

Research Article

Effect of GnRH Administration on Holstein Cows with Follicular Cysts or Ovarian Quiescence as Determined by Ultrasonography at Regular Checkup

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Abstract: Changes in the ovaries were monitored every 2 to 3 weeks using real-time ultrasonography as part of regular reproductive checkups in 178 multiparous and 109 heifer (total 287) cows which had been diagnosed as having follicular cysts (77 cows) or ovarian quiescence (210 cows) and treated once with gonadotropin-releasing hormone, GnRH (fertirelin acetate at 100 µg i.m.). Following the one-time treatment, 96 cows (33.4%) showed estrus and underwent artificial insemination by the next reproductive checkup (Cow AI), 87 cows (30.3%) were given dinoprost as prostaglandin F_{2α} (PG) to induce estrus at the next checkup because of existence of Corpus Luteum (CL) in their ovaries (Cow PG) and there was no change (no effect) in the ovaries of 104 cows (Cow NE, 36.2%). The number of cows which conceived during the estrus cycle following injection of GnRH was 39 (40.6%) in Cow AI and 38 (43.7%) in Cow PG. In conclusion, one-time GnRH administration to cattle with ovarian cysts or quiescence but without CL at a regular reproductive checkup may effectively shorten the time to conception.

Keywords: Cystic ovary, GnRH, ovarian quiescence, return of estrus, ultrasonography

INTRODUCTION

In reproductive management for cows, to improve reproductive performance it is important to detect and treat abnormalities in reproductive organs as soon as possible. Introduction of the use of portable transrectal real-time ultrasonography into reproductive management programs has enabled visualization of ovarian pathologies that are not accurately detected via rectal palpation (Fricke, 2002). Ovarian quiescence and follicular cysts are the most common ovarian disturbances in cows or heifers failing to show signs of estrus after parturition or after reaching breeding age, respectively (Bosu and Peter, 1987; Opsomer *et al.*, 1998). Although gonadotropin-releasing hormone (GnRH) has been shown to be effective for the treatment of ovarian quiescence and follicular cysts (Isobe *et al.*, 2007; Mollo *et al.*, 2012; Peter, 2004), there are no reports showing the effectiveness of GnRH administration in cows with ovarian quiescence or follicular cysts diagnosed by ultrasonographic imaging at regular reproductive checkups. Furthermore, although there are many treatment strategies for ovarian quiescence or follicular cyst, adapting plural remedies at reproductive checkups in the field may complicate conducting these checkups and evaluation of the effects

of these treatments. The objective of this study was to investigate the effect of GnRH given to cows upon diagnosis based on ultrasonographic images at regular reproductive checkups with the aim of improving reproductive performance.

MATERIALS AND METHODS

Cows (multiparous, 178; nulliparous, 109; total, 287) on 17 dairy farms which were conducting regular reproductive checkups including ultrasonographic examination were included in the study. The multiparous cows had not shown sign of estrus at 30 days after parturition and the nulliparous cows had not shown the first estrus by attaining the age of 13 months.

Changes in the ovaries were monitored every 2 to 3 weeks using real-time ultrasonography (50S Tringa equipped with a 7.5-MHz transrectal linear transducer; Sumire Medical Co., Ltd., Tokyo, Japan). Cows were administered GnRH (fertirelin acetate 100 µg i.m.) if corpus luteum (CL) was not detected at two consecutive examinations. If large follicles (diameter>2.5 cm) were detected, the cows were diagnosed as having a follicular cyst (Group FC) and others were diagnosed as showing ovarian quiescence. Furthermore the cows with ovarian quiescence were allocated to Group FOQ

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Table 1: Treatment and effect of GnRH administration

| | Number of each group | Number of cow AI (%) | Number of cow PG (%) | Number of effectiveness (Cow AI+Cow PG) (%) | Number of cow NE (%) |
|-----------|----------------------|----------------------|----------------------|---|----------------------|
| Group FC | 77 | 22 (28.6) | 29 (37.7) | 51 (66.2) | 26 (33.8) |
| Group FOQ | 166 | 62 (37.3) | 47 (28.3) | 109 (65.7) | 57 (34.3) |
| Group OQ | 44 | 12 (27.3) | 11 (25.0) | 23 (52.3) | 21 (47.7) |
| Total | 287 | 96 (33.4) | 87 (30.3) | 183 (63.8) | 104 (36.2) |

Group FC: cows which had cystic follicles at GnRH administration; Group FOQ: cows which had follicle ≥ 8 mm at GnRH administration; Group OQ: cows which had follicle < 8 mm at GnRH administration; Cow AI: cows which showed estrus after GnRH administration and received artificial insemination by the next reproductive checkup; Cow PG: cows which were given prostaglandin $F_{2\alpha}$ at the next reproductive checkup; Cow NE: cows in which there was not any change in their ovaries

(with follicles having a diameter of 8 mm or greater) and Group OQ (with follicles less than 8 mm).

After GnRH administration, all cows were classified into three groups based on the response to GnRH. The Cow AI group included cows that showed estrus and had undergone Artificial Insemination (AI) by the next reproductive checkup, The Cow PG group was given prostaglandin $F_{2\alpha}$ (PG) (Pronalgon F, Pfizer, Tokyo, Japan) at the next reproductive checkup based on the presence of CL and no changes were observed in the ovaries of the Cow NE group. The GnRH treatment was considered to have been effective in the Cow AI and Cow PG groups. The incidence of cows showing estrus within 16 days after GnRH administration was compared among Group FC, Group FOQ and Group OQ. Conception was confirmed by ultrasonography at 41 days or later after artificial insemination.

RESULTS

At the time of GnRH administration, 77 cows (26.8%) belonged to Group FC, 166 cows (57.8%) belonged to Group FOQ and 44 cows (15.3%) belonged to Group OQ (Table 1). After GnRH administration, 96 cows (33.4%) showed estrus and underwent artificial insemination before the next reproductive checkup (Cow AI), 87 cows (30.3%) were given PG at the next reproductive checkup due to the presence of CL in their ovaries (Cow PG) and there were no changes in the ovaries of 104 cows (36.2%) (Cow NE) (Table 1). The number of cows in which GnRH was effective (Cow AI+Cow PG) was 51 (66.2%) in Group FC, 109 (65.7%) in Group FOQ and 23 (52.3%) in Group OQ and there was no significant difference among the groups (Table 1). The number of cows in the Cow AI and Cow PG groups was 22 (28.6%) and 29 (37.7%) in Group FC, 62 (37.3%) and 47 (28.3%) in Group FOQ and 12 (27.3%) and 11 (25.0%) in Group OQ, respectively (Table 1). The number of days from GnRH administration to artificial insemination was 15.0 ± 6.4 days in the Cow AI group and 23.0 ± 8.7 days in the Cow PG group and it was significantly shorter in the Cow AI group than in the Cow PG group ($p < 0.01$) (Table 2). In the Cow AI and Cow PG groups, 39 (40.6%) and 38 (43.7%) cows, respectively, conceived and there was no significant difference between the two groups (Table 2). Of 183 cows in which effectiveness of GnRH was confirmed, 60 cows (32.8%) underwent AI within 16 days of GnRH administration. The

Table 2: Reproductive performance

| | Number of days from GnRH administration to AI (days) | Conception rate (%) |
|--------|--|---------------------|
| Cow AI | 15.0 ± 6.4^a | 40.6 (39/96) |
| Cow PG | 23.0 ± 8.7^b | 43.7 (38/87) |

AI: artificial insemination. There was a significant difference between a and b ($p < 0.01$)

distribution of these cows in each group was 12 (23.5%) in Group FC, 39 (35.8%) in Group FOQ and 9 (39.1%) in Group OQ, respectively and there were no significant differences among the groups.

DISCUSSION

As a result of GnRH administration, 96 cows (33.4%) showed estrus and underwent AI by the time of the next reproductive checkup (Cow AI) and 87 cows (30.3%) showed CL in the ovaries at the next reproductive checkup and received PG (Cow PG). This shows that the GnRH administration was effective in 183 cows (63.8%). The number of days from GnRH administration to artificial insemination was significantly shorter in the Cow AI group than in the Cow PG group. Because the dominant follicle needed to be ovulated before PG was given in the Cow PG group, it might have taken longer to show estrus.

Mollo *et al.* (2012) reported a cure rate of follicular cysts by GnRH of 64.3% and the effective rate of GnRH administration in Group FC (66.2%) was similar. When GnRH is given to cows with follicular cysts, a smaller follicle coexisting with cystic follicle tends to ovulate and form the CL instead of the cystic follicle (Isobe *et al.*, 2007). In this study, 22 out of 77 cows in Group FC (28.6%) were also in the Cow AI group. The smaller follicle coexisting with the cystic follicle may have matured and this follicle may have led to estrus. On the other hand, 29 cows (37.7%) were in the Cow PG group and the follicle coexisting with the cystic follicle may have ovulated and formed the CL in those cows.

There is another mechanism for recovering from cystic ovaries by GnRH treatment. Following GnRH treatment, luteinization of cysts occurs, which is followed by luteolysis and the cows resume a normal estrus cycle (Peter, 2004). Because the luteinization and luteolysis takes about 16 to 18 days (Peter, 2004), the rate of receiving AI within 16 days of GnRH administration in Group FC may have tended to be low.

Furthermore, some cows in Group FC may have been judged as belonging to the Cow NE group, even if GnRH showed effectiveness.

A single GnRH treatment can induce ovulation of dominant follicles (Crowe *et al.*, 1993; Quintans *et al.*, 2004) or restart ovarian cyclic activity (Cavestany and Foote, 1985). In this study, 62 cows (37.3%) in Group FOQ and 12 cows (27.3%) in Group OQ were in the Cow AI group. GnRH may have stimulated the growth of dominant follicles, which resulted in estrus in those cows. The rate at which estrus was induced in Group FOQ tended to be higher than that in Group OQ. It was reported that the smaller follicles are more likely to become atretic follicles than follicles having a diameter of more than 8.5 mm (Roche *et al.*, 1998) and the ovaries were considered to be active when follicles with a diameter greater than 8 mm are present (Sheldon *et al.*, 2000). Furthermore, the larger the follicle which was induced ovulation by GnRH, the higher the conception rate was (Peters and Pursley, 2003). Therefore, GnRH is recommended as the treatment for cows with ovarian quiescence that has a follicle of which the diameter is more than around 8 mm. If not, other formulations which stimulate follicle growth, like eCG, can be used as a pretreatment to the administration of GnRH in order to enhance the effectiveness of GnRH. However, 52.3% of the cows were in the Cow AI or Cow PG groups, even in the absence of a follicle with a diameter of more than 8 mm. The conception rate for the Cow AI group was 40.6% and this rate was the same as for cows in which the natural estrus was detected by the observation of estrus signs (Cavestany *et al.*, 2007; Cordoba and Fricke, 2002; Cavestany *et al.*, 2003; Tóth *et al.*, 2006). Accordingly, the administration of GnRH at the reproductive checkup is recommended for cows with ovarian quiescence.

When cows experience reduced PG level before ovulation, short luteal phases caused by the premature release of PGF_{2α} by the endometrium may occur (Cerri *et al.*, 2011). Ovulation may have progressed in the Cow AI group as follows: the first follicle with a diameter of more than 8 mm was released in the ovulation after GnRH administration, then the CL that developed following the ovulation regressed early and the cows showed estrus. Some cows in Group FOQ might have followed this pathway. The ratio of cows in the Cow PG group was almost that same in Groups FOQ (28.3%) and OQ (25.5%). Although dominant follicles are considered to be luteinized without ovulation, there is a possibility that the estrus signs were overlooked, even if estrus was induced and the rate of reaching the Cow AI group may be increased through more careful observation.

Pretreatment by intravaginal device or hCG aims to raise the blood progesterone level and expose follicles to sufficient progesterone level and the administration of GnRH after that pretreatment can effectively induce ovulation and maintain normal CL function (Mee *et al.*, 1991; Stevenson *et al.*, 2007; Smith *et al.*, 1987). From

these reports, pretreatment by intravaginal device or hCG may be valuable if GnRH is given to cows with ovarian cyst or quiescence but in the absence of CL.

This study shows that the application of ultrasonography improves the effectiveness of the reproductive checkups due to the visualization of the ovarian structure and, furthermore, the administration of GnRH in a one-time administration to cattle with ovarian cyst or quiescence but without CL at regular reproductive checkup may shorten the period to conception.

REFERENCES

- Bosu, W.T.K. and A.T. Peter, 1987. Evidence for a role of intrauterine infections in the pathogenesis of cystic ovaries in postpartum dairy cows. *Theriogenology*, 28: 725-736.
- Cavestany, D. and R.H. Foote, 1985. Reproductive performance of Holstein cows administered GnRH HOM 766 (Buserelin) 26 to 34 days postpartum. *J. Anim. Sci.*, 61: 224-233.
- Cavestany, D., H. Betancour, J.E. Blanc, C. Lemaire, J. Slavica, F. Moreira, J. Piaggio and C. Risco, 2007. Reproductive efficacy in grazing lactating dairy cows under a programmed reproductive management system. *Aust. Vet. J.*, 85: 141-147.
- Cerri, R.L.A., R.C. Chebel, F. Rivera, C.D. Narciso, R.A. Oliveira, M. Amstalden, M.G.M. Baez-Sandoval, L.J. Oliveira, W.W. Thatcher and J.E.P. Santos, 2011. Concentration of progesterone during the development of the ovulatory follicle: II. Ovarian and uterine responses. *J. Dairy Sci.*, 94: 3352-3365.
- Cordoba, M.C. and P.M. Fricke, 2002. Initiation of the breeding season in a grazing-based dairy by synchronization of ovulation. *J. Dairy Sci.*, 85: 1752-1763.
- Crowe, M.A., D. Goulding, A. Baguisi, M.P. Boland and F.J. Roche, 1993. Induced ovulation of the first postpartum dominant follicle in beef suckler cows using a GnRH analogue. *J. Reprod. Fertil.*, 99: 551-555.
- Cvestany, D., J. Cibils, A. Freire, A. Sastre and J.S. Stevenson, 2003. Evaluation of two different oestrus-synchronisation methods with timed artificial insemination and resynchronization of returns to oestrus in lactating Holstein cows. *Anim. Reprod. Sci.*, 77: 141-155.
- Fricke, P.M., 2002. Scanning the future-ultrasonography as a reproductive management tool for dairy cattle. *J. Dairy Sci.*, 85: 1918-1926.
- Isobe, N., K. Yamada and Y. Yoshimura, 2007. Involvement of plasma progesterone, oestradiol-17β and cortisol in ovulatory response to gonadotropin-releasing hormone in dairy cows with cystic follicles. *Reprod. Domest. Anim.*, 42: 370-375.

- Mee, M.O., J.S. Stevenson and J.E. Minton, 1991. First postpartum luteal function in dairy cows after ovulation induced by progestogen and gonadotropin-releasing hormone. *J. Dairy Sci.*, 74: 1573-1581.
- Mollo, A., G. Stradaoli, A. Gloria and F. Cairolì, 2012. Efficacy of different ovarian cysts treatments (GnRH, hCG and PRID) in dairy cows. *J. Anim. Vet. Adv.*, 21: 4058-4063.
- Opsomer, G., M. Coryn, H. Deluyker and A. de Kruif, 1998. An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. *Reprod. Domest. Anim.*, 33: 193-204.
- Peter, A.T., 2004. An update on cystic ovarian degeneration in cattle. *Reprod. Domest. Anim.*, 39: 1-7.
- 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.
- Quintans, G., C. Viñoles and K.D. Sinclair, 2004. Follicular growth and ovulation in postpartum beef cows following calf removal and GnRH treatment. *Anim. Reprod. Sci.*, 80: 5-14.
- Roche, J.F., M. Mihn, M.G. Diskin and J.J. Ireland, 1998. A review of regulation of follicle growth in cattle. *J. Anim. Sci.*, 76(Suppl. 3): 16-29.
- Sheldon, I.M., D.E. Noakes and H. Dobson, 2000. The influence of ovarian activity and uterine involution determined by ultrasonography on subsequent reproductive performance of dairy cows. *Theriogenology*, 54: 409-419.
- Smith, V.G., J.R. Chenault, J.F. McAllister and J.W. Lauderdale, 1987. Response of postpartum beef cows to exogenous progestogens and gonadotropin releasing hormone. *J. Anim. Sci.*, 64: 540-551.
- Stevenson, J.S., M.A. Portaluppi, D.E. Tenhouse, A. Lloyd, D.R. Eborn, S. Kacuba and J.M. DeJarnette, 2007. Interventions after artificial insemination: conception rates, pregnancy survival and ovarian responses to gonadotropin-releasing hormone, human chorionic gonadotropin and progesterone. *J. Dairy Sci.*, 90: 331-340.
- Tóth, F., G. Gábor, M. Mézes, E. Váradi, L. Ozsvári, R.G. Sasser and Z. Abonyi-Tóth, 2006. Improving the reproductive efficiency by zoo-technical methods at a dairy farm. *Reprod. Domest. Anim.*, 41: 184-188.