

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. Eighty 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 P₄ 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 PGF₂ α 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

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 ($p<0.02$). Concentration 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 concentrations 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 transmittion chause 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 Echterkamp, 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).

REFERENCES

- Ahmad, N., S.W. Beam, W.R. Butler, J.E. Fortune and L.S. Jones, 1996. Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *J. Anim. Sci.*, 74 (8): 1943-1952.
- Alacam, E., T. Tekeli and Y. Gokcay, 1986. Improvement of conception rate in dairy cows by means of GnRH injection. Selcuk university Veteriner Fakultesi Desgisi. *S. Vet. Fak. Derg.*, 2 (1): 27-36.
- Ambrose, J.D., M.F. Pires, F. Moreira, T. Diaz, M. Binelli and W.W. Thatcher, 1998. Influence of deslorelin (GnRH-agonist) implant on plasma progesterone, first wave dominant follicle and pregnancy in dairy cattle. *Theriogenology*, 50: 1157-1170.
- Archbald, L.F. and E. Klapstein, 1992. Conception rates in dairy cows after timed insemination and simultaneous treatment with gonadotropin releasing hormone and prostaglandin α . *Theriogenology*, 37: 723-772.
- Archbald, L.F., C. Risco, P. Chavette, S. Constant, T. Tran, E. Klapstein and J. Elliot, 1993. Estrus and pregnancy rate of dairy cows given one or 2 doses of prostaglandin F 2 alpha 8 or 24 h apart. *Theriogenology*, 40: 873-884.
- Bartolom, J., A. Melendez, D. Kelbert, K. Swift, J. Machale, J. Hernandez, F. Silvester, C.A. Risco, A.C. M. Artech, W.W. Thatcher and L.F. Archbald, 2005. Strategic use of GnRH to increase pregnancy and reduce pregnancy loss in lactating dairy cows subjected to synchronization of ovulation and timed insemination. *Theriogenology*, 63: 1026-1037.
- Biger, E., D. Wolfenson, R. Mamluk, N. Levi, Y. Graber and R. Median, 2000. Effects of LH surge patterns on the steroidogenic capacity of bovine follicular cells luteinized *in vitro*. 14th Int. Cong. Anim. Reprod. Stockholm, 1: 27.
- Bloch, A., D. Wolfenson, M. Kaim, Z. Roth, R. Braw-Tal and Y. Folman, 2001. Factors affecting the time intervals between estrus, LH surge and ovulation in high-yield dairy cows. *J. Dairy Sci.*, 84 (Suppl. 1): 464.

- Burke, J.M. and C.R. Staples, 1998. Physiological responses of dairy cows to gonadotropin releasing hormone injection. *J. Dairy Sci.*, 76: 1186-1197.
- Fogwell, R.L., J.L. Cowley, J.A. Wortman, N.K. Ames and J.J. Ireland, 1985. Luteal function following destruction of ovarian follicles at midcycle. *Theriogenology*, 23: 389-398.
- Fortune, J.E., G.M. Rivera, A.C. Ewans and A.M. Turzilo, 2001. Differentiation of dominant versus subordinate follicles in cattle. *Biol. Reprod.*, 65: 648-654.
- Franco, M., P.M. Thompson, A.M. Brad and P.J. Hansen, 2006. Effectiveness of administration of gonadotropin-releasing hormone at Days 11, 14 or 15 after anticipated ovulation for increasing fertility of lactating dairy cows and non-lactating heifers. *Theriogenology*, 66 (4): 945-954.
- Hughes, T.L., A. Villa Godoy, J.S. Kesner and R.L. Fogwell, 1987. Destruction of bovine ovarian follicles: Effects on the pulsatile release of luteinizing hormone and prostaglandin F2 alpha-induced luteal regression. *Biol. Reprod.*, 36: 523-529.
- Humblot, P., 2002. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. *Theriogenology*, 56: 1417-1433.
- Kristula, M., R. Bartholomew and D. Galligan, 1992. Effects of a prostaglandin F2a synchronization program in lactating dairy cattle. *J. Dairy Sci.*, 75: 2713-2718.
- Less, M., D. Wolfenson, A. Shaham-Albalancy, Z. Roth and R. Meidan, 1998. Effects of LH surge patterns on progesterone secretion during development of the corpus luteum and *in vitro* luteinization of granulosa and theca cells in cows. *Ann. Meeting for the Study of Fertility. Glasgow*, pp: 76. (Abstr.).
- Maurer, R.R. and S.E. Echtenkamp, 1985. Repeat-breeder females in beef cattle: Influences and causes. *J. Anim. Sci.*, 61: 624-636.
- Mee, M.O., J.S. Stevenson, B.M. Alexander and R.G. Sasser, 1993. Administration of GnRH at estrus influences pregnancy rates, serum concentration of LH, FSH, estradiol-17 β , pregnancy specific protein B and progesterone, proportion of luteal cell types and *in vitro* production of progesterone in dairy cows. *J. Anim. Sci.*, 71: 185-198.
- Moreira, F., C. Orlandi, C.A. Risco, F. Lopes, R. Mattos and W.W. Thatcher, 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J. Dairy Sci.*, 84: 1646-1659.
- Morgan, W.F. and I.L. Lean, 1993. Gonadotrophin-releasing hormone treatment in cattle: A meta-analysis of the effects on conception at the time of insemination. *Aust. Vet. J.*, 70: 205-209.
- Nakao, T., J. Shirakawa, M. Tsurubayashi, K. Oboshi, T. Abe, Y. Sawamukai, N. Saga, N. Tsunoda and K. Kawata, 1984. A preliminary report on the treatment of ovulation failure in cows with gonadotrophin releasing hormone analog or human chorionic gonadotrophin combined with insemination. *Anim. Reprod. Sci.*, 7: 489-495.
- Opsomer, G., Y.T. Grohn, J. Hertl, M. Coyn, H. Deluyker and A. de Kruif, 2000. Risk factors for post partum ovarian dysfunction in high production dairy cows in belgium: A field study. *Theriogenology*, 53: 841-857.
- Peters, A.R., S.J. Ward, M.J. Warren, P.J. Gordon, G.E. Mann and R. Webb, 1999. Ovarian and hormonal responses of cows to treatment with an analogue of gonadotropin releasing hormone and prostaglandin F2 α . *Vet. Rec.*, 144: 343-346.
- Rajamahendran, R., J.D. Ambrose, E.J. Schmitt, M.J. Thatcher and W.W. Thatcher, 1998. Effects of buserelin injection and deslorelin (GnRH-agonist) implants on plasma progesterone, LH, accessory CL formation, follicle and corpus luteum dynamics in Holstein cows. *Theriogenology*, 50: 1141-1455.
- Risco, C.A., R.L. De La Sota, G. Morris, J.D. Savio and W.W. Thatcher, 1995. Postpartum reproductive management of dairy cows in a large Florida dairy herd. *Theriogenology*, 43: 1249-1258.
- Rosenberg, R.M., Wolfenson, D. and A. Bloch, 2003. Effects of GnRH administered to cows at the onset of estrus on timing of ovulation and conception. *J. Dairy Sci.*, 86: 2012-2021.
- Sangsrivong, S., D.K. Combs, R. Sartori, L.E. Armentano and M.C. Wiltbank, 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17beta in dairy cattle. *J. Dairy Sci.*, 85: 2831-2842.
- Schmitt, J.E.P., T. Diaz, C.M. Barros, R.L. de la Sota, M. Drost and E.W. Fredriksson, 1996. 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.

- Taponen, J., 2003. Ovarian function in dairy cattle after gonadotropin releasing hormone treatments during perioestrus. To be presented, with the permission of the Faculty of Veterinary Medicine, University of Helsinki, for public criticism in Auditorium Maximum, Hämeentie 57, Helsinki.
- Thatcher, W.W., M. Drost, J.D. Savio, K.L. Macmillan, K.L. Entwistle, E.J.P. Schmitt, R.L. De La Sota, G. Morris, 1993. New clinical uses for GnRH and its analogues in cattle. *Anim. Reprod. Sci.*, 33: 27-49.
- Thatcher, W.W. and R. Staples, 1999. Nutrient influences on reproduction of dairy cows. *J. Anim. Sci.*, 87: 986-899.
- Ullah, G., J.W. Fuquay, T. Keawkhong, B.L. Clark, D.E. Pogue and E.J. Murphey, 1996. Effects of gonadotropin releasing hormone at estrus on subsequent luteal function and fertility in lactating Holsteins during heat stress. *J. Dairy Sci.*, 79: 1950-1953.
- Zelinski-Wooten, M.B., J.S. Hutchinson, I. Trinchard-Lugan, D.L. Hess, D.P. Wolf and R.L. Stouffer, 1997. Initiation of periovulatory events in gonadotrophin-stimulated macaques with varying doses of recombinant human chorionic gonadotrophin. *Hum. Reprod.*, 12: 1877-1885.