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1.The Effect of Administration of Different Levels of GnRH on the Day 0, 5 and 12 Post-Insemination on Progesterone Concentration in Dairy Heifers

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Abstract: This study was carried out to evaluate the effects of different levels of GnRH in different days of reproductive cycle on progesterone concentration in dairy heifers. Two hundred heifers were divided into ten experimental treatment: control group with no injection 1) administration of 2.5 mL Gonadorelin (a GnRH analogue) in day of insemination 2) administration of 5 mL GnRH in day of insemination 3) administration of 10 mL GnRH in day of AI insemination 4) administration of 2.5 mL GnRH in day 5 post-insemination 5) administration of 5 mL GnRH in day5 post-insemination 6) administration of 10 mL GnRH in day5 post-insemination 7) administration of 2.5 mL GnRH in day 12 post-insemination 8) administration of 5 mL GnRH in day 12 post-insemination 9) administration of 10 mL GnRH in day 12 post-insemination. Blood samples were collected in days 0, 5, 12 and 19 post insemination (AI = Day 0) for analysis of serum P4 concentration. There was no significant difference among experimental groups on day of insemination. Evaluation of P4 concentration on day 19 illustrated differences in progesterone concentration between groups on day 19 post-insemination. P4 concentration of serum in the day 19 post-insemination significantly increased in groups either by 5 or 10 mL injection of GnRH whether in day 5 or 12 post-insemination versus control group (9.24±2.2, 8.6±1.96, 9.43±2.15 and 9.42±2.14 versus 5.5±0.8, respectively; $p<0.05$). GnRH administration in the day 5 and 12 post-AI significantly increased progesterone concentration that may decline early embryonic death and improve pregnancy rate.

Key words: GnRH, progesterone concentration, early embryonic death, heifers, pregnancy rate

INTRODUCTION

Many factors influence conception rate: among them is cyclicity, energy balance, heat stress, parity, milk production, diet and diseases (Cartmill *et al*., 2001; Gröhn and Rajala-Schultz, 2000; Hansen and Arechiga, 1999; Lucy, 2001; Moreira *et al*., 2001; Santos *et al*., 2004; Starbuck *et al*., 2004). Reasons for the decline in fertility are multifactorial and indirectly associated with an increase in milk production (Thatcher *et al*., 2006). Up to 40% of total embryonic losses are estimated to occur between day 8 and 17 of pregnancy (Thatcher *et al*., 1994). However, it is clear that lactation, as a physiological process, is associated with a lower reproductive rate compared to that of nonlactating heifers (Lucy, 2001). In an extensive review of the literature, Lucy (2001) suggested that pregnancy loss in lactating dairy cattle has increased. A significant proportion of infertility in cattle has been attributed to inadequate functioning of the CL (Lamming *et al*., 1989; Wielbold, 1988; Thatcher *et al*., 1994; Rajamahendran *et al*., 1998; Mann *et al*., 1995; Thatcher *et al*., 2001; Mann and Lamming, 2001). During early pregnancy, progesterone influences the endometrial secretion of nutrients, growth factors, immunosuppressive agents, enzymes, ions and steroids that are essential for embryo development (Graham and Clarke, 1997). The maintenance of progesterone (P4) secretion by a viable Corpus Luteum (CL) is vital to early pregnancy and untimely luteolysis is probably a major cause of embryo loss (Mann and Lamming, 2001; Peters, 2005). Elevated plasma progesterone (P4) levels during the early luteal phases post insemination may have beneficial effects on pregnancy, particularly in subfertile cattle (Butler *et al*., 1996; Ambrose *et al*., 1998; Binellii *et al*., 2001).

Some studies (Schmitt *et al*., 1996; Sianangama and Rajamahendran *et al*., 1996) have used hCG and GnRH at various times after insemination to enhance pregnancy rates. Even though there is evidence that hCG or GnRH could enhance CL function, as evident by higher plasma concentrations of P4, often such increases were due to formation of an accessory CL resulting from the ovulation of the dominant follicle present at the time of hCG/GnRH treatment. Prolonged exposure of follicular

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theca and granulosa cells of the preovulatory follicle to high levels of LH could be crucial for the subsequent development of a robust CL capable of secreting more P4 (Rajamahendran *et al*., 1998; Binellii *et al*., 2001).

Few studies have specifically addressed the use of luteotropic hormones or those that may directly or indirectly modify ovarian follicular dynamics toward an endocrine environment that would be more conducive to embryo development and conceptus survival in heat-stressed dairy cattle. Notably, Ullah *et al*. (1996) observed that GnRH administered at estrus to heat-stressed dairy cows improved pregnancy rates and increased serum concentrations of progesterone. In contrast, studies of supplemental hCG administration post-breeding (which also initiates LH-like, luteotropic effects) have reported varying effects on pregnancy rates in heat-stressed dairy heifers or lactating cows (Schmitt *et al*., 1996). While obvious differences between these two studies exist in the type of hormone treatment (GnRH versus hCG) and the timing of administration (at estrus versus post-estrus), a meta-analysis by Peters *et al*. (2000) suggests that supplemental GnRH treatment administered between 11 and 14 days after AI is a hormonal therapy that increased overall pregnancy rates (Lo´pez-Gatius *et al*., 2006; Peters, 2005). Nevertheless, definitive studies have yet to be conducted to address whether supplemental GnRH treatment post-breeding may be similarly beneficial under heat stress conditions (Kaim *et al*., 2003; Willard *et al*., 2003). In addition the appropriate timing of such treatment has remained questionable. It has been suggested that early post-estrus administration of GnRH (Day 5 or 6 post-estrus) to achieve ovulation and accessory CL formation from the first-wave dominant follicle (Binellii *et al*., 2001; Starbuck *et al*., 2001; Mann and Lamming, 2001) may be an optimal time for hormonal treatment, as opposed to delaying GnRH treatment to mid-diestrus (Days 11-14 ostestrus), which would coincide with subsequent follicular waves, initiation of maternal recognition of pregnancy and the timing of luteolytic mechanisms (Willard *et al*., 2003)

The objective of this study was to evaluate different levels of GnRH administration at various times of reproductive cycle on progesterone concentration in dairy heifers.

MATERIALS AND METHODS

Location of farm and date of experiment: This study was conducted on a 1600-Holstein dairy cow in Kermanshah province of Iran. The farm is located in Ravansar region between Latitudes 34°N and 35°N and between Longitudes 46°E and 47°E. The climate of the area is characterized by an average annual of 14.9°C and 537.9 mm mean rainfall per year. The study period lasted 8 months from April 2006 to November 2006.

Detection of oestruse and insemination: As a routine activity of the Artificial Insemination Unite, animal in the research program were monitored for standing oestrus and inseminated using Frisian semen. Cows were monitored for mounting activities and mucus discharge. Visual detection of oestrus by herdmen was also put in place to improve the reliability of detecting oestrus. Cows with standing oestrus were inseminated 12 h after the onset of oestrus in morning and evening.

Management of cows: Cows were fed a diet formulated to meet nutrient requirements established by NRC (2001). The diet was offered as three equal portions at 9:00, 15:00 and 19:00 only in Total Mixed Ration (TMR).The heifers were housed individually and fed standard food with access to water *ad libitum*.

Program of treatment: Two hundred heifers were randomly divided into ten experimental treatment: control group with no injection 1) administration of 2.5 mL Gonadorelin (a GnRH analogue) in day of insemination 2) administration of 5 mL GnRH in day of insemination 3) administration of 10 mL GnRH in day of AI insemination 4) administration of 2.5 mL GnRH in day5 post-insemination 5) administration of 5mL GnRH in day5 post- insemination 6) administration of 10 mL GnRH in day5 post-insemination 7) administration of 2.5 mL GnRH in day 12 post-insemination 8) administration of 5mL GnRH in day 12 post-insemination 9) administration of 10 mL GnRH in day 12 post-insemination.

Pregnancy diagnosis: Animals were examined for pregnancy determination by palpation, 40 days post insemination and pregnancy rate was defined as percentage of cows conceived after AI in a group.

Blood sampling and progesterone assay: Blood samples (10 mL) were collected by syringe with a 3.5 cm, 18 gauge needle from coccygeal vein. After removing the needle, the blood samples instantly but slowly were poured to the test tube. Samples allowed to clot at 4°C refrigerator for approximately 24 h and then centrifuged at 2000 x g for 10 min before serum was decanted in to vials and stored at -20°C until hormone analysis.

Blood sampling for determination of progesterone concentration was performed from the day of insemination (Day 0) and the days 5, 12 and 19 post-insemination and was always collected prior to the treatments. Then blood

samples stored stably and allowed to clot at 4°C then centrifuged 2000 g for 10 min and separated serum stored at -20°C until progesterone assay. Serum P4 concentrations were determined using an extract single antibody Radioimmunoassay (RIA).

Data and statistical analysis: Mean serum P4 concentration in the Control and treatment group was compared at the Days 0, 5, 12 and 19 using ANOVA by the SPSS 11.0 program. Mean serum P4 concentration are expressed as mean±SD. Differences at a p value less than 5% ($p<0.05$) were considered to be statistically significant using Duncan's multiple range test (Duncan, 1955). Pregnancy rate was defined as the percentage of cows in a group conceived after artificial insemination.

RESULTS

Statistical analysis of serum P4 concentrations, estrus and pregnancy rate are shown in Table 1. The average of plasma progesterone concentrations on the day of insemination was 0.28±0.11 ng mL^{-1} and there was no difference between experimental animals ($p>0.05$).

There is a significant difference in serum P4 concentration of day5 post-insemination in group3 versus control group (5.98±2.28 versus 1.42±0.14 ng mL^{-1}, respectively; $p<0.05$), but serum P4 concentrations were similar in other groups ($p>0.05$).

Table 1: Serum P4 concentration between groups at different time of blooding (Day 0 = AI)

		Serum P4 concentration[b]				
Groups[a]	N[d]	0	5	12	19	Pregnancy rate[c] (%)
Control	20	0.23±0.08	1.42±0.14^{a}	1.70±0.62^{a}	5.50±0.8^{a}	70
1	20	0.24±0.11	1.58±0.72^{a}	2.38±2.2^{ef}	6.40±2.51^{a}	70
2	20	0.25±0.1	5.43±2.38^{ef}	6.02±1.91^{f}	6.45±0.95^{a}	80
3	20	0.33±0.3	5.98±0.28^{f}	6.26±1.45^{df}	7.26±1.18^{ef}	75
4	20	0.30±0.24	2.09±0.02^{a}	2.80±0.3^{ef}	6.85±1.38^{ef}	80
5	20	0.40±0.17	2.03±0.23^{a}	6.94±2.8^{f}	9.24±2.2^{g}	65
6	20	0.30±0.05	1.97±1.73^{a}	6.80±3.8^{f}	8.60±1.96^{fg}	70
7	20	0.26±0.14	1.68±0.2^{a}	2.60±0.86^{df}	5.54±1.57^{a}	70
8	20	0.25±0.17	1.89±0.49^{a}	2.30±0.49^{df}	9.43±2.15^{g}	85
9	20	0.29±0.2	2.03±1.04^{a}	2.60±0.45^{df}	9.42±2.14^{g}	75

[a]Experimental groups include: 1) administration of 2.5 mL GnRH in day of insemination 2) administration of 5 mL GnRH in day of insemination 3) administration of 10 mL GnRH in day of AI insemination 4) administration of 2.5 mL GnRH in day 5 post-insemination 5) administration of 5 mL GnRH in day 5 post-insemination 6) administration of 10 mL GnRH in day 5 post-insemination 7) administration of 2.5 mL GnRH in day 12 post-insemination 8) administration of 5 mL GnRH in day 12 post-insemination 9) administration of 10 mL GnRH in day 12 post-insemination,

[b]Serum P4 concentration in days of insemination (day 0), 5, 12 and 19 post-insemination, respectively.

[c]The pregnancy rate was defined as the number of cows that became pregnant expressed as a percentage of the total number of cows in each group.

[d]Number of cows in each group.

[a, f, g]Values with different superscripts in each days show significant differences between experimental groups ($p<0.05$)

Also, Duncan's multiple range tests shows differences in P4 concentrations between groups 5, 6, 8 and 9 versus control group in the day 19 post-insemination (9.24±2.2, 8.6±1.96, 9.43±2.15 and 9.42±2.14 versus 5.5±0.8 ng mL^{-1}, respectively; $p<0.05$) and there were no significant differences between other groups.

DISCUSSION

Many known biological effects of GnRH are caused by LH release during some hours after GnRH release/administration (Chenault *et al*., 1990). In the present study, LH surge as a consequence of GnRH injection was not measured.

Investigation of plasma P4 concentrations of the day 5 showed greater increase ($p<0.05$) in groups 2 and 3 versus control and other groups. Also dose of 5 mL in day of insemination induced an increasing in serum P4 in group 2 but that's not significantly different versus control group.

Administering GnRH or it's analogues to cows at the time of AI increased PA concentration in some experiments (Mee *et al*., 1990; Stevenson *et al*., 1990; Kaim *et al*., 2003). Improvement of conception following GnRH treatment during estrus has been attributed to the prevention of ovulation failure or to reduced variation in the interval to ovulation (Coulson *et al*., 1980; Nakao *et al*., 1984).

Kaim *et al*. (2003) showed in their study all GnRH-treated cows ovulated within 20 h after AI, whereas 24% of the control cows ovulated later. This delay in ovulation relative to the timing of estrus and AI might reduce fertilization (Santos *et al*., 2000) and pregnancy rates and also delay the establishment of luteal function (Larson and Sirois, 1997) and consequently reduce fertility. Stevenson *et al*. (1993) observed that Injections of GnRH stimulated the transformation of follicular cells to luteal cells, which required at least 2 to 3 days. failure or delay of ovulation might be prevented and conception rate might increase by GnRH administration at AI. On the other hand, if GnRH is administered at the time of AI, frequently, with effects on the timing of ovulation and a prospective increase in plasma progesterone levels in the subsequent luteal phase versus control (Ryan *et al*., 1994).

Investigation of plasma P4 concentrations showed greater increase ($p<0.05$) in day12 in groups 3, 5 and 6 versus control groups and other groups. Also dose of 5 mL in day of insemination in group3 induced an increasing in serum P4 but that's not significantly different versus control group. As discussed previously, administration of GnRH seems reduced variation in the

interval to ovulation (Coulson *et al*., 1980; Nakao *et al*., 1984) and induced rapid luteinization in granulosa cells (Less *et al*., 1998; Binellii *et al*., 2001) and developed era of CL, which continually extended. But in groups 5 and 6 that received 5 and 10 mL of Gonadorelin respectively in the day 5, found in high P4 concentration in day 12 versus control group and other groups. Data from this study are consistent with the hypothesis that GnRH given on the day 5 of the estrous cycle induced ovulation resulting in formation of accessory CL thereby increasing endogenous progesterone concentrations on day 12 (Beck *et al*., 1994; Cam *et al*., 2002). Currently, previous studies indicate that exogenous administration of GnRH on day 5 could initiate endogenous increases in P4 via modulation of ovarian follicular populations and promotion of accessory CL formation (Schmitt *et al*., 1996). Present research about administration of GnRH on day5 is in agreement with previous reports (Schmitt *et al*., 1996) that showed increased progesterone concentrations between days 11 and 16 in Holstein heifers administered GnRH 5 days after estrus. Similarly, Willard *et al*. (2003) observed increased serum progesterone concentrations between days 9 and 19 when heat-stressed dairy cows were administered GnRH 5 days after insemination.

In order to increase progesterone concentrations mid-cycle injection of GnRH agonists was associated with reduced follicular secretion of estradiol 17β from the days 13 to 16 post-AI (Mann *et al*., 1995). Using the available published data, a Meta analysis have been performed by Peter *et al*. (2000) and found that the odds ratios (the relative probability of pregnancy between treated and control cows) varied significantly among studies, ranging from 0 to 22% (Peter *et al*., 2000).

According to previous reports, the effect on fertility rates of GnRH agonist administration between days 11 and 14 post-AI is highly variable. This variation might be attributed to numerous factors such as environment, management, breed, animal age, breeding season, individual farm effects and interval from calving to first service, reproductive/lactational status and type of breeding (Peter *et al*., 2000). However, only a small subset of these variables including environment, management and breed has been commonly examined. Nonetheless, it is clearly known that these factors can significantly influence fertility (MacMillan *et al*., 1986; Sheldon and Dobson, 1993; Drew and Peters, 1994; Jubb *et al*., 1990; Ryan *et al*., 1994). Thatcher *et al*. (1989) proposed an antiluteolytic role of a midluteal injection of a GnRH agonist, in which the surge-like release of LH from the pituitary either luteinized or ovulated midcycle follicles, thus altering follicular secretion of estradiol, which is necessary to initiate the uterine changes in oxytocin receptors and serum concentrations of $PG_{F2\alpha}$ preceding luteolysis (McEvoy *et al*., 1984). Luteinized follicles and (or accessory CL) were observed after LH or GnRH treatments during the estrous cycle in cows (Binellii *et al*., 2001), heifers (Thatcher *et al*., 1989) and sheep (Farin *et al*., 1988), demonstrating that midcycle follicles respond similarly to an ovulatory follicle at estrus when exposed to a surge of FSH and LH. The LH surge induced by GnRH or one of its agonists also might provide a luteotropic stimulus to the CL. The scientific rationale for this treatment is to enhance embryo survival rates by delaying the luteolytic mechanism (Mann *et al*., 1995) that sometimes occurs due to failed maternal recognition of pregnancy. Some studies rebigerported significant improvements of 10-12% in pregnancy rates (MacMillan *et al*., 1995; Sheldon and Dobson, 1993; Drew and Peters, 1994), while others did not (Jubb *et al*., 1990; Ryan *et al*., 1994). Mean concentrations comparison of progesterone between groups in the day 19 post-insemination shows serum P4 concentrations were greater ($p<0.05$) in the groups 5, 8 and 9 versus groups 1, 2, 3, 4, 7 and control group, but were similar to group 6, hence so based on the result of this study, GnRH agonist administration increases mean serum progesterone concentration when administrated either on day 5 or 12 post-insemination at least with 5 mL Gonadorelin; increase in dosage of GnRH administration either in the Day 5 or 12 post-insemination had no significant effect on P4 concentration, so it can be concluded that administration of GnRH during mid-diestrus (Day 12) post-insemination appears to facilitate a protective effect within the endocrine environment of the uterus in order to improve conceptus survival in dairy cattle.

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