et al., 1975).

Previously described that an injection of Gonadorelin resulted in the ovulation of the dominant follicle followed by the formation of a new corpus luteum. Although the

diameter of the induced corpus luteum was smaller than the natural one in cattle (Pursley et al., 1995). A significant rise in progesterone concentration was detected on day 3 followed by the formation of corpus luteum secreting progesterone on day 6 after GnRH injection. This corpus luteum regressed and plasma progesterone concentration dropped significantly after prostaglandin injection (Rastegarnia et al., 2004). The increase in plasma progesterone concentration on day 3 after GnRH injection and the formation of the induced corpus luteum have been also reported in cattle (Thompson et al., 1999). However, the results showed that the progestrone concentration following GnRH injection between days 5 and 7 or 15 and 17 of the estrous cycle did not change indicating that the significant increase in progesterone may not always occures. Previous study in cattle also showed that GnRH injection did not alter the pre-ogesteron concentrations during the estrous cycle. This occurred whether cows (Kohram et al., 1998a, b) or buffaloes were in the early (days 5 to 7) or late (days 15 to 17) phases of the estrous cycle when GnRH treatment was administered.

By design, the buffaloes in our study were either at the beginning (days 5 to 7) or at the end (days 15 to 17) of the estrous cycle, and hence progesterone profilles were completely different at lime of GnRH treatment. As expected (Knopf et al., 1989; Pursley et al., 1995), progesterone concentrations increased when GnRH was given during the early luteal phase, however, the amount of progesterone concentrations was not significantly different between control and GnRH6 groups.

In conclusion, the results presented here indicate that GnRH given at the beginning (days 5 to 7) or at the end (days 15 to 17) of the estrous cycle did not alter the profile of progesterone and estradiol concentration in water buffaloes as previously described in cattle (Kohram et al., 1998a, b).

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