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Effect of GnRH Administered 7 Days after Insemination on Serum Progesterone, Luteinizing Hormone and Duration Estrous Cycle with Pregnancy Rates in Heifers

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Abstract: Serum Progesterone (P4), Luteinizing Hormone (LH) concentrations, estrous cycle lenght ve pregnancy rates were determined in Gonadotropin Releasing Hormone (GnRH; 10.5 μg synthetic gonatotrophin releasing hormone agonist, receptal) administered heifers on day 7 after insemination (n = 9) compared to control heifers (n = 5). All heifers oestrous cycle were synchronised by intramuscular administration of prostaglandin F_2 α (its analog, cloprostenol) twice at 11 days interval. Estrous exhibited heifers were mated naturally. Blood samples were collected every two days from all animals. Serum progesteron and LH concentrations were measured by ELISA method. GnRH administration significantly increased serum LH concentration which reached peak level 2nd h after treatment. However, serum progesterone concentration was not affected. There were no differences in mean progesterone concentrations on days 7-24 after insemination between GnRH administered and control pregnant heifers. However, in non pregnant animals, progesteron concentrations of treated group were significantly lower than control group on 16th day ($p<0.05$). Pregnancy diagnosis was made by B-mode ultrasonography between the 30 and 35th days following insemination. Pregnancy was determined in 5 animals in treated group and in 2 animals in control group. Duration of the estrous cycle in the non-pregnant animals was not affected by the treatment (control, 20.6±0.96 days; treated, 19.5±0.93 days). In conclusion, this study supports the use of GnRH after insemination of 7 day as a method for enhancing pregnancy rates in heifers.

Key words: Pregnancy, heifer, progesterone, LH, GnRH, oestrous cycle length

INTRODUCTION

Progesterone hormone deficiency after insemination has an important place among the causes of early embryonic death. Even though average fertilization rates of heifer and cows are between 88-90, 20% or more of the embryos are lost after insemination before 21th days (Diskin and Sreenan, 1980; Drew and Peters, 1994).

GnRH injections are applied from 4-15 days after insemination for the prevention of early embryonic death which is attached to inadequate progesterone hormone in cattle. These applications aim is to ensure the earlier formation of the corpus luteum and progesterone secretion, encourage a secondary corpus luteum and to increase progesterone levels as a result of this to prevent possible deficiency of progesterone hormone.

Bentele and Humke (1987) reported that on days 9-13 after insemination. Application of GnRH did not affect pregnancy rates but the best effect is related to the decrease of early embryonic death. Ryan *et al.* (1991) reported that effect of environmental stress on the same days GnRH application reduced as a result of the hypothalamus GnRH secreted, probably by increasing the rate of luteal function and ovulation may improve pregnancy rates. Ryan *et al.* (1994) reported that on day 12 application of GnRH in a temperate climate area of in lactating dairy cows with progesterone and LH hormone levels and follicle numbers ovary changed but did not affect the pregnancy rate. Howard *et al.* (2006) reported that of GnRH administration on day 5 after insemination caused accessory corpus luteum formation, serum progesterone concentration increased but not in pregnancy rate. Willard *et al.* (2003) reported that GnRH administration on days 5 or 11 after insemination caused under high temperature stress in dairy cows ovaries to the formation of the more corpus luteum tissue, the elevation

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of serum progesterone concentration and pregnancy rates tend to show a larger increase. In another study, Lopez-Gatius *et al.* (2006) reported that of GnRH application on day 12 post-insemination increased the pregnancy rate did not affect the rate of twin pregnancies but increased incidence of accessory corpus luteum formation. In a similar study also Bartolome *et al.* (2005) reported that of GnRH administration on days 5 or 15 after insemination did not increase pregnancy rate but reduced losses of pregnancy between 27-55 days.

In the same study even GnRH was applied in non-pregnant cows in interestrous range (23 days) in controls (21 days) were found to be longer. Sheldon and Dobson (1993) reported that to increase pregnancy rates GnRH can be applied 1, 2 and later insemination after 11th day and this practice increased pregnancy rate that does not include all embryonic death by reducing early embryonic death. Harvey *et al.* (1994) reported that on day 11-13 after insemination some of the cows in the GnRH applied and that these structures formed in the secondary corpus luteum determined that all pregnant cows were detected.

In this study, it was aimed that effect of GnRH administered 7 days after insemination on serum progesterone, LH concentration and duration of oestrus cycle with pregnancy rates in heifers.

MATERIALS AND METHODS

In this study, a total 14 adult heifers (average body weight 300-350 kg) between 18-24 months of age (8 Simmental, 4 Swiss-Brown and 2 Holstein) were used. The study was conducted at the Research and Implementation Farm Faculty of Veterinary Medicine, Firat University, Turkey. 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 α (Estrumate, Sanofi DIF, Istanbul, Turkey) at 11 days apart. All cows exhibited estrous after the second injection of prostaglandin F_2 α and were mated by bulls approximately 8-10 h after the estrous observation. Then, heifers were assigned randomly into treatment (n = 9) and control (n = 5) groups. On day 7 post-mating (day of estrous = day 0), the heifers in the treatment group were given i.m. injection of 10.5 μg of synthetic GnRH agonist (Receptal, Intervet, İstanbul, Turkey) and the heifers in the control group was given 2 mL (0.9% NaCl solution) saline injection. Pregnancy diagnosis was determined between 30 and 35 days after insemination by transrectal ultrasonography using a real-time, B-mode diagnostic ultrasound scanner (100 Falco, Pie Medical Application Manual, Equipment B.V., Maastricht, Netherlands) equipped with a linear array, 7.5-MHz rectal transducer.

Bloods 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 α 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.

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).

Differences in plasma progesterone and LH concentrations between GnRH treated and control groups on day 7 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 AND DISCUSSION

Serum LH concentrations (Table 1) reached a peak level 2nd h after GnRH administration ($p<0.01$), then declined following the next hours. No significant change in serum LH concentrations was observed in saline injected heifers during the 5 h sampling period but in the beginning to the end of time measurement serum LH concentrations in GnRH administered heifers are significantly higher than saline administered heifers (1-4 h; $p<0.01$, 4-5 h; $p<0.05$), (Fig. 1). The differences in concentrations of serum progesterone, except 1 h, prior to and during 1-5 h after GnRH administered and saline on day 7 post-mating were not significant (Table 1). Also, there were not significant differences in serum progesterone concentrations between GnRH-treated heifers and controls during the same sampling period.

Serum progesterone concentrations in heifers during estrous cycle in control and GnRH treated groups are shown in Table 2. The progesterone concentration on day 16 after mating in non pregnant heifers in control group was significantly higher than GnRH treated groups ($p<0.05$) but the difference was not significant for the other days of estrous cycle. The differences in mean progesterone concentrations in pregnant heifers during 24 days after mating between control and GnRH treated

Table 1: Mean (±SE) serum LH and Progesterone (P4) concentrations (ng mL^{-1}) in heifers before and after administration of GnRH and saline

	Time after treatment					
Parameters	-1 h	1 h**	2 h**	3 h**	4 h**	5 h*
LH (GnRH)	0.70±2.92	9.52±2.48^{a}	23.56±3.40^{a}	16.37±2.38^{a}	10.68±2.60^{a}	5.91±2.25^{a}
LH (Saline)	0.70±2.27	0.70±0.00^{b}	0.92±0.70^{b}	0.70±0.00^{b}	0.70±0.00^{b}	0.70±0.00^{b}
P4 (GnRH)	0.84±0.79	0.45±0.00^{a}	0.65±0.64	0.69±0.70	0.78±0.99	0.54±0.52
P4 (Saline)	1.43±1.22	0.77±0.55^{b}	1.05±1.05	0.53±0.33	1.49±1.33	1.17±1.00

^{a,b}p<0.05 *p<0.05, **p<0.01

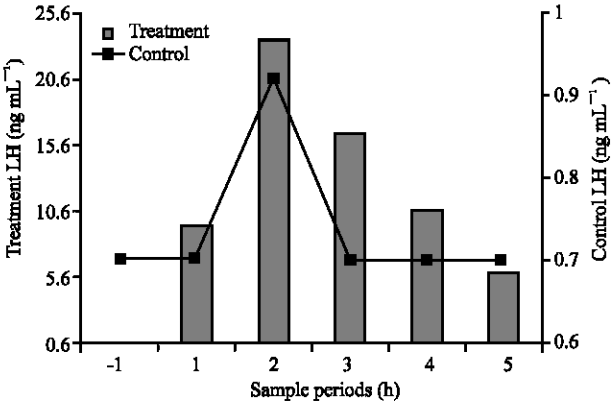

Fig. 1: Mean serum LH concentrations before and after administration of GnRH and saline

groups, except for 4th day (p<0.05) were not significant (Table 2). The differences in duration of the estrous cycle of non-pregnant heifers between GnRH-treated (19.5±0.93 days) and control groups (20.6±0.96 days) were not significant (Fig. 2).

Heifers were regarded to be pregnant when the progesterone concentrations in serum were above 1 ng mL^{-1} 22th day post mating. Five out of nine heifers in GnRH treated group on day 7 post-mating and two out of five heifers control group were recorded to be pregnant. This finding was confirmed using ultrasonography between 30 and 35 days post-mating

Serum LH concentrations reached a peak level with 23.56 ng mL^{-1} 2nd h after GnRH administration in GnRH treated heifers (p<0.01) and then declined during the next hours. No significant change in serum LH concentrations was observed during the 5 h sampling period in saline injected heifers. Treatment of heifers resulted in with 10.5 μg buserelin acetate on day 7 after insemination LH release from the pituitary. Duration of the response and time to LH peak concentrations were within the range of values reported previously using a similar dose of GnRH (8 or 12 μg buserelin) on days 11-14 after insemination (Stevenson *et al*., 1993). Rettmer *et al*. (1992a, b) reported that reached LH peak level about 2nd h after administration 200 μg fertirelin acetate injected heifers on days 11-13 after insemination and then declined and LH peak concentration 19.3 ng mL^{-1}.

Similarly Macmillan *et al*. (1985) reported that 21-30 ng mL^{-1} serum LH concentration of 5 μg buserelin administration on days 10-12 after insemination caused a peak at 2-2.5 h with and within 6 h of injection, serum LH concentrations had returned to pre-injection levels. The serum concentration of LH in cows during 12 h after treatment with 8 μg of receptal peaked (12.6 ng mL^{-1}) at 172 min, which was significantly higher than that of control (0.6 ng mL^{-1}) (Stevenson *et al*., 1993).

The differences in concentrations of serum progesterone except for 1nd h, prior to and during 1-5 h after GnRH administered and saline on day 7 post-mating were not significant (Table 1). Also, there were not significant differences in serum progesterone concentrations between GnRH-treated heifers and controls during the same sampling period. Rettmer *et al*. (1992a, b) reported that concentrations of progesterone in serum increased within 15 min after injection of 200 μg fertirelin acetate and remained elevated until 360 min postinjection on days 11-13 after estrous in heifers. Also, heifers treated with fertirelin acetate had higher (p<0.05) concentrations of progesterone in serum than control heifers from 15-360 min after treatment. Stevenson *et al*. (1993) reported that concentrations of serum progesterone increased during 6-12 h after a single injection of 8 μg receptal on days 11-14 estrus. Seguin *et al*. (1977) reported that serum progesterone concentrations increased within 2 h after administration 100 μg GnRH on days 10-11 after insemination in heifers but by 24 h.

In the study, differences in mean progesterone concentrations in pregnant heifers during 24 days after mating between control and GnRH treated groups, except for 4th day (p<0.05) were not significant. However, mean progesterone concentrations on day 16 after mating in non pregnant heifers in GnRH treated group was significantly lover than that in control group (p<0.05), (Table 2). Rettmer *et al*. (1992a, b) reported that in nonpregnant, control and fertirelin acetate-treated heifers, serum progesterone began to decrease approximately 3-5 days after treatment but heifers injected with fertirelin acetate seemed to have higher concentrations of progesterone at 7 and 8 days after treatment. Also, pregnant heifers treated with 200 μg fertirelin acetate had higher (p<0.05) concentrations of progesterone in serum

Table 2: Mean (±SE) serum progesterone concentrations (ng mL^{-1}) in pregnant and non-pregnant heifers after administration of GnRH and saline

Days of estrous cycle	Pregnant		Non-pregnant	
	GnRH (n = 5)	Saline (n = 2)	GnRH (n = 4)	Saline (n = 3)
0	0.52±0.31	0.45±0.00	0.46±0.14	0.46±0.14
2	0.63±0.37	0.45±0.00	0.58±0.36	0.61±0.34
4	0.87±0.20[a]	0.70±0.26[b]	0.72±0.30	0.74±0.36
6	1.20±0.60	0.65±0.20	0.91±0.37	0.94±0.54
8	1.37±0.45	1.48±1.00	1.02±0.48	1.29±0.73
10	1.80±0.62	1.49±0.78	1.47±0.61	1.65±0.73
12	2.24±0.67	2.19±1.14	1.62±0.75	1.92±0.72
14	2.43±0.78	2.97±1.50	2.02±0.72	2.49±0.50
16	2.45±0.71	2.71±0.82	2.00±0.60[a]	2.62±0.33[b]
18	2.35±0.53	3.09±1.11	1.15±0.47	1.28±0.10
20	2.65±0.58	2.93±0.82	0.73±0.31	0.91±0.40
22	2.51±0.84	2.69±0.65	0.61±0.33	0.71±0.34
24	2.37±1.00	2.45±0.17	0.55±0.36	0.57±0.33

[a,b]: $p<0.05$

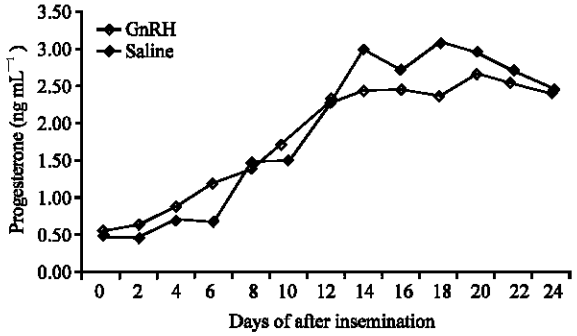

Fig 2: Mean serum progesterone concentrations in pregnant heifers after administration of GnRH and saline

than pregnant controls from 4-12 days after injection, except on day 7. Ryan *et al.* (1994) and Schmitt *et al.* (1996) reported that injection of 8-10 μg buserelin on days 5 or 12 estrous cycle cows increased dioestrous serum progesteron concentrations. Mann and Picton (1995) reported that from 12-17 days the plasma concentration of progesterone was significantly higher ($p<0.05$) in pregnant than in inseminated not-pregnant in both the control and the treated groups (10 μg buserelin administration on day 12 after insemination). Howard *et al.* (2006) reported that serum progesterone concentration of 100 μg gonadorelin treated cows on day 5 after insemination on day 13 higher than serum progesterone concentration in control groups. Leslie *et al.* (1986) reported that progesterone concentrations between days 4 and 8 were significantly greater ($p<0.01$) in pregnant cows compared to nonpregnant cows (250 μg GnRH on day 4 after insemination). Stevenson *et al.* (1993) reported that of 8 μg buserelin administration on days 11-14 after insemination, except 7 days after administration during 12 days progesterone concentrations in treated cows significantly higher ($p<0.01$) than in saline injected cows. In same study, non-pregnant heifers in the case of treated buserelin concentrations of progesterone for 3 days after administration of the non-pregnant controls were found to be higher than concentrations of progesterone. On the contrary, treatment with 100 μg GnRH on day 10 of the estrous cycle caused a reduction in concentrations of serum progesterone on days 12, 14 and 16th of the cycle (Rodger and Stormshak, 1986). Yildiz *et al.* (2009) reported that the differences in mean progesterone concentrations 10.5 μg buserelin treated on day 12 post-mating pregnant cows during 24 days after mating between control and GnRH treated groups were not significant but the progesterone concentration on day 16 after mating in non pregnant cows in control group was significantly higher than that in GnRH treated groups ($p<0.01$).

In this study, the differences in duration of the estrous cycle of non-pregnant heifers between GnRH-treated (19.5±0.93 days) and control groups (20.6±0.96 days) were not significant. Seguin *et al.* (1977) of 100 μg GnRH administration and on days 10-11 after insemination, Coleman *et al.* (1991) of 8 μg buserelin administration and on day 14 after insemination, Rodger and Stormshak (1986) of 100 μg GnRH administration and on days 2 or 10 of estrous cycle and Yildiz *et al.* (2009) of 10.5 μg buserelin administration on day 12 after insemination lenght of estrous cycle did not differ between treated and control groups. However, Ryan *et al.* (1994) reported that of 10 μg buserelin administration on day 12 after insemination increased by 1 day compared with control animals (21.5±0.3 vs. 20.5±0.3). Stevenson *et al.* (1993) reported that of 8 μg buserelin administration on days 11-14 after insemination increased approximately 2.5 day lenght of estrous cycle.

In the present study, pregnancy rates in heifers treated with 10.5 μg buserelin on day 7 after insemination were higher than those in the saline treated group. Pregnancy rates following the treatment with 6-10.5 μg buserelin on 11-14 days after insemination have been

demonstrated to be increased in some experiments (Nakao *et al*., 1995; Lajili *et al*., 1991; Sheldon and Dobson, 1993; Drew and Peters, 1994; Yildiz *et al*., 2009), while no such increase was reported by others (Jubb *et al*., 1990; Ryan *et al*., 1994; Bartolome *et al*., 2005).

CONCLUSION

The results of the study indicate that GnRH could be used a useful method for enhancing fertility rate in heifers.

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