The effect of buserelin injection 12 days after insemination on selected reproductive characteristics in cows

Mehmet B. Ataman[1], Hüseyin Erdem[2], Bülent Bülbül[3], Seyit Ümütlü[3], Mehmet Çolak[3]

[1]Department of Reproduction and Artificial Insemination, [2]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Selçuk, Konya, Turkey
[3]Bahri Dağdaş International Agricultural Research Institute, Konya, Turkey

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Abstract

The aim of this study was to evaluate the effect of buserelin injection on day 12 postinsemination on fertility in lactating dairy cattle. A total of 57 cows were assigned to two groups and four subgroups. In the treatment group, the cows were synchronized with $PGF_{2\alpha}$-$PGF_{2\alpha}$ (group A) or GnRH-$PGF_{2\alpha}$ (group B) protocol, and buserelin was injected on day 12 postinsemination. Cows in the control group were synchronized with $PGF_{2\alpha}$-$PGF_{2\alpha}$ (group C) or GnRH-$PGF_{2\alpha}$ (group D) protocol, saline solution was injected on day 12, and served as controls. Pregnancy rates on day 21 and 45 and embryonic death rates were 85.7%, 71.4% and 16.7%, 85.7%, 85.7% and 0.0%, 73.3%, 62.1% and 27.3% and 85.7%, 71.4% and 16.7% in groups A, B, C and D, respectively. There was no significant difference between synchronization protocols for pregnancy rates, and among groups A, B, C and D for pregnancy rates and embryonic death rates. Mean progesterone concentrations in pregnant cows in groups A and B were higher than that in groups C and D, respectively, on day 18 and 21 ($p < 0.05$). In conclusion, GnRH injection on day 12 postinsemination increased the plasma progesterone concentrations on day 18 and 21 postinsemination. However, it did not alter the pregnancy rates and prevent embryonic deaths.

GnRH, plasma progesterone, pregnancy, embryonic death, cow

For dairy farming to remain a profitable enterprise, more attention and skill need to be devoted to increasing reproductive efficiency (Jobst et al. 2000). Vasconcelos et al. (2006) reported that fertility was influenced by many factors including cyclicity, energy balance, heat stress, parity, milk production, diet and diseases. In addition, early embryonic loss is the main factor affecting fertility (Sheldon 1997). One of the reasons causing early embryonic death is low progesterone (P_4) production, possibly due to poor luteinization after ovulation or deficiency of corpus luteum (CL) (Schmitt et al. 1996).

Gonadotrophin releasing hormone (GnRH) occupies a central role in the reproductive function and has a potential for fertility control in mammals (Diskin et al. 2002). In treatment, GnRH administration at oestrus increased serum concentrations of P_4 and improved pregnancy rates in dairy cows (Ullah et al. 1996).

A functional CL is required for the maintenance of pregnancy (Howell et al. 1994). Inducing formation of an additional CL during the luteal phase of the oestrus cycle with a gonadotropin challenge could be a strategy to increase concentration of P_4 in plasma (Macmillan and Thatcher 1991).

De Rensis and Peters (1999) reported that treatment with GnRH during the luteal phase may stimulate transformation of small luteal cells to large luteal cells and seems to prolong CL lifespan by partially protecting the CL against spontaneous luteolysis. GnRH injection causes a predictable release of luteotrophic hormone (LH) and a significant increase in serum P_4 (Stevenson et al. 1993). In addition, it is reported that GnRH injection on day 5 or 11 (Willard et al. 2003) and 11 to 14 (Hansen 2002) after artificial insemination (AI) increased serum concentrations of P_4 and caused a tendency toward higher pregnancy rates.

Address for correspondence:
Dr. Bülent Bülbül, DVM, PhD
Bahri Dağdaş International Agricultural
Research Institute, 42020, Karatay, Konya
Turkey

Phone: +90 332 3551290
Fax: +90 332 3551288
E-mail: bulbulent@hotmail.com
http://www.vfu.cz/acta-vet/actavet.htm

The aim of this study was to evaluate the effect of GnRH injection on day 12 after the AI on plasma P_4 concentrations, pregnancy rates and to prevent early embryonic deaths in lactating dairy cattle synchronized with GnRH-Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or $PGF_{2\alpha}$-$PGF_{2\alpha}$ combination.

Materials and Methods

Animals

This study was carried out in 57 lactating Brown Swiss cows, aged 3-5 years. The animals were selected taking the criterions listed as followed: no dystochia and retained foetal membranes in previous calving; no purulent discharge during vaginal examination; 50 to 90 day postpartum; have shown oestrus at least once; no AI or mating after previous calving. The cows were fed with a ration composed of corn silage, alfalfa hay and a concentrate-mineral mix, had *ad libitum* access to fresh water and were housed in a free-stall confinement facility.

Experimental protocol

The cows were randomly assigned to two treatments (GnRH treatment or placebo injection 12 day after AI) and four subgroups (Fig. 1). In the treatment group (n = 28), the cows were synchronized with $PGF_{2\alpha}$-$PGF_{2\alpha}$ (group A, n = 14) or GnRH-$PGF_{2\alpha}$ (group B, n = 14) protocol. In the $PGF_{2\alpha}$-$PGF_{2\alpha}$ protocol, the cows were given two intramuscular injections of 0.150 mg d-cloprostenol (Dalmazin, Vetaş, Turkey) 14 days apart at a random stage of the oestrous cycle. In the GnRH-$PGF_{2\alpha}$ protocol, the cows were treated with an intramuscular injection of 20 µg buserelin (Receptal, Intervet, Turkey) (day = -7) at a random stage of the oestrous cycle followed by intramuscular injection of 0.150 mg d-cloprostenol 7 days later.

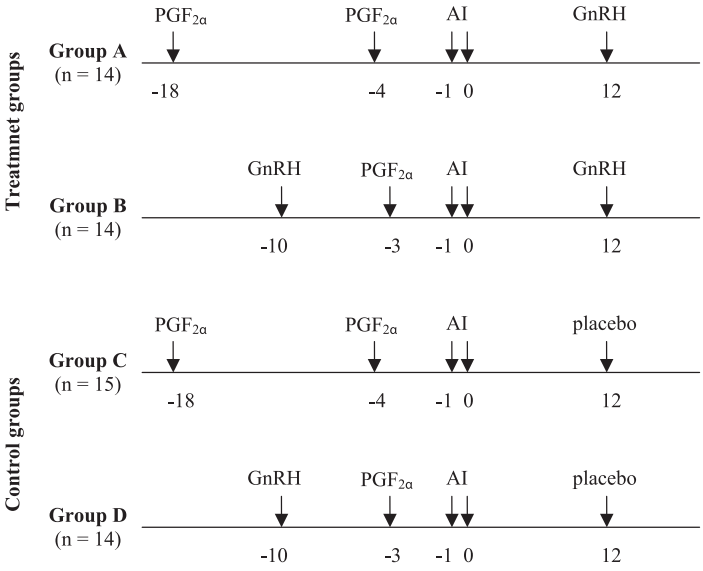

Fig. 1. Experimental design
GnRH = gonadotrophin releasing hormone, $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$, AI = artificial insemination

Cows in the control group (n = 29) were synchronized with $PGF_{2\alpha}$-$PGF_{2\alpha}$ (group C, n = 15) or GnRH-$PGF_{2\alpha}$ (group D, n = 14) protocol described as the treatment group. AIs were performed by a single practitioner twice at 72 and 96 h after the last cloprostenol injection in groups A and C and at 48 and 72 h after cloprostenol injection in groups C and D according to Çoyan (2002) and Yamada et al. (2002). Buserelin at a dose of 10 µg in treatment groups and saline solution at a dose of 2 ml in control groups was also injected intramuscularly on d 12 after the last AI. Pregnancy was determined by serum progesterone concentrations on day 21 and by 5-7.5 MHz transrectal ultrasonography (480 Vet, Pie Medical, Maastrich, The Netherlands) 45 days after the last insemination.

Blood samples

Blood samples were collected on day 0, 3, 6, 9, 12, 15, 18 and 21 from the jugular vein into evacuated 10 ml tubes containing heparin to determine plasma P_4 concentrations in all groups starting from the day of last AI.

Plasma was prepared by centrifugation (3000 x *g* for 5 min) and frozen at -20 °C within 2-4 h for subsequent determinations of P_4 concentrations. The plasma P_4 concentrations were determined by an enzyme immunoassay method (Prakash et al. 1987).

Statistical analyses

Results for mean P_4 concentrations were expressed as mean ± SEM. The x^2 test was used to compare the pregnancy and embryonic loss rates. P_4 levels in groups were evaluated with analysis of variance (ANOVA). All analyses were carried out using a statistical analysis system configured for computer (MINITAB, Release 12.1, Minitab Inc.). The differences were considered significant at $p < 0.05$.

Results

Pregnancy rates on day 21 and 45 and embryonic death rates in all groups are shown in Table 1. There was no significant difference between synchronization protocols GnRH-$PGF_{2\alpha}$ and $PGF_{2\alpha}$-$PGF_{2\alpha}$ for pregnancy rates and plasma P_4 concentrations. Pregnancy rate in cows treated with GnRH 12 days after the insemination was higher on day 21 and 45 but the difference was not significant. The highest and the lowest pregnancy rates were obtained in groups B and C on day 45. Although the differences among groups A, B, C and D were not significant for pregnancy rates both on day 21 and day 45, there was a tendency in the pregnancy rate to be higher in group B than group C on day 45 ($p = 0.06$) (Table 1).

Table 1. Pregnancy rates (%) on day 21 (according to plasma progesterone concentrations) and on day 45 (according to ultrasound examination) and embryonic loss in placebo and gonadotrophin releasing hormone treated cows.

Treatment	Synchronization protocol	Pregnancy rate (%) (on day 21)	Pregnancy rate (%) (on day 45)	Embryonic death rate (%)
	(Group A)	85.7	71.4	16.7
	$PGF_{2\alpha}$-$PGF_{2\alpha}$	(12/14)	(10/14)	(2/12)
GnRH	(Group B)	85.7	85.7	0
	GnRH-$PGF_{2\alpha}$	(12/14)	(12/14)	(0/12)
	Total	85.7	78.6	8.3
		(24/28)	(22/28)	(2/24)
	(Group C)	73.3	62.1	27.3
	$PGF_{2\alpha}$-$PGF_{2\alpha}$	(11/15)	(8/15)	(3/11)
Control	(Group D)	85.7	71.4	16.7
	GnRH-$PGF_{2\alpha}$	(12/14)	(10/14)	(2/12)
	Total	79.3	62.1	17.2
		(23/29)	(18/29)	(5/23)

GnRH = gonadotrophin releasing hormone, $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$

Embryonic death rate in GnRH treated cows was similar to that in placebo injected cows. Although there was no significant difference for embryonic death rate among groups A, B, C and D, it tended to be higher in group C than group B ($p = 0.052$) (Table 1).

Mean P_4 concentrations in plasma (ng/ml) on day 0, 3, 6, 9, 12, 15, 18 and 21 starting from the day of last AI in groups A, B, C and D were summarized in Table 2. Mean P_4 concentration in pregnant cows in group D was higher than that in group B ($p < 0.05$) on day 0. In group C, mean P_4 concentration in nonpregnant cows was higher than that in the pregnant cows in group A ($p < 0.05$), and in group D, mean P_4 concentration in pregnant cows was higher than that in nonpregnant cows ($p < 0.05$) on day 3 (Table 2).

There were significant differences between pregnant and nonpregnant cows in group D, between nonpregnant cows in groups A and B, between nonpregnant cows in groups B and D ($p < 0.05$) for mean P_4 concentration on day 6. Mean P_4 concentration in the nonpregnant

Table 2. Mean P_4 concentrations in cow plasma (ng/ml) on day 0, 3, 6, 9, 12, 15, 18 and 21 starting from the day of last artificial insemination in groups A, B, C and D (± S.E.M.) (according to pregnancy on day 45) (minimum-maximum values are presented in paranthesis).

Days	Group A		Group B		Group C		Group D	
	+ (n = 10)	- (n = 4)	+ (n = 12)	- (n = 2)	+ (n = 8)	- (n = 7)	+ (n = 10)	- (n = 4)
0	0.25 ± 0.05 (0.10–0.48)	0.21 ± 0.07 (0.10–0.35)	0.23 ± 0.04^{b} (0.10–0.50)	0.27 ± 0.08 (0.19–0.35)	0.32 ± 0.03 (0.15–0.48)	0.36 ± 0.03 (0.25–0.48)	0.38 ± 0.04^{a} (0.10–0.50)	0.34 ± 0.04 (0.20–0.44)
3	0.39 ± 0.06 (0.14–0.76)	0.24 ± 0.05^{b} (0.13–0.35)	0.66 ± 0.21 (0.15–2.45)	0.91 ± 0.35 (0.56–1.25)	0.52 ± 0.07 (0.28–0.75)	0.56 ± 0.10^{a} (0.27–0.92)	0.59 ± 0.08^{c} (021–100)	0.34 ± 0.07^{d} (0.20–0.62)
6	1.51 ± 0.31 (0.28–2.91)	0.85 ± 0.38^{d} (0.23–1.96)	2.66 ± 0.67 (0.24–9.25)	3.82 ± 0.12^{ce} (3.70–3.94)	1.30 ± 0.15 (0.75–1.87)	1.40 ± 0.16 (0.79–2.01)	1.67 ± 0.21^{a} (058–257)	0.76 ± 0.23^{bf} (0.26–1.45)
9	3.27 ± 0.39 (1.73–4.90)	2.26 ± 0.96 (0.23–4.30)	3.67 ± 0.39^{b} (0.75–5.38)	5.19 ± 0.32^{ae} (4.87–5.50)	2.95 ± 0.27 (1.83–3.82)	2.67 ± 0.46 (1.25–4.80)	3.28 ± 0.30^{c} (2.25–4.75)	1.81 ± 0.50^{df} (0.49–3.00)
12	6.85 ± 0.45 (4.40–9.06)	5.67 ± 1.47 (2.11–9.28)	6.94 ± 0.46 (4.36–8.97)	7.75 ± 1.16 (6.59–8.90)	6.46 ± 0.87 (2.57–11.00)	5.09 ± 0.86 (1.89–7.44)	6.41 ± 0.73^{a} (3.60–11.05)	2.78 ± 0.88^{b} (0.42–5.15)
15	6.69 ± 0.60 (4.65–9.25)	5.76 ± 0.77 (4.30–7.43)	6.37 ± 0.54 (3.90–9.60)	8.77 ± 1.08 (7.59–9.85)	6.83 ± 0.58 (4.52–9.22)	6.21 ± 1.11 (2.90–11.15)	7.92 ± 0.80 (4.69–12.00)	5.07 ± 1.20 (0.62–7.25)
18	10.41 ± 0.62^{a} (7.52–13.34)	8.65 ± 1.99 (5.18–13.02)	11.31 ± 0.82^{c} (4.82–13.52)	8.05 ± 3.85 (4.20–11.90)	6.11 ± 0.32^{b} (5.29–7.76)	5.18 ± 0.85 (0.68–7.30)	6.88 ± 0.73^{d} (4.53–11.50)	6.55 ± 1.65 (0.84–11.00)
21	12.44 ± 0.42^{c} (9.65–13.85)	8.12 ± 3.03 (0.56–13.98)	10.61 ± 0.96^{e} (4.06–13.88)	5.77 ± 5.23 (0.54–11.00)	6.28 ± 0.90^{ad} (3.65–9.43)	2.25 ± 1.11^{b} (0.51–7.41)	7.55 ± 0.73^{f} (5.23–11.92)	5.00 ± 1.24 (0.74–7.05)

Different superscripts (a,b; c,d; e,f) in lines differ significantly ($p < 0.05$), + pregnant on day 45, - nonpregnant on day 45

cows in group B was higher than that in the pregnant cows in group B and in the nonpregnant cows in group D ($p < 0.05$) on day 9. In group D, mean P_4 concentration in the pregnant cows was higher than that in nonpregnant cows ($p < 0.05$) on day 9 and 12 (Table 2).

There was no significant difference between the groups and pregnant and nonpregnant cows for mean P_4 concentrations on day 15 ($p > 0.05$). Mean P_4 concentrations in the pregnant cows in groups A and B were higher than that in groups C and D, respectively, on day 18 ($p < 0.05$). In group C, mean P_4 concentration was higher in the pregnant cows on day 21 ($p < 0.05$). In groups A and B, mean P_4 concentrations in the pregnant cows were higher than that in groups C and D, respectively, on day 21 ($p < 0.05$) (Table 2).

Discussion

We synchronized the animals with $PGF_{2\alpha}$-$PGF_{2\alpha}$ or GnRH-$PGF_{2\alpha}$ method before AI. In spite of the pregnancy rate being slightly higher in GnRH-$PGF_{2\alpha}$ protocol than that in $PGF_{2\alpha}$-$PGF_{2\alpha}$ group, there was no significant difference between synchronization protocols. In another study, Jobst et al. (2000) obtained similar results by synchronizing the animals with the protocols used in this study. In addition, there

was no significant difference in P_4 concentrations among the synchronization methods. Pregnancy rates in the present study (62.1% and 78.6% in $PGF_{2\alpha}$-$PGF_{2\alpha}$ and GnRH-$PGF_{2\alpha}$ groups, respectively) are in agreement with previous studies reported by Stevenson et al. (1999) and Karaca et al. (2002). In our study, the pregnancy rate in GnRH-$PGF_{2\alpha}$ method was higher than that obtained by DeJarnette et al. (2001) and Yamada et al. (2002) who synchronized the cows with GnRH followed by a $PGF_{2\alpha}$ injection 7 days later and inseminated once at a fixed time. The inconsistency in results between studies could be explained by the fact that in this study double insemination was used.

GnRH also promotes formation of an accessory CL when injected at dioestrus (Stevenson et al. 1996). In a study, GnRH injection at dioestrus promoted formation of an accessory CL by causing ovulation or luteinization (40% ovulation and 60% luteinization) of the existing dominant follicle in the ovaries (Bülbül et al. 2009). Its administration causes dose-releated increases in the serum concentration of gonadotropins (follicle stimulating hormone (FSH) and LH) (Jemmeson 2000). Some researchers reported that GnRH administration on day 5 or 11 (Willard et al. 2003) and 11 to 14 (Stevenson et al. 1993) increased the P_4 concentrations whereas others reported that GnRH injection on day 12 (Macmillan and Thatcher 1991; Çınar 1999) did not. In our study, P_4 concentrations were higher in GnRH treated pregnant cows on day 18 and 21 postinsemination in both synchronization groups. The results obtained in this study indicate that GnRH administration on day 12 postinsemination induced an accessory CL and increased the P_4 concentrations described as Mihm et al. (1996) and Diskin et al. (2002). Injection of GnRH may have stimulated the transformation of small cells to large cells which had a higher basal secretion rate of P_4 (Stevenson et al. 1993; De Rensis and Peters 1999).

Stevenson et al. (1993) reported that P_4 concentrations in cows were increased 3 days after the injection of 8 µg buserelin acetate on day 12 after AI while saline treated cows remained stable. In another study, Schmitt et al. (1996) injected a GnRH agonist on day 5 after AI and concentrations of plasma P_4 were increased 6 days after the injection. In this study, P_4 concentrations were not significantly different between the groups on day 12 and 15 and GnRH administration increased the P_4 concentrations after 3-6 days.

It is reported that embryonic death rate before the 21st day of pregnancy is about 20-30% (Sheldon 1997). One of the most important reasons for embryonic death in these days is CL deficiency and insufficient progesterone secretion from the CL (Kastelic 1994). GnRH injection leads to LH secretion which causes luteinization and then progesterone secretion. For this reason, GnRH treatments have been used to prevent embryonic death because of luteal deficiency (Sheldon and Dobson 1993). Drew and Peters (1992) reported 12% higher pregnancy rate in GnRH treated cows on day 12 after insemination than controls. In a similar research, Çınar (1999) obtained higher pregnancy rate in GnRH treated cows (35% versus 25%). In our study, although GnRH injection increased the progesterone concentrations and we obtained higher pregnancy rates on day 21 and 45, the differences in pregnancy rates were not significant. Embryonic death rate was lower in GnRH treated cows but there was no significant difference between embryonic deaths of GnRH and placebo treated groups. However, there was a tendency to higher pregnancy rate ($p = 0.06$) and to lower embryonic death rate ($p = 0.052$) in group B compared to group C.

Establishment and maintenance of pregnancy and embryo growth in cattle are related to the ability of the CLs P_4 secretion (Schmitt et al. 1996). In an earlier study, Lynch et al. (1999) reported that P_4 administration per vaginum for 10 days from day 2 or 3 (oestrus = day 0) with an injection of a GnRH analog (10 µg buserelin) on day 12 or 13 increased the conception rates. In our study, although P_4 concentrations in treatment groups were significantly higher than the controls, there was no significant difference between control and treatment groups for pregnancy rates. Now it is known that ovarian follicular growth

in cattle occurs in waves and emergence of a cohort of growing follicles is followed by selection of a dominant follicle (Martinez et al. 1999). It is reported that cows generally have 2 or 3 waves in an oestrus cycle and second follicular wave emerges 1 or 2 days earlier in three-wave cycles than in two-wave cycles (Webb and Armstrong 1998). In addition, the effectiveness of GnRH injection on induction of an accessory CL is affected by the stage of follicular development at the time of treatment (Moreira et al. 2000; Taponen et al. 2000). These varied responses may have been associated with different proportions of two and three-wave cycles in different populations of cows. In addition, different results obtained in various studies may be influenced by age, nutrition, suckling and season described as Odde (1990) and Bülbül and Ataman (2009).

In conclusion, although GnRH injection on day 12 after the AI increased the P_4 concentrations on day 18 and 21, it did not alter the pregnancy rates. Furthermore, plasma P_4 concentrations and pregnancy rates did not differ between $PGF_{2\alpha}$-$PGF_{2\alpha}$ or GnRH-$PGF_{2\alpha}$ methods.

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