한국임상수의학회지 15(1) : 131-139 (1998)
Korean J. Vet Clin Med. 15(1) : 131-139 (1998)

Development of Differential Diagnosis and Treatment Method of Reproductive Disorders Using Ultrasonography in Cows I. Response of Ovarian Structures to CIDR Treatment at Day 16 of Estrous Cycle in Dairy Heifers

Hyun-gu Kang[1], Toshihiko Nakao*, Byong-kyu Kang, Han-sun Choi, Guk-hyun Suh and Chang-ho Son*****

*College of Veterinary Medicine, ***Hormone Research Center, Chonnam National University, Kwangju 500-757, Korea*
**Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-0836, Japan*
***National Livestock Research Center, Chonan 330-800, Korea*

초음파검사에 의한 소의 번식장애 감별진단 및 치료법 개발 I. 처녀젖소에서 발정주기의 16일째에 CIDR의 치료에 대한 난소구조물의 반응

강현구[1] · 中尾敏彦* · 강병규 · 최한선 · 서국현** · 손창호***
전남대학교 수의과대학, *일본 酪農學園大學 獸醫學科
**축산기술연구소, ***전남대학교 호르몬연구센터

요 약 : Progesterone을 함유하고 있는 CIDR(Controlled Internal Drug Release)의 질내삽입은 황체기를 인위적으로 연장시킬 수 있다. CIDR의 삽입이, 삽입시 존재했던 우세난포(dominant follicle)의 반응과 난포의 발육반응 그리고 2회 또는 3회의 난포주기를 가지고 있는 처녀우에서 CIDR의 삽입기간동안 난포의 성장 및 발육에 어떠한 영향을 미치는가를 비교검토하기 위하여 배란후 16일째의 처녀우 4마리에 7일동안 CIDR를 삽입하였다. CIDR의 삽입은 발정의 발현을 억제시켰으며 그리고 발정주기의 길이를 정상 발정주기보다 유의성있게 연장시켰다(26.3±0.5 vs 20.8±1.5일, P<0.05). CIDR의 삽입시 혈장 progesterone 농도는 3.6±2.7 ng/ml 이었으며, 17일과 23일 사이에는 2.1~4.4 ng/ml(3.6±1.2 ng/ml) 사이를 유지했다. 혈장 estradiol-17β의 농도는 난포의 발육 및 배란전 배란난포의 성숙을 나타내는 특징적인 변화양상을 나타내었다. 4마리의 처녀우중 2마리는 CIDR 삽입전 발정주기당 2회의 난포주기를 가진 반면, 나머지 2마리는 주기당 3회의 난포주기를 가졌다. 그렇지만 CIDR의 삽입기간동안 모든 처녀우는 주기당 3회의 발정주기를 가졌다. CIDR의 삽입전 발정주기당 3회의 난포주기를 갖는 처녀우에서 CIDR의 삽입은 세 번째 난포주기에서 배란성 우세난포의 우세기(dominant phase)를 연장시켰다. 3회의 난포주기를 갖는 2마리에서 CIDR의 삽입후 배란난포는 존속시간과 우세기가 유의성있게 연장되었다. CIDR의 삽입전 발정주기당 2회의 난포주기를 갖는 다른 2마리의 처녀우에서 CIDR의 삽입후 우세난포는 곧바로 퇴행되었고 새로운 난포주기를 형성하였으며, 우세난포의 우세기와 배란난포의 존속기간을 연장시키지 않았다. CIDR의 삽입은 CIDR의 삽입후 이어지는 발정주기동안 난포의 발육 및 성장에 영향을 미치지 않았으며 발정주기의 길이, 난포주기, 혈장 progesterone 및 estradiol-17β 농도에 영향을

본 연구는 1997년도 농림수산기술개발사업(현장애로)과 1998년도 한국과학재단 호르몬연구센터 연구비(과제번호: HRC-98-0301)의 일부에 의하여 수행되었음

[1]Corresponding author.

미치지 않았다. 결과적으로 황체기 후반부에 CIDR의 삽입은 CIDR 삽입전 발정주기동안 3회의 난포주기를 갖는 처녀우에서 배란성 우세난포의 발육과 배란까지의 기간을 연장시켰고 2회 난포주기를 갖는 처녀우에서는 우세난포를 곧바로 퇴행시킨후, 새로운 난포주기를 형성하였다.

Key words : CIDR, dominant follicle, progesterone, estradiol-17β, heifer

Introduction

Estrus can be synchronized in cattle either by shortening luteal phase with prostaglandin $F_2\alpha$ ($PGF_2\alpha$) or by extending luteal phase with progestogens[2,7,8,14]. An intravaginal administration of progesterone, CIDR (Controlled Internal Drug Release) for example, causes elevation of plasma progesterone concentrations which are maintained above 2 ng/ml during periods of CIDR treatment in ovariectomised cows[4,5,9]. Maturation of a dominant follicle (DF), growth of other follicle, exhibition of estrous signs and ovulation are suppressed during a period of the progesterone treatment. After removal of the device, plasma progesterone levels declined rapidly, the DF matures, produces estrogens to exhibit estrous signs and ovulates, provided that the corpus luteum had already regressed[17,18].

Extension of a period of treatment with CIDR often results in a low fertility at estrus induced after the CIDR removal[10,15]. It has been assumed that a subluteal phase progesterone level achieved by CIDR treatment after spontaneous luteolysis causes persistence of DF and cessation of turnover of follicular waves[17,19]. Meantime, the ovum in DF undergoes an aging process, thus decreasing fertilization capability[17,18].

A currently recommended period of CIDR treatment is 7 days, often combined with $PGF_2\alpha$ injection before or at the time of CIDR removal[3,4]. However, if CIDR is inserted into the vagina for 7 days starting at late-luteal phase when DF of preceding follicular wave starts to undergo atresia and a new follicular wave emerges, the treatment may interfere with atresia of the preceding DF and growth and maturation of a new DF. This has not well been demonstrated. It also remains to be clarified if there is any difference in effect of CIDR on follicular growth between heifers with two or three follicular waves.

Therefore, objectives of the present study are: 1) to show the effect of CIDR on turnover of DF and follicular growth after CIDR insertion on day 16 of estrous cycle and 2) to compare the follicular growth during CIDR treatment between heifers with two or three follicular waves.

Materials and Methods

Animals and experimental design

Four Holstein-Friesian heifers (2 to 3 years old) raised at the Department of Veterinary Obstetrics and Gynecology, Rakuno Gakuen University, were used for this study. The heifers had normal and regular estrous cycles. They were fed hay, concentrate ration and mineral supplement and housed in stanchions during the study and checked for signs of estrus. When they exhibited estrus, ultrasonographic examination was performed every day for detection of ovulation.

The experiment was initiated on day 0 of the estrous cycle. No treatment was given during the first estrous cycle in order to find out follicular wave patterns of each heifer (stage 1). Then on day 16 of the subsequent cycle, silastic devices containing progesterone (called Controlled Internal Drug Release type B or CIDR B, AHI Plastic Moulding Company, Hamilton, New Zealand) were inserted intravaginally for 7 days to examine effects of the treatment on the follicular waves (stage 2). The observation was continued during the estrous cycle following the treated cycle to investigate whether the CIDR treatment affected follicular growth during the following cycle (stage 3).

Ultrasonography of the ovary

Transrectal ultrasound examinations of the ovaries were conducted. The ovaries were monitored with a real-time, B-mode ultrasound scanner equipped with

a 5.0 linear type or 7.5 MHz convex type intrarectal transducer (Hitachi Medical Corporation, EUB-310, Japan). Patterns of growth and regression of individual follicles ≥5 mm were observed using techniques previously described[13]. The transducer was moved intrarectally along the dorsal surface of the reproductive tract, then bilaterally to examine each ovary, as described by Pierson *et al*[12]. Each ovary was scanned several times in at least two planes to assess the maximum diameter of the follicle and corpus luteum. The day of ovulation (Day 0) was determined by the disappearance of the largest follicle that had been present on the day before. Cross section of the follicle and corpus luteum were visualized by real-time imaging, and the sonograms were stored on black-and-white polaroid film (EZU-VP2) used with a Mitsubishi Video Printer. Diameters of nonspherical structures were obtained by averaging length and width.

Blood collection and hormone assays

Blood was collected from the coccygeal or jugular vein daily throughout the study period. Samples were collected into heparinized tubes and immediately centrifuged at 3000 rpm for 15 min. The plasma was transferred to another test tube and stored at -20 °C until analyzed for progesterone and estradiol-17β concentrations.

Plasma progesterone concentrations were determined by an enzyme immunoassay on microtiter plates coated with second antibody. Microtiter plates were coated with affinity-purified goat IgG antirabbit IgG. The assay used antiserum raised in rabbits against progesterone-3(E)-CMO-BSA. Progesterone-3(F)-CMO -HRP was used as tracer. The microtiter plates, antigen and antigen-enzyme conjugate were obtained from Kambegawa Research Institute, Tokyo. Borate-buffered solution (0.05 M, pH 7.8) with 0.2% bovine serum albumin (w/v) was used as assay buffer. Briefly, 500 μl of plasma were transferred into a 13× 100 mm tube, and extracted with 2.5 ml of diethyl ether (recovery rate=68.2±6.7%). Tubes were mixed for 5 min and diethyl ether was decanted into a new tube and evaporated at 50°C in water bath. The residue was redissolved in 500 μl of assay buffer and duplicates of 50 μl were analyzed using an enzyme immunoassay for progesterone. Intra-assay and inter-assay coefficients of variation and minimum detection values of the assay were 9.2%, 17.3% and 0.08 ng/ml, respectively.

Plasma estradiol-17β concentrations were determined by radioimmunoassay, with the following modifications: 1) 1000 μl aliquots of plasma were extracted before assay with 3 ml diethyl ether (recovery rate=78.3±5.4%); 2) water solvable layer was frozened at -50°C; 3) diethyl ether was decanted to the tube for assay; 4) dried up diethyl ether at 50°C in water bath; 5) added 0.5 ml of acetonitrile and 1 ml of n-hexane and mixed for 5 min; 6) centrifuged at 1700×g for 5 min; 7) aspirated n-hexane (the upper layer); 7) put 2 ml of n-hexane on the acetonitrile layer and aspirated n-hexane (repeated 2 times); 8) dried up acetonitrile at 50°C with dry N_2 gas; 9) the volume of extracted plasma used in the assay was 100 (l/tube); 10) estradiol standards (0.3125~320 pg) and assay diluents (1% BSA-0.01 M PBS) used were prepared in our laboratory; and 11) 100 μl estradiol antiserum and 100 μl [^{125}I] estradiol were used per assay tube. Reagents included: 1) 1st Ab sheep A/E_2-17β serum #244 (gifted by Dr. Niswender); 2) ^{125}I-E_2 IM-135 (purchased from Amasham); and 3) 2nd Ab A/sGG Donkey.

Terminology

In all stages, the length of cycle was defined as the interval of time (days) between two successive periods of ovulation. Persistence of a follicle on the ovary was defined as the interval of time elapsed between its appearance and its disappearance as a follicle ≥5 mm. The beginning of a plateau was defined as the first day a follicle did not increase more than 1 mm in diameter in the subsequent 48-h period. The relative diameter of an ovulatory follicle at the time when progesterone first became less than 1 ng/ml was calculated as the ratio of its diameter at that time to its diameter on the day of estrus. The functional dominance phase of a follicle was calculated as the interval of time between the first day a follicle became 2 mm greater than the other follicles in the same wave, and either the day of ap-

pearance of a new follicular wave (for the DF of the first wave), or the day preceding ovulation (for ovulatory follicles).

Statistical analysis

One-way analysis of variance was used for comparisons between more than two means, and when a significant difference was found, a Duncan's multiple range test was used to determine which means were significantly different. Student's t-tests were used for comparisons between two means. Repeated measures analysis of variance using the General Linear Model procedure of Statistical Analysis System[16] was used to compare plasma concentrations of estradiol -17β and progesterone, and to compare effects of treatments on basal levels of estradiol and within groups.

Results

Effects of CIDR on cycle length and plasma progesterone and estradiol-17β concentrations

The three of four heifers in stage 1 had the normal estrous cycle, including progesterone concentration, time of luteolysis, cycle length, and ovulation (Fig. 1A and 1C, 2A and 2C, 3A and 3C and Table 1). The one of four heifers in stage 1 had corpus luteum with large cavity after ovulation and the corpus luteum was ruptured during ultrasonographic examination on 3 day of estrous cycle. The heifer had small corpus luteum after rupture and plasma progesterone concentrations were maintained low levels (0.2~1.7 ng/ml) (Fig. 4A). The presence of CIDR in the vagina containing progesterone prevented for the animals to return to estrus at the normal time and significantly increased cycle length (stage 2), as compared to the preceding cycle (26.3± 0.5 vs 20.8±1.5 days, $P<0.05$). At the time of CIDR insertion (day 16), plasma progesterone concentrations were 3.6±2.7 ng/ml. Between day 17, first day after CIDR treatment, and day 23, when the CIDR was removed, progesterone concentrations ranged between 2.1 and 4.4 ng/ml (3.6±1.2). Plasma estradiol concentrations showed typical patterns reflecting follicular growth and preovulatory matura-

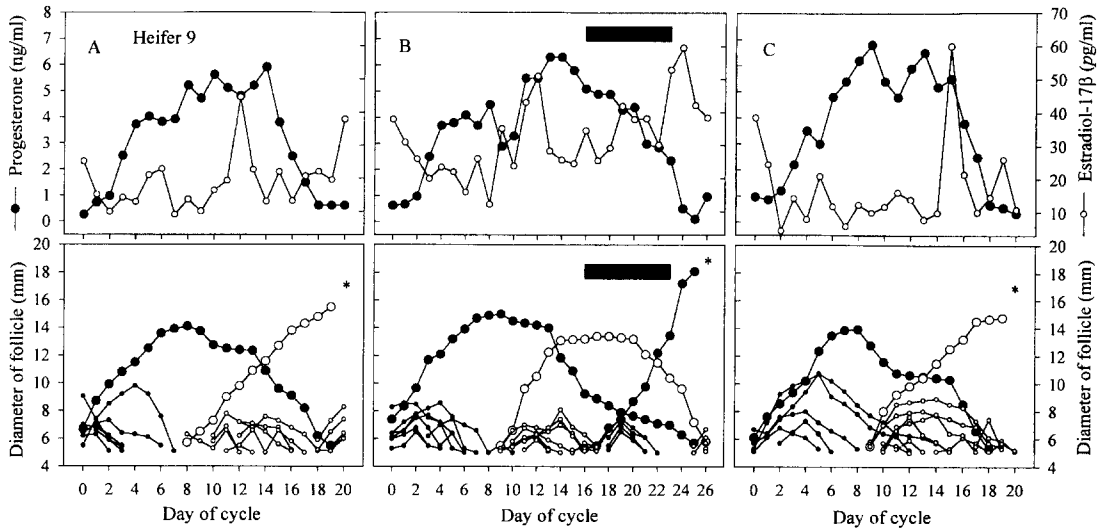

Fig 1. Patterns of follicular development and concentrations of hormones in heifer with two follicular waves per cycle before and after CIDR treatment. The upper graph in each panel describes variations in estradiol-17β (hollowed circle) and progesterone (black circle) concentrations. The character in the upper left corner of each graph indicates each stages. A is estrous cycle before CIDR treatment, B is cycle at CIDR treatment and C is cycle after CIDR treatment. The lower graph in each panel shows patterns of growth and regression of individual follicles (each hollowed and black circle represents an individual follicle). Asterisks indicate the day on which the ovulatory follicle disappeared and black rectangles the interval of time during which the CIDR was present. Follicular diameter represents only the size of the antrum.

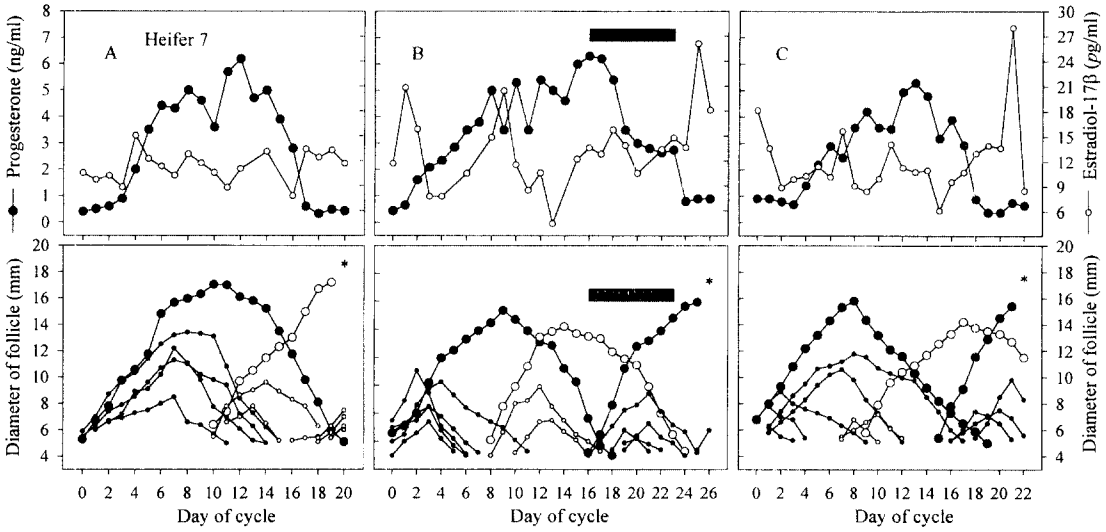

Fig 2. Patterns of follicular development and concentrations of hormones in heifer with two follicular waves per cycle before CIDR treatment had three follicular waves per subsequent cycle after CIDR treatment. The upper graph in each panel describes variations in estradiol-17β (hollowed circle) and progesterone (black circle) concentrations. The character in the upper left corner of each graph indicates each stages. A is estrous cycle before CIDR treatment, B is cycle at CIDR treatment and C is cycle after CIDR treatment. The lower graph in each panel shows patterns of growth and regression of individual follicles (each hollowed and black circle represents an individual follicle). Asterisks indicate the day on which the ovulatory follicle disappeared and black rectangles the interval of time during which the CIDR was present. Follicular diameter represents only the size of the antrum.

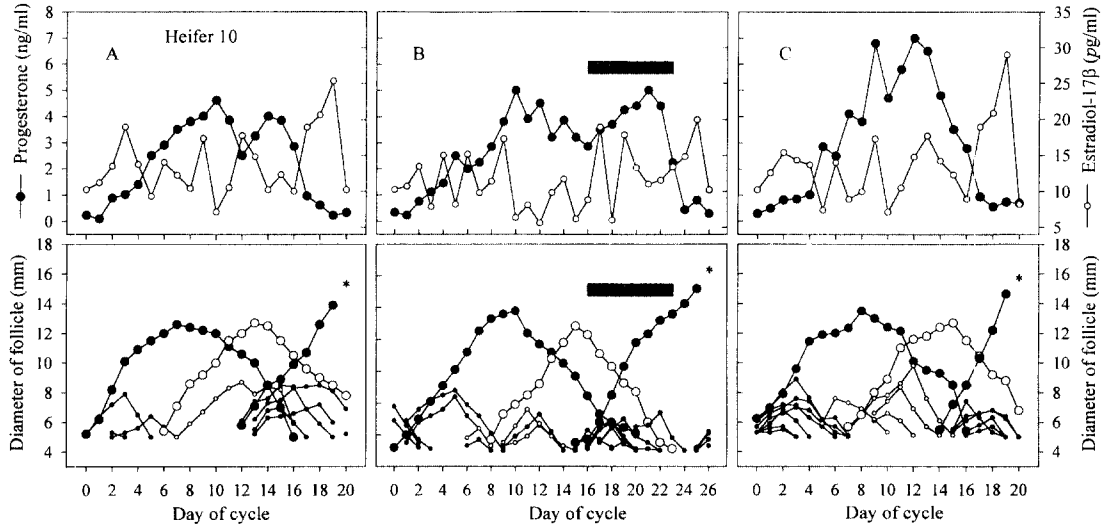

Fig 3. Patterns of follicular development and concentrations of hormones in heifer with two follicular waves per cycle before and after CIDR treatment. The upper graph in each panel describes variations in estradiol-17β (hollowed circle) and progesterone (black circle) concentrations. The character in the upper left corner of each graph indicates each stages. A is estrous cycle before CIDR treatment, B is cycle at CIDR treatment and C is cycle after CIDR treatment. The lower graph in each panel shows patterns of growth and regression of individual follicles (each hollowed and black circle represents an individual follicle). Asterisks indicate the day on which the ovulatory follicle disappeared and black rectangles the interval of time during which the CIDR was present. Follicular diameter represents only the size of the antrum.

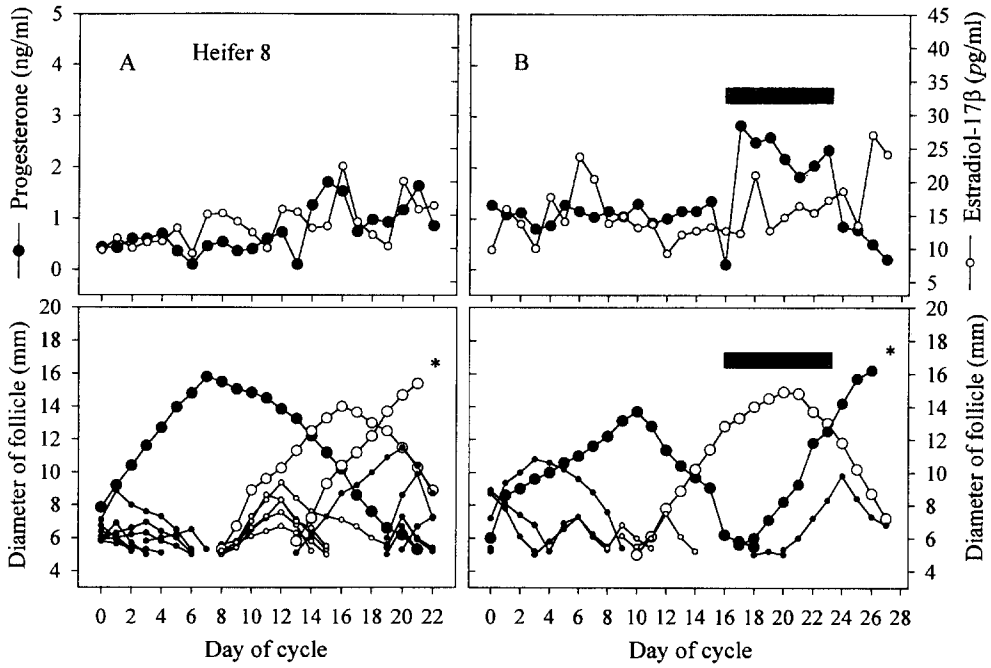

Fig 4. Patterns of follicular development and concentrations of hormones in heifer with three follicular waves per cycle before CIDR treatment. The upper graph in each panel describes variations in estradiol-17β (hollowed circle) and progesterone (black circle) concentrations. The character in the upper left corner of each graph indicates each stages. A is estrous cycle before CIDR treatment and B is cycle at CIDR treatment. The lower graph in each panel shows patterns of growth and regression of individual follicles (each hollowed and black circle represents an individual follicle). Asterisks indicate the day on which the ovulatory follicle disappeared and black rectangles the interval of time during which the CIDR was present. Follicular diameter represents only the size of the antrum.

Table 1. Estrous cycle length, characteristics of the ovulatory follicle according to the number of follicular waves in heifers treated with intravaginal progesterone releasing device (CIDR)

	Stage 1		Stage 2	
	2 waves	3 waves	2 waves*	3 waves*
No. of heifer	2	2	2	2
No. Follicular waves/cycle	2.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0
Cycle length (days)	20.0±0.0[a]	21.0±1.4[a]	26.0±0.0[b]	26.5±0.7[b]
Follicular wave with ovulatory follicle				
No. of follicles ≥5 mm/wave	6.5±2.1[a]	6.0±1.4[a]	5.5±0.7[a]	8.0±1.4[b]
Dominant ovulatory follicle				
Appearance (day)	9.0±1.4[a]	12.5±0.7[b]	16.0±0.0[c]	16.0±1.4[c]
Maximum size (mm)	16.4±1.2[a]	14.7±1.1[b]	17.1±1.5[c]	15.7±0.7[a]
Interval from emergence to ovulation (days)	11.0±1.4[a]	8.0±1.4[b]	9.5±0.7[b]	10.5±0.7[a]
Dominance phase (days)	7.0±1.4[a]	4.0±1.4[b]	5.5±0.7[b]	7.5±0.7[a]
Present as the largest follicle (days)	2.5±0.7[a]	2.0±0.0[b]	3.0±0.0[a]	3.0±0.0[a]

*Follicular waves per cycle before CIDR treatment

[a,b,c]Data with different superscripts within the same row differ significantly (P<0.05; mean±SD)

tion (Fig. 1B, 2B, 3B and 4B). Treatment of CIDR did not affect cycle length, ovulation and plasma progesterone profile in subsequent estrous cycle (Fig. 1C, 2C and 3C).

Effects of CIDR on ovarian follicular dynamics

Ovarian follicular dynamics were characterized by waves of follicular development occurring at different times during the estrous cycle. Each wave generally consisted of the development of one larger follicle and a variable number of follicles (Fig. 1, 2, 3 and 4). In the stage 1, two of four heifers had two follicular waves per estrous cycle (Fig. 1A, 2A and Table 1), whereas two of four heifers had three follicular waves per cycle (Fig. 3A, 4A and Table 1). However, during the cycle of CIDR treatment, all heifers had three follicular waves per cycle (Fig. 1B, 2B, 3B and 4B). In heifer with three follicular waves during estrous cycle before CIDR treatment, treatment with CIDR on day 16 did not affect turnover of DF of preceding follicular wave. However, the CIDR treatment caused an extension of dominant phase of the ovulatory DF selected in the third follicular wave in heifer with three follicular waves per cycle before CIDR treatment. The ovulatory follicle of the two heifers with three follicular waves had a significantly longer persistence and dominant phase after CIDR treatment (Table 1). Whereas two heifers with two follicular waves per cycle before CIDR treatment, after CIDR treatment the DF underwent atresia without delay and followed emergence of the new follicular wave (Fig. 1B and 2B), in which no extension of dominant phase of the DF or ovulatory follicle was shown. The CIDR treatment did not affect the follicular growth during the subsequent cycle (Fig. 1C, 2C and 3C). Estrous cycle length, follicular waves and plasma concentrations of progesterone and estradiol-17β in the following cycle were not altered.

Discussion

The insertion of CIDR into the vagina, extends luteal phase and increases cycle length[4,6,17]. In the present study, four heifers were treated with CIDR at day 16 of the estrous cycle when DF of preceding follicular wave starts to undergo atresia and new follicular wave emerges. The CIDR treatment caused an extension of the length of estrous cycle. The estrous cycle length in the CIDR-treated cycles was longer than in control cycles (26.1±0.8 vs 20.5±1.4 days).

It has already been reported that cattle maintains high plasma progesterone concentrations about 4 days after the CIDR insertion followed by low or subluteal phase progesterone levels[5,6,11]. High plasma progesterone concentrations reduce LH pulse frequencies and as a consequence, growth of DF ceases and DF undergoes atresia [8, 9]. This is followed by an emergence of a new follicular wave. Subluteal phase levels of progesterone, not enough to reduce the LH pulse frequencies to cause atresia of DF, but enough to surpress maturation of DF, LH surge and ovulation are often seen 4 to 5 days after the CIDR treatment and thereafter[1].

In this study heifers were given CIDR at late luteal phase on 16 day of estrous cycle and low progesterone levels were maintained between 20~23 days of the cycle in general. The treatment resulted in an extension of the interval from emergence to ovulation of DF and dominance phase in heifers with three follicular wave pattern before CIDR treatment. On the other hand in heifers with two follicular waves per cycle, after CIDR treatment the DF underwent atresia and followed emergence of the new follicular wave. It was also shown that the CIDR treatment did not affect follicular growth and ovulation in the following estrous cycle.

Under field conditions, CIDR can be inserted in to the vagina at any stage of the ovarian cycle. There could be always some cows treated with CIDR at late-luteal phase. Subluteal phase level or low level of plasma progesterone achieved by CIDR after luteolysis may extend an interval from emergence to ovulation of DF even when CIDR was removed after 7 days of treatment. Extended period of CIDR treatment over 7 days which may further delay ovulation should be avoided to improve the conception rate of the induced estrus.

In conclusion, CIDR treatment may cause an extension of an interval from emergence to ovulation of ovulatory DF in heifer with three follicular waves. Since only four animals were used for this experiment, it is hard to make any conclusion. Heifers with two follicular waves generally have longer in-

terval between emergence and ovulation of DF during estrous cycle than those with three follicular waves. Theoretically, animals with 3 follicular waves might have DF in more advanced stage of its growth than those with the 2 follicular waves at the time of CIDR insertion[4,8]. This could partly explain the different response of the growth of DF to CIDR between two and three follicular wave heifers. In this study, it was not known whether or not an extension of the dominant phase of ovulatory follicles by 3.5 days in heifers treated with CIDR on 16 days of estrous cycle adversely affected fertility. This remains to be clarified by investigating conception rate of the induced estrus after CIDR removal[4,18]. Cows, in general, are considered to have three follicular waves, white heifers tends to have the two waves. It is, therefore, of worth describing possible difference in follicular growth during CIDR treatment between two and three follicular wave animals. Results of the present experiment also indicates that the CIDR treatment does not delay atresia process of DF in preceding cycle if the treatment commenced a late-luteal phase in heifers with two follicular waves per cycle before CIDR treatment.

Conclusion

The luteal phase is able to be artificially lengthened with an intravaginal progesterone-releasing device (CIDR). To show the effect of CIDR on turnover of DF and follicular growth after CIDR insertion and to compare the follicular growth during CIDR treatment between heifers with two or three follicular waves during the preceding cycle, four heifers were given CIDR for 7 days at late luteal phase on 16 day of estrous cycle.

The presence of CIDR in the vagina containing progesterone prevented for the animals to return to estrus at the normal time and significantly increased cycle length, as compared to the preceding cycle (26.3 $\pm$0.5 vs 20.8$\pm$1.5 days, $P<0.05$). At the time of CIDR insertion (day 16), plasma progesterone concentrations were 3.6$\pm$2.7 ng/ml. Between day 17 and 23, progesterone concentrations ranged between 2.1 and 4.4 ng/ml (3.6$\pm$1.2 ng/ml). Plasma estradiol-17β concentrations showed typical patterns reflecting follicular growth and preovulatory maturation.

The two of four heifers had two follicular waves per estrous cycle, whereas other two heifers had three follicular waves. However, during the cycle of CIDR treatment, all heifers had three follicular waves per cycle. The CIDR treatment caused an extension of dominant phase of the ovulatory DF selected in the third follicular wave in heifer with 3 follicular waves before CIDR treatment. The ovulatory follicle of the two heifers with three follicular waves had a significantly longer persistence and dominant phase after CIDR treatment. In other two heifers with two follicular waves per cycle before CIDR treatment, after CIDR treatment the DF underwent atresia without delay and followed emergence of the new follicular wave, in which no extension of dominant phase of the DF or ovulatory follicle was shown. The CIDR treatment did not affect the follicular growth during the subsequent cycle. Estrous cycle length, follicular waves, and plasma concentrations of progesterone and estradiol-17β in the following cycle were not altered.

In conclusion, when CIDR treatment commenced a late luteal phase, CIDR may cause an extension of an interval from emergence to ovulation of ovulatory DF in heifer with three follicular waves. And also, the CIDR treatment does not delay atresia process of DF in preceding cycle and is developed the new follicular waves in heifers with two follicular waves per cycle before CIDR treatment.

References

1. Beal WE. Application of knowledge about corpus luteum function in control of estrus and ovulation in cattle. Theriogenology 1996; 45: 1399-1411.
2. Folman Y, Kaim M, Herz Z, Rosenberg M. Reproductive management of dairy cattle based on synchronization of estrous cycles. J Dairy Sci 1984; 67: 153-160.
3. Kastelic JP, Knopf L, Ginther OJ. Effect of day of prostaglandin $F_2\alpha$ treatment on selection and development of the ovulatory follicle in heifers. Anim Reprod Sci 1990; 23: 169-180.
4. Macmilan KL, Peterson AJ. A new intravaginal progesterone releasing device for cattle (CIDR-B)

for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anestrous. Anim Reprod Sci 1993; 33: 1-25.
5. Macmilan KL, Taufa VK, Barnes DR, Day AM. Plasma progesterone concentrations in heifers and cows treated with a new intravaginal device. Anim Reprod Sci 1991; 26: 25-40.
6. Macmillan KL, Washburn SP, Henderson HV, Petch SF. Effects of varying the progesterone content of CIDR intravaginal devices and multiple CIDR treatments on plasma hormone concentrations and residual hormone content. Proc NZ Soc Anim Prod 1990; 50: 471-472.
7. Mauleon P. New trends in the control of reproduction in the bovine. Livest Prod Sci 1974; 1:117-131.
8. Munro RK, Moore NW. The use of progesterone administered intravaginally and pregnant mares serum gonadotrophin given by injection in controlled breeding programs in beef and dairy cattle. Aust Vet J 1985; 62: 228-234.
9. Munro RK. Concentrations of plasma progesterone in cows after treatment with 3 types of progesterone pessaries. Aust Vet J 1987; 64: 385.
10. Odde KG. A review of synchronisation of estrus in postpartum cattle. J Anim Sci 1990; 68: 817-830.
11. Peterson AJ, Henderson HV. Plasma progesterone concentrations in ovariectomised dairy cows treated with a CIDR-B breeding device. J Reprod Fertil Suppl 1990; 43: 308.
12. Pierson RA, Ginther OJ. Ultrasonic imaging of the ovaries and uterus in cattle. Theriogenology 1988; 29: 723-731.
13. Quirk SM, Hickey GJ, Fortune JE. Growth and regression of ovarian follicles during the follicular phase of the oestrous cycle in heifers undergoing spontaneous and $PGF_2\alpha$-induced luteolysis. J Reprod Fertil 1986; 77: 211.
14. Robinson NA, Leslie KE, Walton JS. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. J Dairy Sci 1989; 72: 202-207.
15. Roche JF. Control of estrous in cattle using progesterone coils. Anim Reprod Sci 1974; 1: 145-154.
16. SAS User's Guide. Statistics. SAS Institute Inc 1985.
17. Sirois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 1990; 127: 916-925.
18. Stock AE, Fortune JE. Ovarian follicular dominance in cattle: Relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 1993; 132: 1108-1114.
19. Swanson LV, Wickman BW, Macmillan KL. Effect of exogenous progesterone (P_4) on follicular waves in dairy/beef heifers. J Dairy Sci 1990; 73(suppl 1): 177.