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Alteration of Follicular Dynamics by GnRH at Two Different Stages of the Estrous Cycle in Water Buffalo

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Abstract: The objective of the present study was to evaluate the ovarian response 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 day 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 day 5 and 7 and GnRH16 buffaloes received a GnRH injection between day 15 and 17 of the estrous cycle (estrus = day 0). Daily, from estrous cycle day 0 to the next estrous cycle day 23, cows had their ovaries scanned by ultrasound. All follicles were classified in to 4-6 mm and ≥7 mm follicles. Data were analyzed using the GLM procedure of SAS. In the 3 treatments, the number of 4-6 mm follicles had a decline trend from day 1 and reached their least amount on day 4 and 5 ($p<0.05$). Thereafter, the number of this class of follicles in the control, GnRH6 and GnRH16 treatments increased until day 8, 10 and 6, respectively ($p<0.05$). In response to an injection of GnRH, the number of small follicles in GnRH6 and GnRH16 treatments increased on day 9 and 18 respectively. Again, the number of small follicles increased in the control and GnRH6 groups from day 15 and 19, respectively, to day 21 of the estrous cycle. The number of ≥7 mm follicles had an increase ($p<0.05$) until day 3 in the control and 5 in the GnRH6 and GnRH16 groups. In the GnRH6 treatment of buffaloes, GnRH injection on day 6 increased ($p<0.05$) the number of large follicles on day 10. The number of ≥7 mm follicles in the two groups (control and GnRH6) increased between day 17 and 19 of the estrous cycle. An increase the number of small and medium sized follicles 2 day after GnRH injection showed that an injection of GnRH at the beginning or later days of the estrous cycle could promote the emergence of a new follicular wave in buffaloes.

Key words: Follicle, buffalo, GnRH, ultrasonography

INTRODUCTION

The buffalo plays a very significant role in the production of milk and meat in rural areas around the world. However, as compared to cattle, the methods used to raise and manage the buffaloes have not been developed well, partly due to inadequate rural facilities. The reproduction management methods may have a considerable effect on the productivity optimization of these animals (Singh *et al*., 2000, 2001). There are few studies done to clarify the pattern of ovarian follicular growth and manipulation of these phenomena in female buffaloes (Awasthi *et al*., 2007; Presiccea *et al*., 2005; Awasthi *et al*., 2006, 2000; Manik *et al*., 1999).

There are reports that showed the use of ovulation synchronization and fixed timed AI in buffaloes have advantages, similar to those found in cattle; however, the application of the AI is more difficult in buffalo (Baruselli *et al*., 1997).

Histological and ultrasonographic studies (Rajakoski *et al*., 1960; Matton *et al*., 1981; Sirois and Fortune, 1988; Savio *et al*., 1988) confirmed that follicular development occurs in two or three waves in cattle. Similarly, It has been reported that the pattern of follicular growth during estrous cycle in buffalo occurs in one, two or three waves (Awasthi *et al*., 2006; Terzano, 2004; Taneja *et al*., 1996).

Various approaches are being developed to alter follicular inventories using ultrasound-guided follicular aspiration of the dominant follicle which has been performed to remove large follicles and induce the emergence of a new follicular wave in cattle (Kohram *et al*.,

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1998). Therapeutic approaches based on the use of steroid hormones (Bo *et al*., 1995; Burns *et al*., 2005) or of GnRH (Arnett *et al*., 2000; Sato *et al*., 2005; Twagiramungu *et al*., 1994) have also been shown to alter follicular development. Today, it is well accepted that injection of a GnRH agonist at any stage of the estrous cycle in cattle: Increases the number of medium-sized follicles within 3 days of treatment, eliminates the large follicles by ovulation or atresia and induces the emergence of a new follicular wave within 2-3 days of treatment (Kohram *et al*., 1998a, b; De Rensis *et al*., 1999).

Based on these observations in cattle, we hypothesized that GnRH can be used to elicit the emergence of a follicular wave in a synchronous fashion in a group of buffaloes at contrasting stages of the estrous cycle. The objective of our present study was to promote the emergence of a follicular wave at a predictable time with the use of GnRH at 2 different stages of the estrous cycle in bufalloes.

MATERIALS AND METHODS

Animals and treatment: The estrous cycles of 15 water buffaloes were synchronized with 2 intramuscular injections of prostaglandin F2α (Synchromate®, 500ig cloprostenol sodium, Bremer pharma GmbH, Germany) given 11 day 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).

Ovarian follicular development was monitored daily by transrectal ultrasonography with a real-time linear scanning ultrasounddiagnostic system (LS-300-A: Tokio Keiki Co., Tokyo, Japan; 7.5 MHz transducer). Ultrasonography was performed once daily from the day that second PGF2α inject until the day of next estrous. All follicles larger than 3 mm were counted and classified according to their diameter in one of the following classes: 4-6 mm and ≥7 mm.

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 in follicular fluid samples in a single modified assay (Ronayne *et al*., 1990) using (1,2,6,7-3H) progesterone (Progesterone RIA, Kavoshyar, Iran). Sensitivity of the assay was 3 pg tube^{-1} and intra-assay coefficients of variation were 7.4 and 7.0% for reference samples containing 7.0 and 26.0 pg tube^{-1}, respectively. Estradiol concentrations were measured as previously described (Prendiville *et al*., 1995) with using a Estradiol MAIA kit (Specteria Estradiol RIA; Orion corporation, Orion Diagnostica, Espoo, Finland). Sensitivity was 0.031 pg tube^{-1}. Intra-assay coefficients of variation were 11.1, 8.4 and 22.9% for samples containing 0.14, 0.68 and 5.16 pg tube^{-1}, 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 number of follicles (4-6 mm and ≥7 mm), estradiol and progesterone 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 pattern of 4-6 mm follicles during the estrous cycle of buffaloes: In the 3 groups, the number of 4-6 mm follicles had a decline trend from day 1 and reached their least amount on days 4 and 5 (4.2±2.1 vs 1.1±1.6 follicles; $p<0.05$). Thereafter, the number of this class of follicles in control, GnRH6 and GnRH16 groups increased until days 8, 10 and 6, respectively ($p<0.05$). The number of small follicles in group GnRH6 on day 6 was 2.6±1.6 follicles, which increased to 7.0±1.7 follicles on day 9, in response to an injection of GnRH on day 6.

The number of small follicles in the control group decreased between days 8 and 15 ($p<0.05$) from 6.2±1.1 to 1.0±1.2 follicles which was observed in ultrasonography examination. The number of this class of follicles in group GnRH6 was between 7 and 5 follicles on days 8-15 and decreased to 2.0±0.59 small follicles on the 19th day of the estrous cycle. In group GnRH16, 1.96±0.69 small follicles were observed between days 7 and 17 while detected changes in the ovaries were not significant.

The number of small follicles increased in the control and GnRH6 groups from days 15 and 19, 21 of the estrous cycle. In group GnRH16, 2 days following GnRH injection an increase from 1.4±1.2 to 5.6±1.2 ($p<0.05$) was observed in this class of follicles.

The pattern of ≥7 mm follicles during the estrous cycle of buffaloes: The number of ≥7 mm follicles had an increase ($p<0.05$) until days 3 in control and 5 in the GnRH6 and GnRH16 groups. Thereafter, the number of this class of follicles in control, GnRH6 and GnRH16 deacreased ($p<0.05$) until days 5, 7 and 8, respectively (Fig. 1).

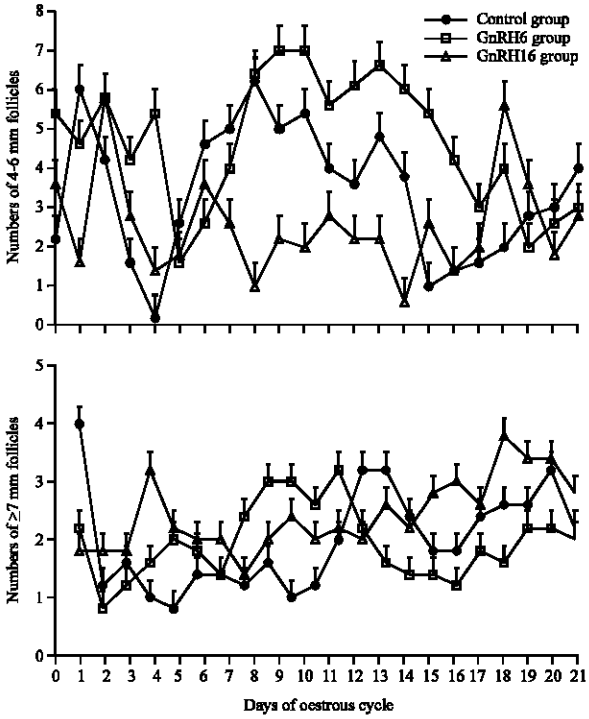

Fig. 1: Mean±SEM number of 4-6 and ≥7 mm follicles monitored by ultrasonography during the estrous cycle in buffaloes

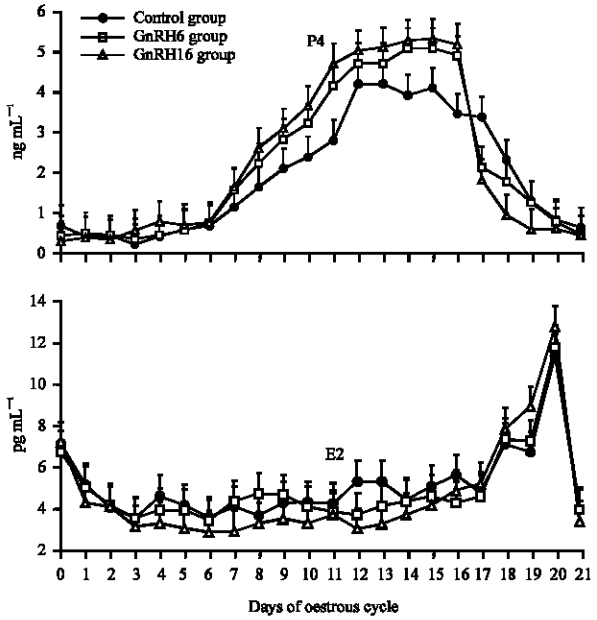

Fig. 2: Mean±SEM progesterone and estradiol concentrations during the estrous cycle in buffaloes

The number of large follicles in the GnRH6 group on day 6 was 1.6±0.5 follicle. In this group of buffaloes, GnRH injection on day 6 increased ($p<0.05$) the number of large follicles to 3.1±0.6 follicles on day 10. An increase trend in groups control and GnRH16 was observed by ultrasonography examination between days 11-14 and 8-10, respectively, of the estrous cycle. The number of large follicles decreased to 1.4±0.2 follicle in the control and GnRH6 groups between days 9-11 and 12-17, respectively. The number of large follicles in group GnRH16 had little change between days 11 and 15 (1.6±0.3) while an increase from 2.6±0.4 to 2.8±0.5 was observed following GnRH injection.

The plasma progesterone and estradiol concentrations: Up to day 6, the plasma progesterone levels in the 3 groups were low (1.0±0.16 ng mL^{-1}) then they gradually increased until day 12 (4.62±0.41 ng mL^{-1}). They maintained high levels (4.99±0.49 ng mL^{-1}) 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±0.11 ng mL^{-1} on day 20 in all groups of buffaloes. No significant differences were observed in the plasma level of estradiol concentrations of the 3 groups duringhe estrous cycle. The peak levels of plasma estradiol were observed on day 20 and the differences were not significant ($p<0.05$) (11.34±1.77, 11.74±1.91 and 12.72±2.49 pg mL^{-1} in the control, GnRH6 and GnRH16 groups, respectively (Fig. 2).

DISCUSSION

The results of daily ovarian ultrasonography in control group showed that ovarian follicles developed in a wave like pattern with two or three follicular wave in Iranian water buffaloes, similar to those observed in Murrah buffaloes (Taneja *et al*., 1995, 1996; Baruselli *et al*., 1997; Manik *et al*., 1998) and cattle (Savio *et al*., 1988; Ginther *et al*., 1989a).

Decrease in the number of 4-6 mm follicles and increase in the number of ≥7 mm follicles in the initiation days of the estrous cycle in the 3 groups showed that the first follicular wave emereged before day 0 of the estrous cycle in the buffaloes. Previous reports (Awasthi *et al*., 2006; Baruselli *et al*., 1997; Terzano *et al*., 2004; Taneja *et al*., 1996) also showed that the emeregence of the first follicular wave occurred between days 0 and 1 of the estrous cycle. The increase in FSH concenterations at the end of the estrous cycle may induce the emergence of first follicular wave in the next estrous cycle. Therefore, it would be expected that the emergence of the first follicular wave occurres before day 0 of the estrous cycle.

In the present study, the second follicular wave emerged with a decrease in the number of = 7 mm follicles and an increase in the number of 4-6 mm follicles on days 4 and 5 of the estrous cycle of buffaloes. However, there are reportes that the second follicular wave emerged on day 7 (Taneja *et al*., 1996) or days 9-11 (Awasthi *et al*., 2006; Baruselli *et al*., 1997; Terzano *et al*., 2004) of the estrous cycle. In the present study, earlier initiation of the first follicular wave likely caused an early commencement of the second follicular wave in the estrous cycle of the buffaloes.

Increase in the number of small and medium sized follicles after GnRH injection in groups GnRH6 and GnRH16 of the buffaloes, shows that an injection of GnRH at the beginning or later days of the estrous cycle could influence the beginning of a new follicular wave in buffaloes. This result is consistent with other studies carried out on cattle (Kohram *et al*., 1998; Ginther *et al*., 1989b; Sato *et al*., 2005). The results also showed that the second follicular wave seems to emerge before GnRH injection in group GnRH6. Interestingly, these follicular waves had more small and medium size follicles in response to GnRH injection compared to control and GnRH16 groups.

Earlier reports showed that the initiation of supperovulation treatment in the beginning of a new follicular wave increase the superovuation responses in terms of the number of large follicles at estrus, CL and transferable embryo at embryo collection in cattle (Kohram *et al*., 1998a, b; Bergfelt *et al*., 1997; Critser, 1980) and buffalo (Misra, 1993; Singh *et al*., 1988). The results showed that an injection of GnRH at 2 different stages of the estrous cycle induce a new follicular wave in buffaloes in which the number of small and medium size follicles increase 2-3 days following GnRH injection. Therefore, initiation of superovulation treatment 2-3 days following GnRH injection may increase the superovulation responses. However, the quality of such follicles and oocytes induced by GnRH injection could evaluate for fertiization ability and embryo production.

The third follicular wave emerged on day 15 of the estrous cycle in the control group by decreasing the number of ≥7 mm follicles and increasing 4-6 mm follicles. It was also previously reported that the third follicular wave begins between days 16 and 17 in buffaloes (Baruselli *et al*., 1997; Taneja *et al*., 1995, 1996). The decrease in progesterone levels result in increasing LH and FSH pulses frequencies late in the estrous cycle leading eventually, ovulation of the dominant follicle of this follicular wave in buffaloes.

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

The concentration of blood progesterone during estrus in our study was 0.49 ng mL^{-1} and remains close to 1 ng mL^{-1} for the next 3-4 days which is in agreement with (Arora and Pandey, 1982) the reported blood concentration of progesterone (0.1-0.3 ng mL^{-1}). 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 4.81 ng mL^{-1} which is in agreement with (Bachlaus *et al*., 1979; Arora and Pandey, 1982; Takkar *et al*., 1983) the reported peak progesterone concentration (4.0-5.1 ng mL^{-1}).

Circulating estradiol concentrations in our study remains low during the luteal phase with minor fluctuations (3-6 pg mL^{-1}) around days 4 and 16 which is in agreement with the previously reports (Batra and Pandey, 1982; Samad *et al*., 1988). The mean estradiol peak concentrations were 11.93 pg mL^{-1} on day 20 of the estrous cycle, followed by a decline to 3-5 pg mL^{-1} within one day. Other studies report the mean of estradiol peak concentrations of 30-35 pg mL^{-1} 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.

CONCLUSION

In conclusion, the results presented here indicated that 2 days after GnRH injection an increase the number of small and medium sized follicles occured. An injection of GnRH can be given at different stages of the estrous cycle in water buffalo to promote the emergence of a follicular wave at a predictable time.

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