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The Efficacy of a Reduced Dose of Gonadotropin Releasing Hormone on the Time and Incidence of Ovulation in Holstein Cows

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Abstract: The objectives were to determine the effect of a reduced dose of GnRH (50 μg) on the incidence and time of ovulation following an ovulation synchronization protocol (Ovsynch) in lactating Holstein cows. Fifty-five days postpartum (14 day) cows received (i.m.) 25 mg $PGF_{2\alpha}$. Fourteen days later (0 day) cows were assigned to1 of 2 treatment groups. Cows in Group 1 received half of the recommended dose of GnRH (HD; n = 17) and received 50 μg of GnRH on d 0, $PGF_{2\alpha}$(25 mg) on day 7 and GnRH (50 μg) on day 9. Cows in Group 2 were assigned to a full dose of GnRH (100 μg; FD; n = 17) and were synchronized using the same protocol. Ovarian activities were monitored by ultrasonography on day 0, 7 and 9 and also at 12 h, 20 h and then every 3 h until 39 h after the second GnRH injection. Blood samples were collected on day 7 and analyzed for serum Progesterone (P4). Mean BW was not different between groups. On day 7, mean serum P4 was above 1 ng mL^{-1} and did not differ between the groups. On day 9, concentrations of P4 decreased to below 1 ng mL^{-1} and did not differ between groups. Mean diameter of the ovulatory follicles in HD and FD were similar. Incidence of ovulation and mean ovulation time relative to the second GnRH did not differ between the groups. None of the cows exhibited estrus during the study period. Cows that ovulated developed a Corpus Luteum (CL) which was visualized using ultrasonography 7 d after ovulation. These results indicated that a reduced dose of GnRH did not affect ovulation time relative to the second GnRH injection and did not compromise the incidence of ovulation and luteal development when used in an Ovsynch breeding protocol.

Key words: GnRH, dose, ovulation, cattle, timed AI

INTRODUCTION

In the U.S., 70-80% of the dairy farms currently use Artificial Insemination (AI) (Pursley *et al.*, 1997a, b) and many operations use hormones to control the estrous cycle in order to administer AI (Caraviello *et al.*, 2006). Systematic breeding programs provide an organized and efficient approach to administering AI and improving reproductive efficiency in dairy herds. To alleviate the difficulties associated with detection of estrus and to increase the AI submission rate, timed AI programs such as Ovsynch (injections of GnRH given 7 day before and 48 h after $PGF_{2\alpha}$; Pursley *et al.*, 1995) have been developed. The cost of hormones used in synchronizing dairy cows has become a limiting factor in many breeding programs. For example, the estimated cost per pregnancy, including semen, in Ovsynch ranged between 30.25- 46.10 $ (Nebel and Jobst, 1998).

During the last few years systematic breeding programs have become more complex, usually through the incorporation of additional drug treatments. Consequently, the high cost of drugs associated with these protocols has become a concern with many dairy producers. Researchers have begun to address the cost of hormones by using a reduced dose of GnRH (50 μg) in timed breeding programs (Fricke *et al.*, 1998; Yamada *et al.*, 2002). Although, use of the half dose of GnRH (50 μg) has reduced the cost associated with synchronization and has been shown to result in a similar a conception rate (Fricke *et al.*, 1998), veterinarians, producers and herd managers have expressed that the half dose (50 μg) is not as effective as a full dose of GnRH (100 μg) (Day, 2005, personal communication). Therefore, many dairy managers and even researchers (Stevenson *et al.*, 2007; Chebel *et al.*, 2006; Bartolome *et al.*, 2005; Santos *et al.*, 2004; Peters and Pursley, 2003) still use a full dose of GnRH (100 μg) in the Ovsynch program. The reason for the lack of adoption of the reduced dose of GnRH (50 μg) among producers and researchers is not clear.

Several factors may be involved in the perceived inadequacy of the reduced dose of GnRH, including the

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accuracy of delivery of a smaller volume of the hormone. Further, a possible change in LH peak magnitude and peak duration following a reduced dose of GnRH may affect the incidence and time of ovulation, thereby necessitating a shift in the time of AI relative to the second GnRH administration and ovulation. The effect of a reduced GnRH dose in Ovsynch on incidence and time of ovulation has not been fully investigated. Therefore, the objective of this study was to investigate whether a reduced dose of GnRH alters the incidence and time of ovulation following implementation of Ovsynch in lactating Holstein cows.

MATERIALS AND METHODS

Animals and treatment: The University of Idaho institutional animal care and use committee approved all of the procedures used in this experiment. Thirty-four lactating Holstein cows (57±1.7 days postpartum) were used in this study. Animals were housed in a freestall barn and fed a total mixed ration formulated to meet or exceed (NRC, 2001) requirements for lactating dairy cows. Based on transrectal palpation by the herd veterinarian, experimental cows had no abnormalities of the reproductive tract at the initiation of the experiment. On d 0 and upon detection of a CL cows were weighed and assigned randomly to one of two treatment groups. Cows in group 1 were assigned to receive half of the recommended dose of GnRH (HD; n = 17) and received 50 μg GnRH (Cystorelin, Merial, Athens, GA, USA) on day 0, $PGF_{2\alpha}$ (25 mg; Pfizer Animal Health, New York) on day 7 and GnRH (50 μg) on day 9. Cows in group 2 were assigned to a full dose of GnRH (FD; n = 17) and were synchronized using the same protocol and received 100 μg GnRH per injection. Both GnRH and $PGF_{2\alpha}$ were administered i.m. in the thigh muscle (semimembranosus muscle) with 5 mL syringe using a 20 gauge by 3.8 cm needle. To monitor estrous activity, animals were equipped with an electronic pressure-sensing device (HW ®, HeatWatch, Cow Chips, LLC, Denver, CO).

Ultrasonogrpahy and blood collection: On day 0, 7 and 9 ovaries were examined via transrectal ultrasonography (7.5 mHz. probe; Sonovet 600, Alliance Medical, Bedford Hills, NY) and structures were recorded. Time of ovulation after the second injection of GnRH (d 9) was determined by ultrasonography conducted at 12 and 20 h after GnRH and then at least every 3 h thereafter until either ovulation or 39 h, whichever occurred first. Ovulation was defined as the disappearance of any antral follicle ≥10 mm in diameter at the time of an ultrasound examination compared with the previous ultrasound examination (Kaneko *et al.*, 1991). Time of ovulation was defined as the number of hours from the time of treatment to the midpoint of two ultrasound examinations between which ovulation had occurred (Walker *et al.*, 1996). On day 16 (7 day after the second GnRH administration), ultrasonographic examinations of the ovaries were performed to confirm the occurrence of ovulation, as evidenced by the presence of a CL ipsilateral to the site of ovulation. Incidence of ovulation was calculated as the number of cows that ovulated at least one follicle as a percentage of the total number of cows subjected to the Ovsynch protocol.

Blood samples were collected via coccygeal venipuncture on day 7 and 9 for measurement of Progesterone (P4) concentrations to determine the ovarian response to first GnRH and $PGF_{2\alpha}$. Blood samples were immediately placed in ice and stored at 4°C for a minimum of 20 h to allow clotting. All samples were centrifuged at 4°C for 30 min at 2750 × g. Serum was harvested and stored at -20°C until assayed for P4. Serum progesterone was quantified using a solid-phase radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). The assay was conducted under equilibrium conditions. The standard curve ranged from 0.1-40 ng mL^{-1} and all sample were assayed in duplicate. The intra-assay CV was 6.4%.

Statistical analysis: Incidence of ovulation was analyzed by logistic regression with the model including the effect of group. Differences in BW, time of ovulation, size of the ovulatory follicles and P4 concentrations between groups were analyzed using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Serum P4 data were analyzed by least-squares analysis of variance using the GLM procedure of SAS. The statistical model included treatment and separate analyses were performed for each sampling day (day 7 and 9) to determine ovarian response to the first GnRH and $PGF_{2\alpha}$.

RESULTS AND DISCUSSION

There was no difference in mean BW between treatments (875±13.1 kg vs 886.1±13.1 kg, FD vs HD, respectively). The incidence of ovulation and time of ovulation after the second GnRH injection in FD and HD groups is depicted in Table 1. The percentages of cows ovulating within 39 h after the second GnRH injection were 100 and 94% for FD and HD, respectively. One cow in HD did not ovulate within 39 h after the second GnRH

Table 1: Time and incidence of ovulation and size of ovulatory follicle in lactating Holstein cows treated with a full dose (100 μg) or half dose (50 μg) of GnRH in the Ovsynch protocol

Treatment	Time of ovulation[1], h	Incidence of ovulation[2],%	Diameter of ovulatory follicle[3], mm
Full Dose (n = 17)	26.8±0.9	100 (17/17)	18.2±0.7
Half Dose (n = 17)	25.9±0.9	94 (16/17)[4]	19.1±0.7

[1]Time interval from the second GnRH injection to the disappearance of ovulatory follicle. One cow in the half dose ovulated after the last ultrasonography session (39 h post-GnRH) and was not included in analysis. [2]Number of cows that ovulated within 39 h following the second GnRH injection/total number of cows submitted to the protocol. [3]Size of the ovulatory follicle 3 h before ovulation. [4]Based on presence of CL ipsilateral to the presumptive ovulatory follicle (7 d after second GnRH injection) all 17 cows ovulated in the half dose group

Table 2: Serum progesterone concentration (ng mL^{-1}) on day 7 and 9 in lactating Holstein cows subjected to the Ovsynch protocol using either 100 or 50 μg of GnRH

	Day of Experiment[1]	
	Least squares means±SEM	
Treatment	7	9
100 μg GnRH (n = 17)	4.5±0.67	0.4±0.1
50 μg GnRH (n = 17)	4.2±0.71	0.2±0.1

[1]d 7 = Mean serum progesterone on the day of $PGF_{2\alpha}$ injection; d 9 = Mean serum progesterone 48 h after $PGF_{2\alpha}$ injection

administration. Nevertheless, this cow developed a CL ipsilateral to the presumptive ovulatory follicle 7 d after the second GnRH, indicating that this cow ovulated some time after the last ultrasonogrpahy session. The cow that ovulated after 39 h was not included in the calculation of time of ovulation and size of ovulatory follicle as these measurements were unknown. Time of ovulation was not different between the FD and HD groups (Table 1). The mean time interval from the second GnRH injection to ovulation was 26.8±0.9 h for FD (range of 21.5-27.5 h) and 25.9±0.9 h for HD (range of 12-30.5 h). The size of the ovulatory follicle did not differ between FD and HD groups, as it was 18.2±0.7 mm in the FD group and 19.1±0.7 mm in the HD group (Table 1). None of the animals exhibited estrus during the trial according to the HeatWatch system.

Serum P4 was measured on day 7 to determine whether the treatment of 50 μg of GnRH caused a change in serum progesterone compared with 100 μg of GnRH. On day 7, mean serum P4 concentrations were not different between the FD and HD groups (Table 2). On day 9, serum P4 concentrations were less than 1 ng mL^{-1} in both groups and were not different.

Although, producers using Ovsynch have achieved reasonable pregnancy rates compared with other breeding programs, the cost per pregnancy has been estimated to range between $30.25-$46.10 (Nebel and Jobst, 1998). Taken together with the concern of producers, managers and veterinarians that a half dose of GnRH was perceived to be less effective than a full dose, we conducted this experiment to investigate whether a reduced dose of GnRH alters the incidence and time of ovulation following implementation of Ovsynch in lactating Holstein cows. The use of a half dose of GnRH (50 μg) in the Ovsynch protocol did not affect the incidence of ovulation, the time interval from the second GnRH injection to ovulation, or serum P4 concentrations at $PGF_{2\alpha}$ injection (7 and day 9). The use of 50 μg compared to 100 μg of GnRH did not alter the time interval from the second GnRH to ovulation (25.9±0.9 in HD vs 26.8±0.9 in FD). Our results agree with Pursley *et al.* (1995) and previous work in our laboratory (Sellars *et al.*, 2006). Sellars *et al.* (2006) reported a similar time interval to ovulation following a full dose of GnRH in the Ovsynch protocol. Further, the results described here are consistent with the time of ovulation in natural and $PGF_{2\alpha}$-induced estrus, which was 27.6±5.4 h after the onset of estrus (Walker *et al.*, 1996). Therefore, it is apparent that 50 μg is sufficient to induce ovulation without shifting ovulation time from the second GnRH injection. If the interval from the second GnRH (using 50 μg) to ovulation had changed, then the time of AI would need to be adjusted in order to achieve maximum fertility. However, the time of ovulation was not affected by the reduced dose of GnRH. Therefore, there is no evidence that the generally accepted optimal range of time to AI cows (~8-20 h) after the second GnRH of Ovsynch should be modified.

It is apparent that 50 μg of GnRH used in the HD group was sufficient to release LH to induce ovulation in lactating Holstein dairy cows. Yamada *et al.* (2002) have shown that a half dose of GnRH analogue (fertirelin acetate, 50 μg) did not affect plasma LH concentration compared to a full dose (100 μg). Further, Yamada *et al.* (2002) described that after both the 50 and 100 μg GnRH injection plasma LH concentration increased within 120 min to reach a peak over 5 ng mL^{-1} and then returned to an initial level within 360 min.

The incidence of ovulation observed in the present study is similar to the findings of Pursley *et al.* (1995), Vasconcelos *et al.* (1999), Cartmill *et al.* (2001) and Sellars *et al.* (2006) who found ovulation rates ranging from 87-100% in dairy cows submitted to the Ovsynch protocol (using a 100 μg dose of GnRH). In a preliminary study (Ahmadzadeh *et al.*, 2002) similar results were observed when lactating Jersey cows (435±14 kg BW) received 50 μg GnRH in an Ovsynch protocol. Although Holstein cows were used in the current study, on a dose per kg BW basis, the Holsteins actually received a lower dose of GnRH. Nevertheless, the reduced dose of GnRH did not affect ovulation time relative to the second GnRH and did not compromise the incidence of ovulation. Lastly, an early study (Seguin *et al*., 1976) reported that treating ovarian follicular cysts with 50 μg of GnRH was as effective as treatment with 100 μg. Taken together, it appears that 50 μg GnRH is as effective in inducing ovulation as 100 μg.

CONCLUSION

For the first time it has been shown that a reduced dose of GnRH (50 μg) in the Ovsynch protocol did not affect the time interval from the second GnRH injection to ovulation. Furthermore, a reduced GnRH dose did not compromise the incidence of ovulation. Therefore, 50 μg GnRH is as effective in synchronizing ovulation in the Ovsynch protocol as the full dose (100 μg GnRH) regimen. Lastly, in lactating dairy cows the use of a reduced dose of GnRH (50 μg), when administered properly, will decrease the cost associated with the Ovsynch protocol without compromising its effectiveness in synchronizing ovulation.

REFERENCES

Ahmadzadeh, A., R. Manzo, C.B. Sellars, L. Palmer and R.L. Nebel, 2002. The efficacy of a reduced dose of GnRH on ovulation rate and time of ovulation in Jersey and Holstein dairy cows. J. Dairy Sci., 85: 306.

Bartolome, J.A., F.T. Silvestre and S. Kamimura *et al*., 2005. Resynchronization of ovulation and timed insemination in lactating dairy cows I: Use of the Ovsynch and Heatsynch protocols after non-pregnancy diagnosis by ultrasonography. Theriogenology, 63: 1617-1627.

Caraviello, D.Z., K.A. Weigel, P.M. Fricke, M.C. Wiltbank, M.J. Florent, N.B. Cook, K.V. Nordlund, N.R. Zwald and C.L. Rawson, 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. J. Dairy Sci., 89: 4273-4735.

Cartmill, J.A., S.Z. El-Zarkouny, B.A. Hensley, G.C. Lamb and J.S. Stevenson, 2001. Stage of cycle, incidence and timing of ovulation and pregnancy rates in dairy cattle after three timed breeding protocols. J. Dairy Sci., 84: 1051-1059.

Chebel, R.C., J.E.P. Santos, R.L.A. Cerri, H.M. Rutigliano and R.G.S. Bruno, 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization Protocols. J. Dairy Sci., 89: 4205-4219.

Day, J., 2005. Personal Communication Dairy Health Services/MPS. 105 Country Lane, Jereome, Idaho 83338, USA.

Fricke, P.M., J.N. Guenther and M.C. Wiltbank, 1998. Efficacy of decreasing the dose of GnRH used in a protocol for synchronization of ovulation and timed AI in lactating dairy cows. Theriogenology, 50: 1275-1284.

Kaneko, H., T. Terada, K. Taya and G. Watanabe *et al*., 1991. Ovarian follicular dynamics and concentrations of oestradiol-17 beta, progesterone, luteinizing hormone and follicle stimulating hormone during the periovulatory phase of the oestrous cycle in the cow. Reprod. Fertil. Dev., 3: 529-535.

National Research Council (NRC), 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Edn. National Academy Press. Washington, DC., pp: 258.

Nebel, R.L. and S.M. Jobst, 1998. Evaluation of systematic breeding programs for lactating dairy cows: A Rev. J. Dairy Sci., 81: 1169-1174.

Peters, M.W. and J. R. Pursley, 2003. Timing of final GnRH of the Ovsynch protocol affects ovulatory follicle size, subsequent luteal function and fertility in dairy cows. Theriogenology, 60: 1197-1204.

Pursley, J.R., M.O. Mee and M.C. Wiltbank, 1995. Synchronization of ovulation in dairy cattle using $PGF_{2\alpha}$ and GnRH. Theriogenology, 44: 915-923.

Pursley, J. R., M. R. Kosorok and M. C. Wiltbank. 1997a. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci., 80: 301-306.

Pursley, J.R., M.C. Wiltbank, J.S. Stevenson, J.S. Ottobre, H.A. Garverick and L.L. Anderson, 1997b. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrous. J. Dairy Sci., 80: 295-300.

Santos, J.E.P., S.O. Juchem and R.L.A. Cerri *et al*., 2004. Effect of bST and reproductive management on reproductive performance of Holstein dairy cows. J. Dairy Sci., 87: 868-881.

Seguin, B.E., E.M. Convey and W.D. Oxender, 1976. Effect of gonadotropin-releasing hormone and human chorionic gonadotropin on cows with ovarian follicular cysts. Am. J. Vet. Res., 37: 153-157.

Sellars, C.B., J.C. Dalton, R. Manzo, J. Day and A. Ahmadzadeh, 2006. Time and incidence of ovulation and conception rates after incorporating estradiol cypionate into a timed artificial insemination protocol. J. Dairy Sci., 89: 620-626.

Stevenson, J.S., M.A. Portaluppi and D.E. Tenhouse, 2007. Ovarian traits after gonadotropin-releasing hormone-induced ovulation and subsequent delay of induced luteolysis in an ovsynch protocol. J. Dairy Sci., 90: 1281-1288.

Vasconcelos, J.L.M., R.W. Silcox, G.J.M. Rosa, J.R. Pursley and M.C. Wiltbank, 1999. Synchronization rate, size of the ovulatory follicle and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology, 52: 1067-1078.

Walker, W.L., R.L. Nebel and M.L. McGilliard, 1996. Time of ovulation relative to mounting activity in dairy cattle. J. Dairy Sci., 79: 1555-1561.

Yamada, K., T. Nakao, K. Nakada and G. Matsuda, 2002. Influence of GnRH analogue (fertirelin acetate) doses on synchronization of ovulation and fixed-time artificial insemination in lactating dairy cows. Anim. Reprod. Sci., 74: 27-34.