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The Effect of GnRH Injection on Plasma Progesterone Concentrations, Conception Rate and Ovulation Rate in Iranian Holstein Cows

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Abstract: Ovarian functional disorders are reported as an important cause of infertility in dairy cattle. This experiment was carried out to determine the effects of GnRH injection at the time of insemination on ovulation rate, conception rate and plasma progesterone concentrations on day 5 post AI. Egithy cows were randomly assigned into 2 experimental groups: Treatment group (n = 40) which received 15 ug of GnRH and control group (n = 40) without any hormonal treatment. Cows were palpated per rectum to ascertain the occurrence of ovulation 24 h post AI. Blood samples were drawn on the day of insemination and on the day 5 post AI. Plasma concentrations of P_4 were measured by RIA and analyzed by means of the GLM procedure of the SAS. Differences between the two experimental groups were analyzed by one way ANOVA. Ovulation rate and conception rate were analyzed by means of Binary Models of the SPSS. Results of this study indicated that the injection of GnRH increased the ovulation rate insignificantly (65% vs 47.5%, $p>0.05$) and increased conception rate significantly (55% vs 25%, $p<0.03$). GnRH administration caused an insignificant increase in the concentration of plasma progesterone on day 5 post AI (6.03 vs 5.27 ng mL^{-1}, $p<0.02$). In general results of this study indicate that GnRH injection at the time of artificial insemination improves ovulation and pregnancy rates.

Key words: GnRH, pregnancy rate, ovulation rate, holstein cows

INTRODUCTION

Reproductive efficiency is a critical component of a successful dairy herd management, whereas a reproductive inefficiency is 1 of the most costly problems facing the dairy industry today. Therefore, the fertility of dairy cows is a growing concern (Kristula *et al*., 1992; Archbald and Klapstein, 1992; Archbald *et al*., 1993; Ahmad *et al*., 1996). Calving interval is a major component which involves the days from calving to the initiation of the next pregnancy, usually referred as open days and the fixed effect of gestation length (Risco *et al*., 1995; Thatcher *et al*., 1993). Open days depend on the days from calving to the first insemination or mating and fertilization and associated with conception rate. Pregnancy rate for 21 days periods, which is the product of conception rate and estrus detection rate, is the most commonly used parameter to evaluate reproductive performance in dairy herds (Alacam *et al*., 1986; Bartolom *et al*., 2005).

After fertilization, Corpus Luteum (CL) development, follicular growth and Progesterone (P4) and estrogen concentrations influence embryo survival and CL maintenance (Thatcher and Staples, 1999). Corpus luteum development results in secretion of P4, which influences embryo development, interferon-τ production and inhibition of the luteolytic cascade. Destruction of ovarian follicles by electro cauterization during mid-cycle reduced estrogen concentrations, extended luteal function (Fogwell *et al*., 1985), increased CL weight and reduced the efficacy of PGF2α induced luteolysis (Hughes *et al*., 1987). The detrimental effect of low P4 and high estrogen concentrations on early embryo development may be exacerbated in high-producing dairy cows subjected to synchronization of ovulation. Low concentrations of P4 during diestrus increase luteinizing hormone (LH) pulses, which stimulate follicular growth and resulting in elevated concentration of estradiol. In fact, the lifespan of the dominant follicle is extended and its size increased in lactating compared to nonlactating dairy cows (Sangsritavong *et al*., 2002; Biger *et al*., 2000).

Early embryonic mortality, indicated by cows returning to estrus before 24 days after insemination, is approximately 20.5-43.6% and late embryonic mortality with cows returning to estrus after 24 days is approximately 8.0-17.5% (Humbolt, 2002). Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies

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and sources of embryonic mortality in ruminants, Late embryonic mortality in cows subjected to synchronization of ovulation protocols was 14.6% measured between pregnancy diagnosis on Days 32 and 74 after Timed Artificial Insemination (TAI) (Moreira *et al*., 2001). The ovulation of dominant follicles and formation of accessory CL increase P4 and reduce estrogen concentrations and There is a paucity of information available on the effect of GnRH administered coincident with the presence of the dominant follicle of the first (Day 5) and second (Day 15) follicular wave in cows subjected to synchronization of ovulation and TAI (Schmitt *et al*., 1996; Burke and Staples, 1998). This experiment was carried out to determine the effects of GnRH injection at the time of insemination on ovulation rate, conception rate and plasma progesterone concentrations on day 5 post AI.

MATERIALS AND METHODS

Animals and treatment: Eighty cows were randomly assigned into 2 experimental groups: Treatment 1 (n = 40) which received 15ug of GnRH (Gonadrolin, 3 mL, IM) and treatment 2 (control group, n = 40) without any hormonal treatment. Cows were palpated per rectum to ascertain the occurrence of ovulation 24 h post AI. The estrus was detected by 2 experienced workers and cows were inseminated by 2 technicians. They were fed by TMR ration and were collected 3 times per day (5am, 13pm and 20pm).

Blood sampling: Blood samples were collected from the jugular vein on the day of insemination and 5 days after insemination into heparinized tubes. Plasma was separated within 1 h of collection and stored at -20°C for progesterone or estradiol assay. Progesterone concentrations were measured in follicular fluid samples in a single modified assay using [1, 2, 6, 7-3H] progesterone (Progesterone RIA, Kavoshyar, Iran). Sensitivity of the assay was 3 pg $tube^{-1}$ and intra-assay coefficients of variation were 7.4 and 7.0% for reference samples containing 7.0 and 26.0 pg $tube^{-1}$, respectively.

Statistical analysis: Progesterone concentrations on insemination day and 5 day after insemination were compared by least squares analysis of variance, using the General Linear Model (GLM) procedure of SAS. The multivariate analysis included sources of variation due to groups, days (repeated measures) and their interactions (MANOVA SAS). Differences between the 2 experimental groups were analyzed by 1 way ANOVA. Pregnancy rate and ovulation rate were analyzed by means of Binary models (Logit regression) of the SPSS13.

RESULTS AND DISCUSSION

Results of this study indicated that the injection of GnRH had not significant effects on the ovulation rate ($p>0.05$). Ovulation rate in cows that receive GnRH was 65% and it was 47.5% in the cows that received physiological serum. Absence of irregular cycles after parturition may related to low secretion of LH, delay in ovulation and little secretion of progesterone after estrus (Taponen, 2003). The treatment of GnRH decreased the time interval between estrus to the LH surge. LH surge caused to decrease production of Miotic inhibitor factor in granulosa cells (Thatcher *et al*., 1993). Following GnRH injection metabolic change occurred in follicular layer and maturation of nucleus and cytoplasm (Rosenberg *et al*., 2003). Nakao *et al*. (1984) showed that GnRH injection at time of estrus prevented from delay in ovulation. Result of present study though non significant showed positive effect of GnRH injection at time of insemination on ovulation rate that probably related to LH surge and occurred ovulation.

In agreement with results of present study Rosenberg *et al*. (2003) reported in cows that receive GnRH, all cows ovulated within 30 h of injection and estradiol concentrations in these cows had a positive correlation with LH surge, therefore GnRH injection can causes to suddenly LH surge.

GnRH injection at the time of insemination had significant effect on concentration of plasma progesterone ($p<0.02$). Concenteration of plasma progesterone in the cows that received GnRH and in cows that received physiological serum was 6.03 and 5.27 ng mL^{-1}, respectively. More production of progesterone in treatment group than control group is related to hypertrophy and hyperplasia of CL cells and probably increasing in LH and FSH pulses. In agreement with results of present study Ullah *et al*. (1996) showed that GnRH injection at time of estrus increased concenterations of plasma progesterone and improved fertility. GnRH injection at the time of estrus causes LH surge and following ovulation. LH increased blood flow to ovary and caused to ovary hyperemia. High blood flow, increased transmition chanse of steroid hormones into systemic blood flow and LDL into ovary that cholesterol in them is necessary for progesterone synthesis. Therefore, CL forming occurred rapidly and progesterone product highly (Rosenberg *et al*., 2003). Follicular structure changes with ovulation and theca granulose cells transform to CL, therefore in day 5 post AI plasma progesterone concentrations in ovulated cows was higher than cows that not ovulated (Fortune *et al*., 2001).

Recently, possible relationships between low LH surge and low *in vitro* secretion of progesterone by luteinized granulosa cells and between a low GnRH-induced LH surge and low plasma progesterone concentrations at mid-luteal phase were reported (Less *et al*., 1998; Biger *et al*., 2000). A similar relationship between low LH surge and low postovulation progesterone concentration was found in primates (Zelinski-Wooten *et al*., 1997). In agreement with these findings, hCG administered to cows on day 5 of the cycle induced a greater increase in progesterone concentrations than the GnRH analogue Buserelin, that is known to induce a narrow LH surge (Schmitt *et al*., 1996) and the potent GnRH implant Deslorelin induced a higher surge of LH and higher postovulation progesterone concentrations than Buserelin (Ambrose *et al*., 1998; Rajamahendran *et al*., 1998).

GnRH injection at the time of insemination had significant effect on pregnancy rate ($p<0.03$). Pregnancy rate in the cows that received GnRH and in cows that received physiological serum was 55 and 25%, respectively. Treatment with GnRH increase LH pulse frequency and decrease interval between LH surge and ovulation in late estrous (Mee *et al*., 1993; Morgan and Lean, 1993). Ovulation was low in the most cows that received GnRH less than 30 h after injection. This delay in ovulation was caused to forming CL and progesterone production. GnRH injection increased pregnancy rate in time of insemination via improved endocrine responses (Opsomer *et al*., 2000). High yielding cows have a longer interval between estrus and ovulation that related to low LH surge, whenever estradiol concentrations were low in follicular phase. This research showed that there was significant and positive correlation between estradiol concentrations before estrus and high LH surge (Bloch *et al*., 2001). They had lower progesterone concentrations that were related with low fertility in cows. There is probably a direct relation between low concentrations of plasma progesterone reducing LH pulse frequency and low pregnancy rate (Rosenberg *et al*., 2003; Franco *et al*., 2006). Reducing of GnRH concentration, decreases LH secretion and progesterone concentrations in mid of luteal phase (Rosenberg *et al*., 2003). These results indicate that at least some cows with low preovulatory LH surge have a late ovulation after estrus and may have low progesterone concentration in the subsequent luteal phase. In light of reports that repeat breeder cows and heifers had lower plasma progesterone levels than control animals in the estrous cycle subsequent to AI (Maurer and Echternkamp, 1985), the GnRH-induced increase in blood progesterone levels in repeat breeder cows (Mee *et al*., 1993) might be one of the causes of the increased conception rates in these cows, as well as of those found in other experiments in which cows exhibited low progesterone concentrations (Ullah *et al*., 1996).

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