



Effect of GnRH injection at day 6 and 12 after insemination on fertility of Holstein dairy cows during the warm season

E. Dirandeh¹, A. Rezaei Roodbari², B. Shohreh¹

¹Sari Agricultural Sciences and Natural Resources University, Department of Animal Science, Sari, Iran, PO BOX, 578.

²University of Tehran, College of Agricultural and Natural Resources, Department of Animal Science, Karaj, Iran, PO BOX, 4111.

ABSTRACT

The objective of this study was to determine the effects of two different time of GnRH injection after artificial insemination (A.I) (d 6 and d 12) on reproductive performances of Holstein cows during the warm season. Cows randomly assigned to treatments 1) No GnRH injection (control, n = 300), 2) GnRH injection at day 6 after A.I (G6, n=300) and 3) GnRH injection at day 12 after A.I (G12, n=300). Ultrasonography was used to detect pregnancy and late embryonic/fetal mortality between Days 32 and 60 after AI. Data were analyzed using Proc GLM of SAS. Results showed treatment affected the percentage of cows diagnosed pregnant at 32±1 d and 60±3 d after resynchronized AI. A greater percentage of G6 (25.0%) cows were pregnant at 32 d after resynchronized AI than control cows (19.3 %) and G12 cows (20.0 %). The risk of pregnancy loss between d 32 and 60 of gestation was affected by treatments and decreased in cows received GnRH injection after AI Compared to control cows. Percentage of cows that showed estrus at 19-24 d after AI was highest in G6 cows and lowest in control cows compared to other treatments. In summary, administration of a GnRH on Day 6 after AI improved reproductive performance in dairy cows.

Keywords: GnRH, Fertility, Warm season, Holstein cows

INTRODUCTION

Reproductive efficiency in lactating dairy cows is not optimal, encouraging the development of several different reproductive management strategies focused on improving the use of AI and pregnancy rates in dairy herds (Thatcher et al., 2006). In cattle, a GnRH or its agonists have been used to treat reproductive disorders. Administration of GnRH during the bovine estrous cycle causes regression or ovulation of the dominant follicle and initiates the emergence of a new wave of follicular growth within two to three days

following treatment (Kohram et al., 1998a; Silcox et al., 1993). Atresia or ovulation of the dominant follicle depends on the status of the dominant follicle (growing, static or regressing) at the time of GnRH injection (Twagiramungu et al., 1994; Dirandeh et al., 2009). 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 P4 and caused a tendency toward higher pregnancy rates. 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 reported significant improvements of 10–12% in pregnancy rates (Lopez et al., 2006), while others did not (Szenci et al., 2006). Studies on the ability of a GnRH agonist to induce ovulation to form new (or accessory) CL are contradictory. Treatment with GnRH induces luteinization and (or) atresia of large follicles or causes ovulation. The ultrasonographic changes in appearance of the borders of follicular antra and opaqueness of follicular fluid (i.e., cloudy follicles) have been reported (Rettmer et al., 1992). Schmitt et al. (1996a) reported that new CL were formed in 91% of the cows treated with hCG and 93% of the animals treated with Buserelin on Day 5 of the estrous cycle. In another study, Schmitt et al. (1996b) removed the induced CL formed by GnRH or hCG. They reported that the new CL formed following GnRH or hCG were responsible for the increase in P4 concentrations after GnRH or hCG. Dirandeh et al. (2009) reported the emergence of a new wave in dairy cows in which a GnRH agonist is injected on Day 6 of estrous cycle. The aim of this study was to investigate the effects of single administration of GnRH at day 6 and 12 after A.I on reproductive performances of Holstein cows during the warm season.

MATERIAL AND METHODS

2.1. Animals and Treatment

The herds were maintained on a weekly reproductive health program. This involved examining the reproductive tract of each animal by palpation per rectum within 30–36 days postpartum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time such as endometria, pyometra or ovarian cysts were treated until resolved.

Multiparous high producing Holstein dairy cows (≥ 40 kg/d) with no overt clinical illnesses. Cows averaged 2.7 ± 1.3 (mean \pm SEM) in parity and 3.3 ± 0.2 (mean \pm SEM) in body condition score at calving (based on a 1 (thin) to 5 (obese) scale). Cows randomly assigned to one of three treatments. 1) No GnRH injection (control, n = 300), 2) GnRH injection at day 6 after A.I (G6, n=300) and 3) GnRH injection at day 12 after A.I (G12, n=300).

2.2. Reproductive Management

Ovarian status of cows synchronized for ovulation beginning on d 30 postpartum with two intramuscular injections of PGF 2α (Synchromate®, 150 μ g cloprostenol sodium, Aburaihan company, Tehran, Iran) given 14 d apart. Estrus was detected using a combination of behavioral observations, pedometer activity monitoring and ultrasonography. Non-pregnant cows were injected with 500 mg of Prostaglandin F 2α (cloprostenol, 250 mcg cloprostenol/mL, i.m.; parnell technologies, PTY. LTD., Alexandria, Australia) and then injected with 100 μ g of GnRH (gonadorellin acetate, 100 mcg gonadorelin/mL, i.m.; parnell

technologies PTY. LTD., Alexandria, Australia) 56 h later. A timed artificial insemination (TAI) was performed 16 h after the GnRH. Cows were examined per-rectum ultrasonography 32 d after the second TAI. Pregnancy was evaluated at 40 d after AI, and pregnant cows had their pregnancy reconfirmed at 60 d after AI and pregnancy losses determined. Artificial inseminations were conducted by one technician in all groups.

2.3. Pregnancy Diagnosis

Cows were examined by ultrasonography (Aloka scanner equipped with a 5-MHz linear array transducer (Aloka Co., Ltd., Tokyo, Japan) at 32 d after AI. Pregnancy was characterized by the presence of fluid, an embryo, and a heartbeat. Cows diagnosed pregnant were reexamined 4 wk later, at 60±3 d after AI to reconfirm pregnancy status and to identify pregnancy loss.

2.4. Statistical analysis

All of the reproductive responses (binary responses), were analyzed GLIM MIX procedure of SAS with a binomial distribution and a logit link function. To examine effects of treatments in period on length of time, such as heat interval, survival analysis was performed. Significance and tendencies were declared at $P < 0.05$ and $P < 0.15$, respectively, unless otherwise indicated.

RESULTS

Treatment affected the percentage of cows diagnosed pregnant at 32±1 d ($P = 0.03$) and 60±3 d ($P = 0.01$) after resynchronized AI. A greater ($P < 0.05$) percentage of G6 (25.0%) cows were pregnant at 32 d after resynchronized AI than control cows (19.3 %) and G12 cows (20.0 %). Percentage of conception rate was no difference between control and G12 cows at 32d after AI. At 60±4 d after TAI, P/AI was similar in cows received GnRH injection after AI but was greater compared to control cows ($P = 0.01$, Table 1). The risk of pregnancy loss between d 32 and 60 of gestation was affected by treatments ($P = 0.04$) and decreased in cows received GnRH injection (2.9% and 5.0 % in G6 and G12, respectively) after AI Compared to control cows (10.3 %), (Table 1). Percentage of cows that showed estrus at 19-24 d after AI was highest in G6 cows and lowest in control cows compared to other treatments (Table 2). Percentage of cows that did not show estrus until day 35 after AI was highest in control cows (7.6 %) compared to other treatments (1.6 % and 3 % in G6 and G12 cows).

Table 1. Effect of GnRH injection at day 6 and 12 after AI fertility during warm season

	Treatments			P
	Control	G6	G12	
Conception rate at 32 d after AI (%)	19.3 (58/300) ^a	22.6 (68/300) ^b	20.0(60/300) ^a	0.03
Conception rate at 60 d after AI (%)	17.3 (52/300) ^a	22.0 (66/300) ^b	19.0(57/300) ^c	0.01
Pregnancy loss (%)	10.3 (6/58) ^a	2.9 (2/68) ^b	5.0(3/60) ^b	0.04

a, b, c: Means in the rows with different letters are significantly different

G6: GnRH injection at day 6 after AI; G12: GnRH injection at day 12 after AI.

Table 2. Effect of GnRH injection at day 6 and 12 after AI on heat interval during warm season

Heat interval (day)	Treatments			P
	Control	G6	G12	
<19	20.0 (60/300) ^a	13.3 (40/300) ^b	12.6(38/300) ^b	0.04
19-24	39.0 (117/300) ^a	47.6 (143/300) ^b	44.0(132/300) ^c	0.01
24-35	30.0 (100/300) ^a	37.3 (112/300) ^b	33.3(120/300) ^c	0.02
>35	7.6 (23/300) ^a	1.6 (5/300) ^b	3.0(10/300) ^b	0.04

a, b, c: Means in the rows with different letters are significantly different

G6: GnRH injection at day 6 after AI; G12: GnRH injection at day 12 after AI

DISCUSSION

Our data showed significant difference in fertility between the GnRH-treated and control cows. These results confirmed the results of Lopez et al. (2006) and Ataman et al. (2011) and were in contrast with Szenci et al. (2006) and Rettmer et al. (1992). Beckett and Lean (1997) performed a meta-analysis and reported injection of a single dose of GnRH in post-partum dairy cows failed to show any significant effect on the time to first estrus or on the odds ratio at first service. 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 (Peters et al., 2000). 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). Progesterone concentrations were higher in GnRH treated pregnant cows on day 18 and 21 post insemination in both synchronization groups (Ataman et al., 2011). GnRH administration on day 12 post insemination induced an accessory CL and increased the P4 concentrations described as 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 P4 (Stevenson et al., 1993; De Rensis and Peters 1999). Percentage of cows that showed estrus at 19-24 d after AI was higher in cows received GnRH injection compared to control treatments. Dirandeh et al. (2009) reported an injection of GnRH on day six of the estrous cycle could promote the emergence of a new follicular wave earlier in cows and alter the estrous cycles shifting two waves into three follicular waves. Moreover, the interval from detection of dominant follicle to ovulation and duration of dominance was shorter in animals with three waves (Celik et al., 2005). In this study, the risk of pregnancy loss decreased in cows received GnRH injection at day 6 and 12 after AI compared to cows did not receive GnRH injection that was similar to results of Drew and Peters (1992), Ataman et al. (2011). Drew and Peters (1992) reported 12% higher pregnancy rate in GnRH treated cows on day 12 after insemination than controls. In a similar research, Ataman et al. (2011) reported embryonic death rate was lower in GnRH treated cows but there was no significant difference between embryonic deaths of GnRH and placebo treated groups. 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). 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). In addition to increased progesterone concentrations (Rettmer et al., 1992), mid-cycle injection of GnRH agonists was associated with reduced follicular secretion of 17 β -estradiol from Days 13 to 16 after AI. Low estradiol concentrations in conjunction with high progesterone levels have been associated with inhibited up-regulation of oxytocin receptors and consequent inhibition of PGF₂ α secretion (Mann et al., 1995). Therefore, GnRH injection at Day 6 and 12 post-AI expected to increase conception rates.

Conclusion

Administration of a GnRH on Day 6 after AI improved reproductive performance in dairy cows and can be use as a strategy for improving fertility in dairy cows during warm season.

REFERENCES

- Ataman MB, Erdem H, Bulbul B, Umutlu S, Çolak M(2011). The effect of buserelin injection 12 days after insemination on selected reproductive characteristics in cows. ACTA. VET. BRNO. 80:171–177.
- Beckett SD, Lean IJ (1997). Gonadotrophin-releasing hormone in postpartum dairy cattle: a meta-analysis of effects on reproductive efficiency. Anim. Reprod. Sci. 48:93–112.
- Bulbul B, Kırbaş M, Köse M, Dursun Ş, Çolak M (2009). The effects of ovsynch started in different phases of oestrus cycle on oestrus synchronization in cows. İstanbul Üniv Vet FakDerg 35:7-17.
- Celik HA, Aydin I, Sendag S, Dinc DA (2005). Number of follicular waves and their effect on pregnancy rate in the cow. Reprod. Domest. Anim. 40(2):87-92.
- De Rensis F, Peters AR (1999). The control of follicular dynamics by PGF₂ α , GnRH, hCG and oestrus synchronization in cattle. Reprod. Dom. Anim. 34:49-59
- Dirandeh E, Kohram H, ZareShahneh, A (2009). GnRH injection before artificial insemination (AI) alters follicle dynamics in Iranian Holstein cows. African. J. Biotech. 8 (15):3672-3676.
- Diskin MG, Austin EJ, Roche JF (2002). Exogenous hormonal manipulation of ovarian activity in cattle. Dom. Anim. Endocrin. 23:211-228.
- Drew SB, Peters AR (1992). The effect of treatment with a gonadotrophin releasing hormone analogue on the fertility of dairy cows. Proceedings of the 12th International Congress on Animal Reproduction, Hague, Netherlands, 319 p.
- Hansen PJ (2002). Embryonic mortality in cattle from the embryo's perspective. J. Anim. Sci. 80:33-44.
- Kastelic JP (1994). Noninfectious embryonic loss in cattle. Vet. Med. 6:584-589.

Kohram H, Bousquet D, Durocher J, Guilbault LA. (1998) Alteration of follicular dynamics and superovulatory responses by gonadotropin releasing hormone and follicular puncture in cattle: a field trial. *Theriogenology*. 49(6):1165-74.

Lopez-Gatius F, Santolaria P, Martino A, Dele'tang F, De Rensis F(2006). The effects of GnRH treatment at the time of AI and 12 days later on reproductive performance of high producing dairy cows during the warm season in northeastern Spain. *Theriogenology*. 65:820–830.

Mann GE, Lamming GE, Fray MD (1995). Plasma oestradiol and progesterone during early pregnancy in the cow and the effects of treatment with Buserelin. *Anim. Reprod. Sci.*37:121–31.

Moreira F, De La Sota RL, Diaz T, Thatcher WW (2000).Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. *J. Anim. Sci.* 78:1568-1576.

Peters AR, Martinez TA, Cook AJC (2000). A meta-analysis of studies of the effect of GnRH 11–14 days after insemination on pregnancy rates in cattle. *Theriogenology*.54:1317–26.

Rettmer I, Stevenson JS, Corah LR (1992). Endocrine responses and ovarian changes in inseminated dairy heifers after an injection of a GnRH agonist 11 to 13 days after estrous. *J. Anim. Sci.* 70:508-517.

Schmitt EJ, Diaz T, Barros CM, de la Sota RL, Drost M, Fredriksson EW, Staples CR, Thorner R, Thatcher WW. les CR, Thorner R, Thatcher (1996a).Differential response of the luteal phase and fertility in cattle following ovulation of the first-wave follicle with human chorionic gonadotropin or an agonist of gonadotropin-releasing hormone. *J. Anim. Sci.*74:1074-1083.

Schmitt EJ, Barros CM, Fields PA, Fields MJ, Diaz T, Kluge JM, Thatcher WW (1996b). A cellular and endocrine characterization of the original and induced corpus luteum after administration of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin on day five of the estrous cycle. *J. Anim. Sci.* 74:1915-1929.

Sheldon M, Dobson H (1993). Effects of gonadotrophin releasing hormone administered 11 days after insemination on the pregnancy rates of cattle to the first and later services. *Vet. Rec.* 133:160-163.

Silcox RW, Powell KL, Kiser TE (1993). Ability of dominant follicles (DF) to respond to exogenous GnRH administration is dependent on their stage of development. *J. Anim. Sci.* 1993, Suppl. 71, 513.

Stevenson JS, Phatak AP, Rettmer IMMO, Stewart RE (1993).Post insemination administration of reeptal: follicular dynamics, duration of cycle, hormonal responses, and pregnancy rates. *J. Dairy. Sci.* 76:2536-2547.

Szenci O, Taka E, Sulon J,Melo de Sousa N, Beckers JF(2006). Evaluation of GnRH treatment 12 days after AI in the reproductive performance of dairy cows. *Theriogenology*, 66:1811–1815.

Thatcher WW, Bilby TR, Bartolome JA, Silvestre F, Staples CR, Santos JE(2006).Strategies for improving fertility in the modern dairy cow. *Theriogenology*. 65:30–44.

Twagiramungu H, Guilbault LA, Proulx JG, Dufour JJ (1994). Influence of corpus luteum and induced ovulation on ovarian follicular dynamics in postpartum cyclic cows treated with buserelin and cloprostenol. *J. Anim. Sci.* 72(7), 1796-805.

Willard S, Gandy S, Bowers S, Graves K, Elias A, Whisnant C (2003). The effects of GnRH administration post insemination on serum concentrations of progesterone and pregnancy rates in dairy cattle exposed to mild summer heat stress. *Theriogenology.* 59:1799-1810.