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Full Length Research Paper

GnRH injection before artificial insemination (AI) alters follicle dynamics in Iranian Holstein cows

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The objective of the present study was to evaluate the ovarian response of cows to gonadotropin-releasing hormone (GnRH) injection on day 6 of the estrous cycle. The estrous cycles of 10 cows were synchronized with 2 intramuscular (im) injections of prostaglandin F2α given 11 d apart. The cows were randomly assigned to 1 of 2 treatments. Cows in the control treatment received no treatment, whereas GnRH6 cows received a GnRH injection on d 6 of the estrous cycle (estrus = d 0). Daily, from estrus d 0 to the next estrus d 23, cows had their ovaries scanned by ultrasound. Profiles of the mean number of follicles (that is, 4 - 6 mm and ≥ 7 mm) and the diameter of the largest follicle (F1) were compared by least squares analysis of variance using the general linear model (GLM) procedure of SAS. In response to an injection of GnRH, the number of small follicles in group GnRH6 on day 6 (1.5 ± 0.6 follicles) increased (P ≤ 0.05) on day 8 (5.0 ± 0.7 follicles) and the number of medium and large follicles (≥ 7 mm) on day 6 (2.0 ± 0.5 follicle) decreased (P ≤ 0.05) on day 9 (0.8 ± 0.6 follicles). In conclusion, an injection of GnRH on day 6 of the estrous cycle could promote the emergence of a new follicular wave in cows.

Key words: Ultrasonography, follicle, GnRH, Iranian Holstein cows.

INTRODUCTION

Several studies (Pierson and Ginther, 1987 a, b; Sirois and Fortune, 1988; Savio et al., 1988) confirmed the hypothesis initially proposed by Rajakoski (1960) that follicular growth in cattle occurs in waves. Estrous cycles with two (Ginther et al., 1989a; Rajamahendran and Walton 1988; Ahmad et al., 1997; Townson et al., 2002; Celik et al., 2005), three (Sirois and Fortune 1988; Savio et al., 1988; Burns et al., 2005; Sartori et al., 2004) or four (Rhodes et al., 1995) follicular waves have been reported in cattle. The follicular waves are first detectable as a group of 4 - 5 mm follicles on approximately day 0 (the day of ovulation) and day 10 for two-wave interovulatory intervals and on approximately days 0, 9, 16 for three-wave intervals (Pierson and Ginther, 1987a; Ginther et al., 1989b).

During each wave, around 7-11 small follicles (4 mm in diameter) enter a common growth phase of about 3 days (Ginther et al., 2003). Following the common growth period, one follicle of the cohort grows rapidly to attain an ovulatory diameter (dominant follicle) (Ginther et al., 2003), suppressing the growth of other follicles (subordinate follicles) and preventing emergence of a new follicular wave (Armstrong and Webb, 1997). Each antral follicular wave is stimulated by an increase in follicle-stimulating hormone (FSH) secretion (Adams et al., 1992) caused by the regression of the dominant follicle of the previous wave (Ginther et al., 2002). The dominant follicle subsequently acquires luteinizing hormone (LH) dependency for its own continued growth and suppresses FSH secretion, starving subordinate follicles of sufficient FSH support (Adams et al., 1992; Ginther et al., 2002; Austin et al., 2002).

Several methods of initiating a new follicular wave and controlling follicle “turnover” have been revealed which can be combined with traditional methods of controlling estrous cycle length. Ablation of ovarian follicles (≥5 mm), altering the endogenous release of LH and FSH or administration of exogenous steroids or gonadotropins can cause regression of a dominant follicle and emergence of a new follicular wave (Roche et al., 1997; Kohram et al., 1998 a, b). Administration of GnRH during the bovine estrous cycle causes regression or ovulation of the dominant follicle and initiates the emergence of a

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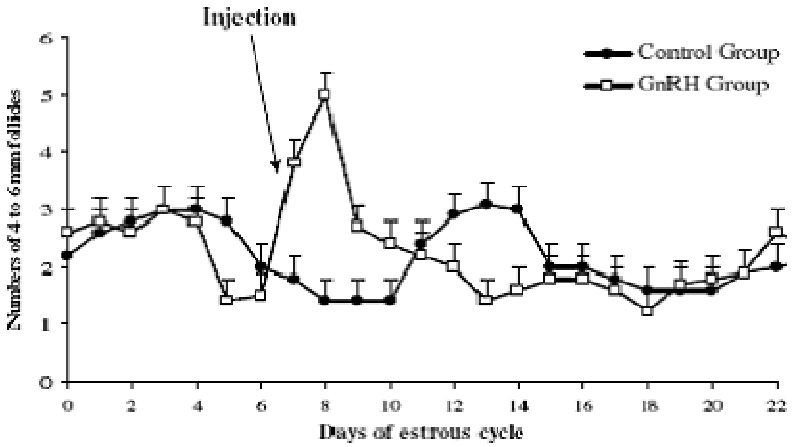

Figure 1. Mean ± SEM number of 4 to 6 mm follicles monitored by ultrasonography during the estrous cycle.

new wave of follicular growth an average of 2.5 d following treatment (Pursley et al., 1995; Kohram et al. 1998). Atresia or ovulation of the dominant follicle depends on the status (growing, static or regressing) of the dominant follicle at the time of GnRH injection (Silcox et al., 1993; Twagiramungu et al., 1994). 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 cows at d 6 of the estrous cycle. The objective of the present study was to evaluate the ovarian response of cows to GnRH injections on Day 6 of the estrous cycle.

MATERIALS AND METHODS

Animals and treatments

The estrous cycles of 10 cows were synchronized with 2 intramuscular injections of Prostaglandin F2α (Synchromate®, 150 µg cloprostenol sodium, Aburaihan company, Tehran, Iran) given 11 days apart. The cows were randomly assigned to 1 of 2 groups. In control group of Animals no injection of GnRH was performed. GnRH administered (Gonadorelin, 5 ml, intramuscular, made by Aburaihan company, Tehran, Iran) on Day 6 of the estrous cycle (estrus = Day 0).

Ultrasonography examination

Ovarian follicular development was monitored daily by transrectal ultrasonography with a real-time linear scanning ultrasound diagnostic system (B mode; Piemedical, Falco 100; 8 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: small (4 to 6) mm, and medium (≥7 mm). The diameter of the largest (F1) follicle was also recorded as described previously by Kohram et al. (1998a).

Statistical analyses

Profiles of the mean number of follicles (that is, 4 to 6 mm and ≥ 7 mm), the diameter of the largest follicle (F1), were compared by least squares analysis of variance using the general linear model (GLM) procedure of SAS software (SAS Institute, Cary, NC, Version 8.2). The multivariate analysis included sources of variation due to groups, days (repeated measures) and their interactions (MANOVA SAS; 17). The significant differences among the treatments were determined when $p < 0.05$. Data are expressed as mean ± SEM.

RESULTS

The pattern of 4 to 6 mm follicles during the estrous cycle of cows

In the two groups, the number of 4 to 6 mm follicles had a decline trend from day 4 until day 6 and 10 in GnRH and control group respectively ($p < 0.05$). The number of small follicles in group GnRH on day 6 was 1.5 ± 0.6 follicles, which increased to 5.0 ± 0.7 follicles on day 8, in response to an injection of GnRH on day 6.

The number of small follicles in the GnRH6 group decreased between days 8 and 18 ($p < 0.05$) from 5.0 ± 0.7 to 1.2 ± 0.2 follicles which were observed in ultrasonography examination. The number of this class of follicles in control group increased between d 10 to 12 and then decreased to 3.1 ± 0.5 small follicles on the 13th day of the estrous cycle. In control group, 1.5 ± 0.6 small follicles were observed between days 13 to 18 while detected changes in the ovaries were not significant (Figure 1).

The number of small follicles increased in the control and GnRH6 groups from days 18, to day 20 (NS) and 22 ($p < 0.05$) of the estrous cycle, respectively (Figure 1).

The pattern of ≥ 7 mm follicles during the estrous cycle of cows

The number of ≥ 7 mm follicles had an increase ($p < 0.05$) from day 3 until days 7 in control and 6 in the GnRH6 groups. Thereafter, the number of this class of

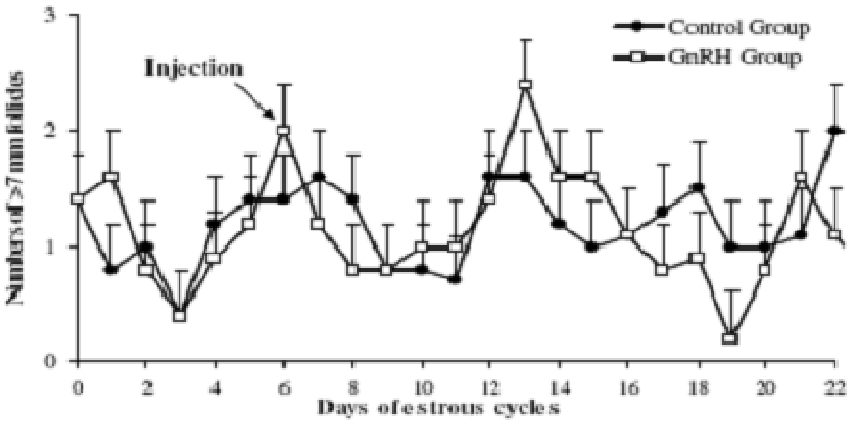

Figure 2. Mean ± SEM number of ≥ 7 mm follicles monitored by ultrasonography during the estrous cycle.

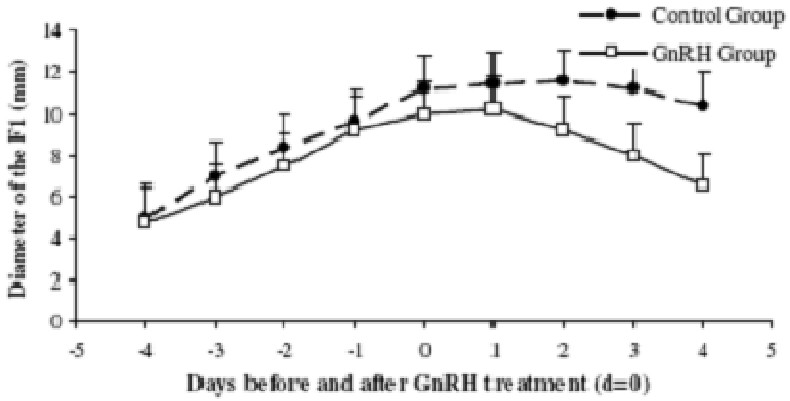

Figure 3. Diameter of the F1 before and after GnRH treatment (day 0).

follicles in control and GnRH decreased ($p < 0.05$) until days 11 and 9, respectively (Figure 2).

The number of large follicles in the GnRH6 group on day 6 was 2.0 ±0.5 follicles. In this group of cows, GnRH injec-tion on day 6 decreased ($p < 0.05$) the number of large follicles to 0.8 ± 0.6 follicles on day 9. The number of large follicles decreased to 1.0 ± 0.2 follicles in the control and 0.2 ± 0.2 in GnRH6 groups between days 13 to 15 and 13 to 19, respectively. The number of large follicles in control group had little change between days 15 and 20 (1.2 ± 0.3) while in group GnRH6 an increase from day 19 to 21 was observed (Figure 2).

Diameter of the F1 before and after GnRH treatment

Over the 4d period prior to GnRH treatment (Figure 3), the diameter of the F1 was increasing (from 4.7 ± 0.9 - 10.0 ± 0.6 mm) in cows treated with GnRH on Days 6 of the estrous cycle. The diameter of the F1 decreased ($P < 0.05$) within 3 day of GnRH treatment and this occurred regardless of whether the F1 was in the growing or plateau phase at time of treatment. In control group the diameter of the F1 increased over the 4-day period prior to GnRH treatment (from 5.0±0.9 - 11.2 ± 0.6 mm) while it remained constant (11.2 ± 0.3 - 11.2 ± 0.3 mm) between days 0 and 3 of the estrous cycle (Figure 3).

DISCUSSION

The results of daily ovarian ultrasonography showed that ovarian follicles developed in a wave like pattern with two or three follicular wave in Iranian Holstein cows, similar to those observed in other cows (Burke et al., 2000; Ahmad et al., 1997). 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 two groups showed that the first follicular wave emerged at day 0 of estrous cycles.

Previous reports (Townson et al., 2002; Sartoreli et al., 2005; Sato et al., 2005; Ginther et al., 1989b) also show-

ed that the emergence of the first follicular wave occurred between days 0 and 1 of the estrous cycle. The increase in FSH concentrations 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 occurred before day 0 of the estrous cycle.

Injection of a GnRH agonist 6 day after estrus induced ovulation of the first-wave dominant follicle, increases in the number of small and medium sized follicles after injec-tion and could emergence a new follicular wave in estrous cycle. This result is consistent with other studies carried out on cattle (Kohram et al., 1998 a, b; Peri et al., 2005; Sato et al., 2005). Several reports indicate that a GnRH agonist will ovulate a dominant follicle and cause atresia or luteinization of follicular structures of smaller size (Macmillan and Thatcher, 1991; Twagiramungu et al., 1994; Wolfenson et al., 1994). Continuous growth of the dominant follicle under a low-progestin environment preserves its ability to ovulate after exposure to a pre-ovulatory surge of LH (Rajamahendran and Taylor, 1991). A first-wave dominant follicle grown in a low-progesterone environment had more LH receptors (Cupp et al., 1993) and was more estrogenic (that is, increased estradiol in follicular fluid; De la Sota et al., 1993) than a dominant follicle of similar age grown in a high-progesterone environment.

Injection of a GnRH agonist (Buserelin) induces concurrent surges of FSH and LH (Chenault et al., 1990). Increased FSH induces recruitment of a follicular wave and programs emergence of a new ovulatory follicle (Adams et al., 1992; Badinga et al., 1992). A new dominant follicle is observed by ultrasonography 3 to 4 days after GnRH treatment. This follicle ovulates after a prostaglandin injection 6 to 7 days later (Twagiramungu et al., 1995). Administration of a GnRH agonist elicits the release of LH and FSH within 2 - 4 h from the pituitary with a response similar to the preovulatory surge in cyclic cows (Chenault et al., 1990; Rettmer et al., 1992). Administration of 10 µg of Buserelin during the luteal phase of the estrous cycle in heifers increased LH concentrations in serum for approximately 5 h (Chenault et al., 1990). A normal period of LH secretion during the preovulatory surge of LH is 10 h in cattle (Chenault et al., 1975).

In the present study, the second and third follicular wave emerged on day 8 and 18 of the estrous cycle in GnRH group. Ginther et al., (1989 b) monitored the temporal association between follicular development and luteal regression and concluded that the viable dominant follicle present at the time of luteolysis continues to grow to become the ovulatory follicle. Further, heifers and cows with shorter luteal phase duration are more likely to have two waves of follicular development than Animals with longer cycles (extended luteal function) that will have three waves (Ginther et al., 1989b; Rajamahendran and Taylor, 1991).

Early induction of ovulation with GnRH led to a smaller total growth of a dominant follicle and a smaller follicle at ovulation. In addition, the follicular growth rate was slower after GnRH treatment, possibly indicating that GnRH retards follicular growth (Bleach et al., 2004; Kulik et al., 1999). Although the mean diameter of the ovulatory follicle at ovulation was smaller in the GnRH-treated groups than in the control groups due to earlier ovulations induced with GnRH, the sizes of ovulatory follicles were within the range of normal ovulatory follicles during induced and spontaneous estrus described above (Celik et al., 2005; Townson et al., 2002). Hence, it is obvious that the size and growth of the ovulatory follicle provide sufficient capacity for further normal development after ovulation (Burns et al., 2005; Kulick et al., 1999). An increase the number of small and medium sized follicles 2 day after GnRH injection showed that an injection of GnRH at the day 6 of the estrous cycle could promote the emergence of a new follicular wave in cows.

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