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—Full Paper—

Comparison of Two Types of CIDR-based Timed Artificial Insemination Protocols for Repeat Breeder Dairy Cows

Ui-Hyung KIM[1], Guk-Hyun SUH[2], Tai-Young HUR[2], Seog-Jin KANG[2], Hyun-Gu KANG[1], Soo-Bong PARK[2], Hyeon-Shup KIM[2] and Ill-Hwa KIM[1]

[1]Laboratory of Theriogenology, College of Veterinary Medicine, Chungbuk National University, Cheongju 361-763 and [2]Dairy Cattle Research Division, National Livestock Research Institute, RDA, Cheonan 330-800, Korea

Abstract. This study compared two types of controlled internal drug release (CIDR)-based timed artificial insemination (TAI) protocol for treatment of repeat breeder dairy cows. In the first trial of the experiment, 55 repeat breeder cows were randomly assigned to the following two treatments. (1) In the EB group, a CIDR device was inserted into the cows, and then the cows were administered an injection of 1 mg estradiol benzoate (EB) plus 50 mg progesterone (P4; Day 0). On Day 7, they were given an injection of $PGF_{2\alpha}$ and the CIDR device was removed. The cows were given an injection of 1 mg EB on Day 8 and were subjected to TAI 30 h later (n=27). (2) In the gonadotrophin releasing hormone (GnRH) group, a CIDR device was inserted into the cows, and then the cows were administered an injection of 250 μg gonadorelin (GnRH; Day 0). On Day 7, they were given an injection of $PGF_{2\alpha}$ and the CIDR device was removed. The cows were given an injection of 250 μg GnRH on Day 9 and were subjected to TAI 17 h later (n=28). In the second trial, 41 repeat breeder cows that were confirmed as not pregnant in the first trial were randomly assigned to the same two treatments used in the first trial (an EB group of 20 cows and a GnRH group of 21 cows). The ovaries of 15 cows from each group were examined by transrectal ultrasonography in order to observe the changes in ovarian structures, and blood samples were collected for analysis of serum P4 concentrations. The pregnancy rates following TAI in the first (18.5 vs. 32.1%) and second (40.0 vs. 38.1%) trials and the combined rates (27.7 vs. 34.7%) did not differ between the EB and GnRH groups. The proportions of cows with follicular wave emergence within 7 days did not differ between the EB (12/15) and GnRH groups (13/15). The interval to wave emergence was shorter ($P<0.01$) in the GnRH group than in the EB group, but there was no difference in the mean diameters of dominant follicles on Day 7 between the groups. Moreover, the proportions of cows with synchronized ovulation following a second EB or GnRH treatment did not differ between the groups. In conclusion, treatment with either EB or GnRH in a CIDR-based TAI protocol results in synchronous follicular wave emergence, follicular development, synchronous ovulation, and similar pregnancy rates for TAI in repeat breeder cows.

Key words: Controlled internal drug release (CIDR), Estradiol benzoate, Gonadotrophin releasing hormone (GnRH), Pregnancy rate, Repeat breeder dairy cows

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Correspondence: I.-H. Kim (e-mail address: illhwa@chungbuk.ac.kr)

Increased milk production in dairy cows induced by continuous genetic manipulation, improved nutritional management, and larger herd size is associated with decreased fertility [1–3].

Repeat breeding is an important cause of decreased fertility and results in severe economic losses due to increased breeding number (wasted semen and insemination costs), an extended calving interval, and increased culling incidence and veterinary services for dairy cattle [4]. Ten to 24% of reproductive cows on dairy farms are repeat breeders [4, 5]. Some of the diverse causes of infertility associated with repeat breeding include reproductive tract abnormality, estrous detection errors, anovulation, endocrine dysfunction, fertilization failure, and embryonic mortality [6–14]. Therefore, various treatments have been used to improve conception rates for repeat breeders. These include gonadotrophin releasing hormone (GnRH) or human chorionic gonadotrophin (hCG) administration at the time of insemination [15–17], multiple artificial inseminations during estrus [18], insulin treatment prior to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injection for estrous induction [19], and post-insemination supplementation with a progesterone releasing intravaginal device (PRID) [11]. However, these treatments have only been somewhat successful.

Timed artificial insemination (TAI) protocols such as Ovsynch, which is composed of GnRH, $PGF_{2\alpha}$, and GnRH injections, and progesterone (P4)/progestogen-based regimens have been successfully used in the past to enhance the fertility of normal estrous and anestrus cows and cows with ovarian cysts [20–25]. These TAI protocols induce a new follicular wave, timely follicular development, and synchronous ovulation, resulting in acceptable pregnancy rates. Similarly, a recent report demonstrated that the Ovsynch protocol results in a conception rate of 21% in repeat breeder dairy cows [26]. However, the efficacy of P4-based TAI protocols remains undetermined in repeat breeder cows. In P4-based regimens, induction of a new follicular wave is necessary in order to prevent development of a persistent follicle. Thus, exogenous treatments with estrogen (and P4) or GnRH have been most commonly used to induce synchronous wave emergence [22]. Injection of estradiol (and P4) or GnRH in the melengestrol acetate (MGA)-based TAI protocol for beef cattle is followed by emergence of a new follicular wave. After injection of $PGF_{2\alpha}$ and removal of the MGA, the second injection of estradiol or GnRH results in acceptable pregnancy rates following TAI, although synchronous ovulation of the grown dominant follicles has not been demonstrated via ultrasonography [27]. We speculated that treatment with either estradiol benzoate (EB) or GnRH in a controlled internal drug release (CIDR)-based TAI protocol might induce timely ovulation of the preovulatory follicles following emergence of a new follicle under fine endocrine regulation during treatment, which might contribute to improved fertility in repeat breeder dairy cows. Therefore, we evaluated the effects of two types of CIDR-based TAI protocol, i.e., EB/P4+CIDR-$PGF_{2\alpha}$-EB and GnRH+CIDR-$PGF_{2\alpha}$-GnRH, on follicular wave emergence, follicular development, synchronous ovulation, serum P4 concentrations, and pregnancy rate in repeat breeder dairy cows.

Materials and Methods

Animals

This study was performed at the National Livestock Research Institute (Cheonan City, Chungnam, Korea). Fifty-five non-lactating repeat breeder Holstein-Friesian cows from the same herd were used for this study. The animals had no gross abnormalities of the reproductive tract as determined by rectal palpation and transvaginal ultrasonography (Sonoace 600 with a 7.5 MHz linear array transducer; Medison, Seoul, Korea). These repeat breeder cows had normal estrous cycles but failed to conceive after repeated inseminations (more than three times). The number of lactations, body condition score (BCS, point scale from 1 to 5; Edmonson *et al.* [28]), postpartum interval, and number of previous inseminations (mean ± SEM) of the selected repeat breeders were 1.8 ± 0.1, 4.4 ± 0.1, 761.9 ± 90.7 and 7.2 ± 0.1, respectively. Therefore, the experimental cows in this study had a highly extended postpartum interval. All experiments were performed with the approval of the Animal Ethics Committee at the College of Veterinary Medicine, Chungbuk National University (Cheongju, Chungbuk, Korea).

First trial of the experiment

In the first trial of the experiment (December 2005 to February 2006), 55 repeat breeder cows were allocated to one of two treatment groups with balancing by lactation number and postpartum interval. (1) Treatment for the EB group consisted of insertion of a CIDR device containing 1.9 g of P4 (CIDR™; InterAg, Hamilton, New Zealand) with an injection of 1 mg EB (SY Esrone; Samyang, Seoul, Korea) plus 50 mg P4 (SY Ovaron; Samyang; Day 0), an injection of $PGF_{2\alpha}$ (Lutalyse; Pharmacia & Upjohn, Puurs, Belgium) and removal of the device on Day 7, an injection of 1 mg EB on Day 8, and TAI 30 h later (n=27). (2) Treatment for the GnRH group consisted of insertion of a CIDR device with an injection of 250 μg gonadorelin (GnRH; Fertagyl, Intervet, Holland) (Day 0), an injection of $PGF_{2\alpha}$ and removal of the device on Day 7, an injection of 250 μg GnRH on Day 9, and TAI 17 h later (GnRH group, n=28). All hormone injections were intramuscularly administered. Pregnancy diagnosis was determined at 45 to 60 days after TAI using both ultrasonography and rectal palpation. Pregnancy rate per TAI was defined as the percentage of cows confirmed to be pregnant in a single pregnancy diagnosis after one TAI.

Second trial of the experiment

In the second trial of the experiment (April to June 2006; 31.7 (± 5.6) days after pregnancy diagnosis in the first trial), 41 repeat breeder cows that were confirmed as not pregnant in the first trial were randomly assigned to the same two treatments used in the first trial (an EB group of 20 cows and a GnRH group of 21 cows).

The ovaries of subsets of cows from both groups (15 cows per group) were examined by transrectal ultrasonography in order to observe the changes in ovarian structures [follicles and corpora lutea (CL)]. This was conducted every 24 h from Days 0 to 8 and on Days 10 and 13 for the EB group and every 24 h from Days 0 to 9 and on Days 11 and 14 for the GnRH group. All visible follicles (antral diameter ≥4 mm) and CLs were measured and mapped individually for each cow. Dominant follicle of a wave was defined as the follicle reaching the largest diameter. Emergence of a new follicular wave was identified on the day that the dominant follicle was retrospectively identified to have a diameter of 4 to 5 mm [29]. The ovaries of the cows in the EB and GnRH groups were assessed via ultrasonography on Day 10 (53 h after the second EB injection) and on Day 11 (40 h after the injection of the second GnRH), respectively, to determine whether ovulation had occurred [30, 31]. Ovulation was diagnosed when the preovulatory follicle disappeared and was confirmed by the appearance of a new CL on Day 13 in the EB group and on Day 14 in the GnRH group.

Blood samples were collected from the tail veins of the cows in the above subsets (15 cows per group) 5 min before insertion of the CIDR device (Day 0), 5 min before injection of $PGF_{2\alpha}$ and removal of the device (Day 7), and 24 h (Day 8) and 48 h (Day 9) after the $PGF_{2\alpha}$ injection, respectively, for analysis of serum P4 concentrations. After 2 h at 5 C, the samples were centrifuged at 2,500 × *g* for 10 min, and the sera were collected, immediately frozen, and thereafter kept at –20 C until assay. P4 concentrations were determined by fluoroimmunoassay (1234 Delfia Fluorometer; Wallac, Turku, Finland). The sensitivity of the assay was 0.25 ng/ml. The intra- and inter-assay coefficients of variation for the serum P4 analyses were 8.7, 8.5, 6.7, 8.5, 11.5 and 9.6%, and 4.0, 5.4, 6.6, 6.9, 10.5 and 8.1% for the standard concentrations of 0, 0.31, 1.26, 3.14, 12.6 and 37.7 ng/ml, respectively. Recovery rates ranged between 87 and 97%. The serum P4 concentrations of the cows during spontaneous estrus were between 0.5 and 0.9 ng/ml, as measured by the present method (reference values).

Pregnancy was determined as in the first trial of the experiment.

Statistical analyses

Statistical analyses were performed using SAS [32]. The pregnancy rates following TAI in the first and second trials of the experiment and the combined pregnancy rates were compared between the groups using *Chi*-square analysis. Emergence of a new follicular wave within 7 days and synchronized ovulation following the second EB and GnRH injections were compared between the groups using Fisher's exact test. The interval from initial treatment to follicular wave emergence was analyzed using the Wilcoxon rank-sum test. The mean diameters of dominant follicles on Day 7 and mean serum P4 concentrations on Day 0 and on Days 7 to 9 were compared between the groups using the Student's *t*-test. A probability level of $P<0.05$ was considered significant.

Results

The pregnancy rates following TAI in the first (18.5 vs. 32.1%) and second (40.0 vs. 38.1%) trials of the experiment and the combined pregnancy rates (27.7 vs. 34.7%) did not differ (P>0.05) between the EB and GnRH groups, respectively (Fig. 1). The mean serum P4 concentrations on Day 0 (10.0 ± 2.5 vs. 8.5 ± 1.1 ng/ml), Day 7 (9.1 ± 2.2 vs. 12.9 ± 1.8 ng/ml), Day 8 (3.2 ± 0.8 vs. 2.8 ± 0.4 ng/ml), and Day 9 (2.6 ± 0.6 vs. 2.6 ± 0.6) did not differ between the EB and GnRH groups, respectively (P>0.05).

The proportion of cows with follicular wave emergence within 7 days of the experiment did not differ (P>0.05) between the groups (Table 1). The interval from initial treatment to wave emergence was shorter (P<0.01) in the GnRH group than in the EB group, but there was no difference in the mean diameters of the dominant follicles on Day 7 (P>0.05) between the groups (Table 1). The proportion of cows with synchronized ovulation did not differ (P>0.05) between the EB and GnRH groups (Table 1).

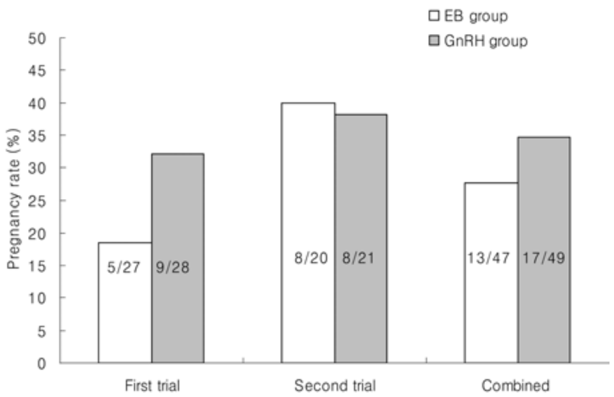

Fig. 1. The pregnancy rates following TAI in the first and second trials of the experiment and the combined rates of the EB and GnRH groups. No significant differences were observed in the pregnancy rates between the groups (P>0.05).

Discussion

This study compared the effects of two types of CIDR-based TAI protocol, the EB/P4+CIDR-$PGF_{2\alpha}$-EB and GnRH+CIDR-$PGF2_{2\alpha}$-GnRH protocols, on follicular dynamics, serum P4 concentrations, and pregnancy rate in repeat breeder dairy cows with highly extended postpartum intervals. Treatment with either EB or GnRH in a CIDR-based TAI protocol results in synchronous follicular wave emergence, timely follicular development, synchronous ovulation following a second EB or GnRH, and similar pregnancy rates for TAI (27.7 vs. 34.7%).

The pregnancy rates in the first and second trials of the experiment and the combined rates did not differ between the EB and GnRH groups, with an overall pregnancy rate of 31.3%. The overall pregnancy rate was slightly higher than the results of Kasimanickam *et al*. [26], who reported a 21.0% pregnancy rate following treatment with the Ovsynch protocol in repeat breeder dairy cows. We believe that fine regulation of folliculogenesis

Table 1. Comparison of new ovarian follicular wave emergence, follicular development and synchronized ovulation in repeat breeder dairy cows treated with two types of CIDR-based TAI protocol

Variables	Treatment group*	
	EB	GnRH
No. of cows with emergence of a new follicular wave	12/15	13/15
Interval (in days) to new follicular wave emergence		
Mean	4.4^{a}	2.6^{b}
SEM	0.2	0.4
Range	3–6	1–5
Diameter of dominant follicle on Day 7 (mm)	9.8 ± 0.5	10.8 ± 0.6
Proportion of cows with synchronized ovulation**	11/15	13/15
Pregnancy rate	6/15	6/15

Values are means ± SEM.
Rows with different superscripts differ significantly. $^{a, b}$, P<0.01.
* EB: CIDR+EB plus P4-$PGF_{2\alpha}$-EB protocol. GnRH: CIDR+GnRH-$PGF_{2\alpha}$-GnRH protocol.
** Synchronized ovulation: ovulation by 53 h after EB injection (Day 10) in the EB group and by 40 h after the GnRH injection (Day 11) in GnRH group.

and synchronous ovulation resulting from the two types of CIDR-based TAI protocol effectively improves the fertility of repeat breeder cows, as demonstrated in the second trial of the present study. However, the overall pregnancy rate in the repeat breeder cows in this study was lower than the pregnancy rate (65.0%) recently reported in normal breeding dairy cows following treatment with the GnRH+CIDR-$PGF_{2\alpha}$-GnRH TAI protocol [33]. The reason for this lower pregnancy rate in repeat breeder cows is unclear, but may be due in part to the suprabasal P4 concentrations during the estrus and periovulatory periods. In the present study, the mean serum P4 concentrations of the EB and GnRH groups 24 h (Day 8) and 48 h (Day 9) after $PGF_{2\alpha}$ injection were at suprabasal levels; however, in normal breeding cows, the mean serum P4 concentrations around the estrus and periovulatory periods following luteolysis during a CIDR-based TAI regimen were at basal levels [33]. Suprabasal P4 concentrations may cause incomplete maturation of preovulatory follicles and subsequent final development in an elevated P4 environment [34]. Båge *et al.* [9] reported that suprabasal P4 levels during estrus in repeat breeders could either be due to incomplete luteolysis or release from sources other than the ovary. The pregnancy rates reported in this study indicate that both types of CIDR-based TAI protocol are effective treatment regimens for repeat breeder dairy cows, particularly in cows with a highly extended postpartum interval. On the other hand, fine regulation of P4 levels during the estrus and periovulatory periods might be necessary to improve fertility in repeat breeder cows.

We compared emergence of a new follicular wave, subsequent follicular development, and synchronized ovulation between the EB and GnRH groups, because we assumed that follicular turnover and timely ovulation might influence the pregnancy rate. Emergence of a new follicular wave occurred within 7 days of the experiment in 12/15 cows in the EB group and in 13/15 cows in the GnRH group in the present study. These findings are similar to those of Diskin *et al.* [35] following treatment with EB plus P4 and Ambrose *et al.* [36] following treatment with GnRH in normal breeding cows. Injection of GnRH induces release of FSH and LH from the pituitary; this results in ovulation and induction of new wave emergence 1–2 days later [35]. On the other hand, administration of EB plus P4 results in atresia of the dominant follicle followed by a new follicular wave at an interval of 4.3 days from treatment [22]. The mean intervals from treatment to a new follicular wave in this study were 4.4 and 2.6 days in the EB and GnRH groups, respectively. These results indicate that insertion of a CIDR and injection of EB plus P4 or GnRH in repeat breeder dairy cows can induce synchronous follicular wave emergence comparable to that observed in normal breeding cows. Despite the longer interval from treatment to a new follicular wave in the EB group compared with the GnRH group, the sizes of the dominant follicles on Day 7 did not differ between the groups. The duration of CIDR treatment was sufficient to allow growth of later emerging dominant follicles in the cows of the EB group such that the follicles were comparable to those in the GnRH group. Synchronous ovulation occurred within 53 h of the second EB injection and within 40 h of the second GnRH injection in the majority of cows in both groups. This is consistent with the results of previous studies [30, 33]. These results, along with the previous observations on follicular turnover and subsequent follicular development, indicate that treatment with EB plus P4 or with GnRH in CIDR-treated, repeat breeder dairy cows induces synchronous follicular wave emergence, subsequent timely follicular development, and following a second injection of EB or GnRH, synchronous ovulation comparable to that observed in normal breeding cows.

In conclusion, treatment with either EB or GnRH in a CIDR-based TAI protocol results in synchronous follicular wave emergence, follicular development, synchronous ovulation after a second EB or GnRH injection, and similar pregnancy rates for TAI in repeat breeder cows. In practice, both types of CIDR-based TAI protocol, EB/P4+CIDR-$PGF_{2\alpha}$-EB and GnRH+CIDR-$PGF_{2\alpha}$-GnRH, effectively improve fertility through fine regulation of folliculogenesis and synchronous ovulation in repeat breeder cows, particularly in cows with a highly extended postpartum interval.

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References

1. **Spalding RW, Everett RW, Foote RH**. Fertility in New York artificial inseminated Holstein herds in dairy herd improvement. *J Dairy Sci* 1975; 58: 718–723.
2. **Emanuelson U, Oltenacu PA**. Incidences and effects of diseases on the performance of Swedish dairy herds stratified by production. *J Dairy Sci* 1998; 81: 2376–2382.
3. **Lee JY, Kim IH**. Advancing parity is associated with high milk production at the cost of body condition and increased periparturient disorders in dairy herds. *J Vet Sci* 2006; 7: 161–166.
4. **Bartlett PC, Kirk JH, Mather EC**. Repeated insemination in Michigan Holstein-Friesian cattle: Incidence, descriptive epidemiology and estimated economic impact. *Theriogenology* 1986; 26: 309–322.
5. **Kimura M, Nakao T, Moriyoshi M, Kawata K**. Luteal phase deficiency as a possible cause of repeat breeding in dairy cows. *Br Vet J* 1987; 143: 560–566.
6. **Pursley JR, Silcox RW, Wiltbank MC**. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *J Dairy Sci* 1998; 81: 2139–2144.
7. **Hawk H, Brinsfield T, Turner G, Whitmore GE, Norcross MA**. Embryo survival in first-service and repeat-breeder cattle after ovariectomy and hormone therapy. *J Dairy Sci* 1963; 46: 1397–1401.
8. **Gustafsson H, Larsson K, Kindahl H, Madej A**. Sequential endocrine changes and behaviour during estrus and metoestrus in repeat breeder and virgin estrus and metoestrus heifers. *Anim Reprod Sci* 1986; 10: 261–273.
9. **Båge R, Gustafsson H, Larsson B, Forsberg M, Rodríguez-Martínez H**. Repeat breeding in dairy heifers: follicular dynamics and estrous cycle characteristics in relation to sexual hormone patterns. *Theriogenology* 2002; 57: 2257–2269.
10. **O'Farell KJ, Langley OH, Hartigan PJ, Sreenan JM**. Fertilization and embryonic survival rates in dairy cows culled as repeat breeders. *Vet Rec* 1983; 112: 95–97.
11. **Villarroel A, Martino A, BonDurant RH, Dèletang F, Sischo WM**. Effect of post-insemination supplementation with PRID on pregnancy in repeat-breeder Holstein cows. *Theriogenology* 2004; 61: 1513–1520.
12. **Katagiri S, Takahashi Y**. Changes in EGF concentrations during estrous cycle in bovine endometrium and their alterations in repeat breeder cows. *Theriogenology* 2004; 62: 103–112.
13. **Heuweiser W, Oltenacu PA, Lednor AJ, Foote RH**. Evaluation of different protocols for prostaglandin synchronization to improve reproductive performance in dairy herds with low estrus detection efficiency. *J Dairy Sci* 1997; 80: 2766–2774.
14. **Santos JEP, Thatcher WW, Chebel RC, Cerri RLA, Galvao KN**. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim Reprod Sci* 2004; 82/83: 513–535.
15. **Swanson LV, Young AJ**. Failure of gonadotropin-releasing hormone or human chorionic gonadotropin to enhance the fertility of repeat-breeder cows when administered at the time of insemination. *Theriogenology* 1990; 34: 955–963.
16. **BonDurant RH, Revah I, Franti C, Harman RJ, Hird D, Klingborg D, McCloskey M, Weaver L, Wilgenberg B**. Effect of gonadotropin-releasing hormone on fertility in repeat-breeder California dairy cows. *Theriogenology* 1991; 35: 365–374.
17. **Archbald LF, Sumrall DP, Tran T, Klapstein E, Risco C, Chavatte P**. Comparison of pregnancy rates of repeat-breeder dairy cows given gonadotropin releasing hormone at or prior to the time of insemination. *Theriogenology* 1993; 39: 1081–1091.
18. **Singh B, Saravia F, Bage R, Rodriguez-Martinez H**. Pregnancy rates in repeat-breeder heifers following multiple artificial inseminations during spontaneous oestrus. *Acta Vet Scand* 2005; 46: 1–12.
19. **Selvaraju S, Agarwal SK, Karche SD, Srivastava SK, Majumdar AC, Shanker U**. Fertility responses and hormonal profiles in repeat breeding cows treated with insulin. *Anim Reprod Sci* 2002; 73: 141–149.
20. **Pursley JR, Mee MO, Wiltbank MC**. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. *Theriogenology* 1995; 44: 915–923.
21. **Bartolome JA, Archbald LF, Morresey P, Hernandez J, Tran T, Kelbert D, Long K, Risco CA, Thatcher WW**. Comparison of synchronization of ovulation and induction of estrus as therapeutic strategies for bovine ovarian cysts in the dairy cow. *Theriogenology* 2000; 53: 815–825.
22. **Bo GA, Adams GP, Pierson RA, Mapletoft RJ**. Exogenous control of follicular wave emergence in cattle. *Theriogenology* 1995; 43: 31–40.
23. **Kim IH, Suh GH, Kim UH, Kang HG**. A CIDR-based timed AI protocol can be effectively used for dairy cows with follicular cysts. *Anim Reprod Sci* 2006; 95: 206–213.
24. **Ahuja C, Montiel F, Canseco R, Silva E, Mapes G**. Pregnancy rate following GnRH + $PGF_{2\alpha}$ treatment of low body condition, anestrous *Bos Taurus* by *Bos indicus* crossbred cows during the summer months in a tropical environment. *Anim Reprod Sci* 2005; 87: 203–213.
25. **Yamada K, Nakao T, Mihara N**. Synchronization of ovulation and fixed-time insemination for improvement of conception rate in dairy herds with

poor estrus detection efficiency. *J Reprod Dev* 1999; 45: 51–55.

26. **Kasimanickam R, Cornwell JM, Nebel RL**. Fertility following fixed-time AI or insemination at observed estrus in Ovsynch and Heatsynch programs in lactating dairy cows. *Theriogenology* 2005; 63: 2550–2559.
27. **Martinez MF, Kastelic JP, Adams GP, Mapletoft RJ**. The use of GnRH or estradiol to facilitate fixed-time insemination in an MGA-based synchronization regimen in beef cattle. *Anim Reprod Sci* 2001; 67: 221–229.
28. **Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G**. A body condition scoring chart for Holstein dairy cows. *J Dairy Sci* 1989; 72: 68–78.
29. **Ginther OJ, Knopf L, Kastelic JP**. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *J Reprod Fertil* 1989; 87: 223–230.
30. **Cartmill JA, El-Zarkouny SZ, Hensley BA, Lamb GC, Stevenson JS**. Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. *J Dairy Sci* 2001; 84: 1051–1059.
31. **Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE**. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_{2\alpha}$ for ovulation control in postpartum suckled beef cows. *J Anim Sci* 2001; 79: 2253–2259.
32. SAS. SAS System, Release 8.1, SAS Inst Inc, Cary, NC, USA 1999.
33. **Kim UH, Suh GH, Nam HW, Kang HG, Kim IH**. Follicular wave emergence, luteal function and synchrony of ovulation following GnRH or estradiol benzoate in a CIDR-treated, lactating Holstein cows. *Theriogenology* 2005; 63: 260–268.
34. **Moreira F, Risco CA, Pires MFA, Ambrose JD, Drost M, Thatcher WW**. Use of bovine somatotropin in lactating dairy cows receiving timed artificial insemination. *J Dairy Sci* 2000; 83: 1237–1247.
35. **Diskin MG, Austin EJ, Roche JF**. Exogenous hormonal manipulation of ovarian activity in cattle. *Domest Anim Endocrinol* 2002; 23: 211–228.
36. **Ambrose DJ, Schmitt EJ-P, Lopes FL, Mattos RC, Thatcher WW**. Ovarian and endocrine responses associated with the treatment of cystic ovarian follicles in dairy cows with gonadotropin releasing hormone and prostaglandin $F_{2\alpha}$, with or without exogenous progesterone. *Can Vet J* 2004; 45: 931–937.