EFFECT OF POST-MATING GNRH TREATMENT ON SERUM PROGESTERONE, LUTEINIZING HORMONE LEVELS, DURATION OF ESTROUS CYCLE AND PREGNANCY RATES IN COWS

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ABSTRACT

Pregnancy rate, estrous cycle lenght, serum progesterone and luteinizing hormone (LH) concentrations were determined in gonadotropin releasing hormone (GnRH; 10.5 μg synthetic gonadotrophin releasing hormone agonist, receptal) administered cows on day 12 post-mating (n=9) compared to control cows (n=8). Their oestrous cycles were synchronised by intramuscular administration of prostaglandin F_2 alpha (its analog, cloprostenol) twice at 11 days interval. Estrous exhibited cows were mated naturally. Blood samples were collected every two days from all animals. Serum progesterone and LH concentrations were measured by ELISA method. GnRH administration significantly increased serum LH concentration which reached peak levels 2-3 h after treatment. However, serum progesterone concentration was not affected. There were no differences in mean progesterone concentrations on days 12 to 24 post-mating between GnRH administrated and control pregnant cows. However, in non pregnant animals, progesterone concentrations on days 16 in the treated group were lower than control group (P<0.01). Pregnancy diagnosis in animals made by B-mode ultrasonography between the 30th and 35th day showed that 77.7% of treated cows were pregnant compared to 50% in control group. Duration of the estrous cycle in the non-pregnant animals was not affected by the treatment (control, 21.3 ± 0.8 days; treated, 22.5 ± 0.5 days). In conclusion, this study supports the use of GnRH on day 12 post-mating as a method for enhancing pregnancy rates in lactating dairy cattle.

Key words: GnRH, progesterone, luteinizing hormone, pregnancy, cows.

INTRODUCTION

İn cattle, major of embryonic loss occurs in preimplantation stage i.e during the first 20 days of pregnancy, when 75-80% of fertilized eggs are lost (Sreenan et al., 1996). One of the causes of embryonic loss is thought to be the inadequate luteal function (Willard et al., 2003). Various hormonal treatments have been attempted to reduce embryonic loss or improve pregnancy rates in cattle. Recent interest has been focused on gonadotrophin releasing hormone (GnRH) and human chorionic gonadotropin (HCG) hormones. However, their effects on pregnancy rates are inconsistent. Some researchers have reported increased conception rate in lactating dairy cattle (Macmillan et al., 1986; Drew and Peters, 1992; Anjum et al., 2009), wherease, others have reported no effect (Ryan et al., 1991; Coleman et al., 1991). Use of GnRH or its agonists to increase conception rates should be based on an understanding of GnRH-induced biological effects on the reproductive-endocrine system. This effect may occur through GnRH-stimulated LH surge, stimulating production of progesterone by corpus luteum (Thatcher et al., 1993; Gaja et al., 2008).

It is reported that injection of GnRH agonist, buserelin, between days 11 and 13 after insemination in lactating dairy cows resulted in extended interestrous intervals and elevated concentration of progesterone in serum (Rettmer et al., 1992; Stevenson et al., 1993). On the contrary, Young and Swanson (1988) and Coleman et al. (1991) could not confirm such effects.

It was stated that injection of a GnRH on mid-cycle after insemination induces sufficient release of LH and FSH to increase the life span of the corpus luteum by counteracting luteolysis through distruption of normal follicular growth and secretion of estrogen, thereby permitting maternal recognition of pregnancy to occur (Roberts et al., 1990; Willard et al., 2003). Macmillan et al. (1986) suggested that GnRH had luteotrophic and luteoprotective effects, thereby enabling maternal recognition of pregnancy.

The objectives of the present study were to determine the changes in LH and progesterone prior to and 1, 2, 3, 4 and 5 h after injection of 10.5 μg buserelin acetate and to monitor concentrations of progesterone during estrous cycle in GnRH-treated and control groups. Also, it was aimed to investigate effects of GnRH treatment on pregnancy rates and duration of estrous cycle in cows.

MATERIALS AND METHODS

Experimental design

In this study, a total of 17 adult cows (average body weight 400-450 kg) between 3-6 years of age (6 Holstein, 7 Swiss-Brown and 4 Simmental) were used. The study was conducted at the Research and Implementation Farm, Faculty of Veterinary Medicine, Fırat University, Turkey, between September and December, 2002.

All animals were kept outdoor under the same care and feeding conditions, fed hay and concentrate twice daily and were provided with water *ad libitum*. Estrous in these animals was synchronised by twice intramuscular administration of prostaglandin F_2 alpha (Estrumate, Sanofi DİF, İstanbul, Turkey) at 11 days apart (first injection given between 50 and 60 days postpartum). All cows exhibited estrous after the second injection of prostaglandin F_2 alpha and were mated by bulls approximately 8 to 10 h after the estrous observation. Then, the cows were assigned randomly into treatment (n=9) and control (n=8) groups. On day 12 post-mating (day of estrous=day 0), the cows in the treatment group were given i.m. injection of 10.5 µg of synthetic GnRH agonist (Receptal, Intervet, Istanbul, Turkey) and the cows in the control group was given 2 ml (0.9% NaCl solution) saline injection. Estrous and insemination dates were recorded. Pregnancy status was determined between 30 and 35 days after insemination by transrectal ultrasonography using a real-time, B-mode diagnostic ultrasond scanner (100 Falco, Pie Medical Application Manual, Equipment B.V., Maastricht, Netherlands) equipped with a linear array, 7.5-MHz rectal transducer.

Blood collection

Blood samples were collected into vacutainer tubes by jugular venipuncture from all animals at two days intervals during the oestrous cycles induced following the second injection of prostaglandin F_2 alpha to measure serum concentrations of progesterone. In addition, blood samples were collected via jugular catheters at 1 h intervals from -1 to +5 h after injecting saline or receptal, serum was harvested and stored at -20˚C to measure serum concentrations of progesterone and LH.

Hormone assay

Serum samples were analyzed using a double-antibody ELISA technique for determination of progesterone (Prakash *et al.*, 1987; Ali *et al.*, 2009) and LH (Mutayoba *et al.*, 1990). All assays were carried out in 96 well microtitre plates (Nunc-Immunoplate, Cat. No. 439454, Brand Products, Denmark), with standards, samples and controls were analyzed in duplicate. The range of standards for progesterone and LH was from 0.25 to 16.0 ng/ml and 0.8 to 50 ng/ml,

respectively. Intensity of colour was measured at 450 nm with an 8-channel microtitration plate photometer (Tecan, Spectra III, A 5082, Austria) and results were evaluated using EasyWin Kinetics software supplied by Tecan. The sensitivities of assays were 0.11 ng/ml for progesterone and 0.11 ng/ml for LH. Intra- and inter assay coefficients of variation were 7 and 14% for progesterone and 10 and 15% for LH, respectively.

Statistical analysis

Differences in plasma progesterone and LH concentrations between GnRH treated and control groups on day 12 post-mating were analyzed with ANOVA and concentrations of progesterone during estrous cycle in the GnRH treated and control groups were compared by unpaired student t test. Unpaired student t test was also performed for duration of the estrous cycle in the non-pregnant animals.

RESULTS

Serum LH concentrations (Table 1) reached a peak at about 2-3 h after GnRH administration (P<0.01), then declined following the next 2 h. No significant change in serum LH concentrations was observed in cows injected with normal saline during the 5 h sampling period.

The differences in concentrations of serum progesterone prior to and 1, 2, 3, 4 and 5 h after GnRH administration and saline on day 12 post-mating were non significant (Table 1). Also, there were non significant differences in serum progesterone concentrations between GnRH-treated cows and controls during the same sampling period.

Serum progesterone concentrations in cows during estrous cycle in control and GnRH treated groups are presented in Table 2. The progesterone concentration on day 16 after mating in non pregnant cows in control group was significantly higher than that in GnRH treated groups (P<0.01), but the difference was not significant for the other days of estrous cycle. Also, the diferences in mean progesterone concentrations in pregnant cows during 24 days after mating between control and GnRH treated groups were non significant. Similarly, the differences in the duration of the estrous cycle of non-pregnant cows between GnRH-treated (22.5 ± 0.5 days) and control groups (21.3 ± 0.8 days) were non significant.

Cows were regarded to be pregnant when the progesterone concentrations in serum were above 1.6 ng/ml 24th day post mating. Pregnancy rates in cows treated with GnRH were 77.7% (7/9) compared with 50% in control group. This finding was confirmed using ultrasonography between 30 and 35 days post-mating.

DISCUSSION

In the present study, pregnancy rates in cows treated with 10.5 µg GnRH on day 12 post mating were higher than those in the saline treated group. Pregnancy rates following the treatment with GnRH on 11-12 days post-mating have been demonstrated to be increased in some experiments (Macmillan *et al*., 1986; Drew and Peters, 1992; Peters *et al*., 2000), while no such increase was reported by others (Ryan *et al*., 1991; Coleman *et al*., 1991; Thatcher *et al*., 1993).

Serum LH concentrations reached a peak level at about 2-3 h after GnRH administration (P<0.01), and then declined during the next 2 h. No significant changes in serum LH were observed in cows injected with normal saline during the 5 h sampling period. Treatment of lactating cows with 10.5 µg buserelin acetate on day 12 after insemination results in LH release from the pituitary. GnRH stimulates the production of progesterone in corpus luteum on the ovary. Duration of the response and time to peak LH concentrations were within the range of values reported previously using a similar dose of GnRH (8 or 12 µg buserelin) on days 11 to 14 after insemination (Stevenson *et al*., 1993) but less than those observed after administration of 10 or 20 µg buserelin during midcycle (Chenault *et al*., 1990). The serum concentration of LH in cows during 12 hours after treatment with 8 µg of receptal peaked (12.6 ng/ml) at 172 minute, which was significantly higher than that of control (0.6 ng/ml) (Stevenson *et al*., 1993).

Differences in the concentrations of serum progesterone prior to and 1, 2, 3, 4 and 5 h after GnRH administration on day 12 post-mating were non significant. Similarly, serum progesterone concentrations in GnRH-treated group were non significantly different when compared with those in control group during the same sampling period (Table 1). Coleman *et al*. (1991) also reported that concentrations of progesterone for 3 hours after 8 µg receptal injection in cattle were not different when compared with those in saline treated animals. In contrast, concentrations of progesterone in serum increased during 6 to 12 hours after injection of 8 or 12 µg of receptal administered on days 11 to 14 after estrous (Stevenson *et al*., 1993).

In the current study, differences in mean progesterone concentrations on day 12 to 24 post-mating in pregnant cows in control and in GnRH treated groups were non significant (Table 2). However, progesterone concentrations on day 16 of estrous cycle in control non pregnant cows were higher than those in GnRH treated non pregnant cows (P<0.01). Treatment of dairy heifers with GnRH during the mid (days 9-12) stages of the estrous cycle did not significantly affect serum progesterone concentrations, nor the duration of the estrous cycle (Lucy and Stevenson 1986; Young and Swanson, 1988; Coleman *et al*., 1991). However, injection of GnRH on days 11 to 14 after artificial insemination in lactating cows increased serum progesterone level (Rettmer *et al*., 1992; Stevenson *et al*., 1993; Tefera *et al*., 2001; Howard *et al*., 2006). In contrast, treatment with GnRH on day 10 of the estrous cycle caused a reduction in concentrations of serum progesterone on days 12, 14 and 16 of the cycle (Rodger and Stormshak, 1986). The finding that GnRH had no effect on progesterone levels is consistent with the reports of Young and Swanson (1988) and Coleman *et al*. (1991).

In this study, the difference in the duration of the estrous cycle of non-pregnant cows between GnRH-treated (22.5 ± 0.5 days) and control groups (21.3 ± 0.8 days) was non significant. Lenght of the estrous cycle in cows treated with GnRH on day 10 of the estrous cycle (21.1 days) did not differ from that of control (20.7 days) animals (Rodger and Stormshak, 1986). Similarly, Coleman *et al*. (1991) reported that treatment with 8 µg buserelin acetate on day 14 after insemination had no effect on cycle lenght. However, it has also been shown that the length of the estrous cycle was extended by up to 2 days following the injections of receptal compared with controls (Macmillan *et al*., 1985; Stevenson *et al*., 1993).

In conclusion, the results of our study indicate that GnRH could be used as a method for improving fertility rate in cattle.

Table1: Mean (± SE) serum LH and progesterone (P4) concentrations (ng/ml) in cows before and after administration of GnRH and saline

Parameters	Time after treatment					
	-1 h	1 h	2 h	3 h	4 h	5 h
LH (GnRH)	0.70 ± 0.01 [a]	2.52 ± 0.34 [ab]	9.05 ± 1.53 [d]	9.51 ± 1.50 [d]	6.74 ± 1.13 [cd]	3.90 ± 0.72 [cd]
LH (saline)	0.70 ± 0.01 [a]	0.70 ± 0.01 [a]	0.70 ± 0.01 [a]	0.70 ± 0.01 [a]	0.70 ± 0.01 [a]	0.70 ± 0.01 [a]
P4 (GnRH)	1.56 ± 0.32 [a]	1.51 ± 0.14 [a]	1.60 ± 0.35 [a]	2.70 ± 0.73 [a]	2.06 ± 0.47 [a]	2.51 ± 0.43 [a]
P4 (saline)	1.93 ± 0.12 [a]	1.62 ± 0.24 [a]	1.67 ± 0.18 [a]	2.03 ± 0.21 [a]	1.82 ± 0.37 [a]	1.76 ± 0.28 [a]

Means in the same row with different superscripts differ significantly (P<0.01).

Table 2: Mean (± SE) serum progesterone concentrations (ng/ml) in pregnant and non-pregnant cows of GnRH-treated and control groups

Days of estrous cycle	Pregnant		Non-pregnant	
	GnRH (n=7)	Saline (n=4)	GnRH (n=2)	Saline (n=4)
0	0.61 ± 0.03	0.50 ± 0.02	0.35 ± 0.06	0.42 ± 0.01
2	0.71 ± 0.08	0.62 ± 0.07	0.61 ± 0.02	0.54 ± 0.04
4	1.04 ± 0.12	1.04 ± 0.33	0.93 ± 0.08	1.05 ± 0.06
6	1.44 ± 0.22	1.81 ± 0.09	1.63 ± 0.17	1.18 ± 0.16
8	1.72 ± 0.34	2.02 ± 0.16	2.18 ± 0.51	1.63 ± 0.22
10	1.78 ± 0.11	2.32 ± 0.17	2.41 ± 0.35	2.13 ± 0.48
12	2.24 ± 0.09	3.07 ± 0.77	2.34 ± 0.28	1.76 ± 0.18
14	2.77 ± 0.32	2.87 ± 0.62	1.77 ± 0.14	2.18 ± 0.21
16	2.14 ± 0.45	2.92 ± 0.18	1.84 ± 0.29 [a]	2.77 ± 0.61 [b]
18	2.33 ± 0.14	2.57 ± 0.21	2.53 ± 0.24	2.09 ± 0.15
20	2.94 ± 0.43	2.67 ± 0.46	1.51 ± 0.12	1.44 ± 0.33
22	2.48 ± 0.32	1.93 ± 0.25	0.85 ± 0.17	0.65 ± 0.06
24	2.82 ± 0.15	2.63 ± 0.19		

Means in the same row with different superscripts differ significantly (P<0.01).

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