Journal of Reproduction and Development, Vol. 53, No. 4, 2007

—Full Paper—

Ovsynch Plus CIDR Protocol for Timed Embryo Transfer in Suckled Postpartum Japanese Black Beef Cows

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Abstract. We compared synchronization and pregnancy rates, and the increase in blood progesterone concentrations during luteal development, between (1) Ovsynch plus an intravaginal controlled internal drug release (CIDR) device protocol followed by timed embryo transfer (timed ET), and (2) a conventional estrus synchronization method using $PGF_{2\alpha}$ and ET in suckled postpartum Japanese Black beef cows. Cows in the PGF group (n=18) received a $PGF_{2\alpha}$ analogue when a CL was first palpated per rectum at 10-d intervals after 1 to 2 month postpartum. Cows (n=11), which showed estrus (Day 0) within 5 d of the $PGF_{2\alpha}$, and had a CL on Day 7, received ET. Cows in the Ovsynch+CIDR group (n=19) underwent the Ovsynch protocol plus a CIDR for 7 d (GnRH analogue and CIDR on Day–9, $PGF_{2\alpha}$ analogue with CIDR removal on Day–2, and GnRH analogue on Day 0), with ET on Day 7. The ovulation synchronization (100%) and embryo transfer (100%) rates in the Ovsynch+CIDR group were greater ($P<0.01$) than the estrus synchronization (66.7%) and the embryo transfer (61.1%) rates in the PGF group. The postpartum interval at ET in the Ovsynch+CIDR group (62.5 ± 2.5 d) was shorter ($P<0.01$) than in the PGF group (74.9 ± 3.9 d). The pregnancy rate in the Ovsynch+CIDR group (57.9%) did not differ significantly from that in the PGF group (50.0%). Plasma progesterone concentrations were not significantly different in the two groups on Days 0, 1, 2, 5, 7, 14 and 21. In summary, higher synchronization and transfer rates, and shorter postpartum interval to ET, can be achieved with timed ET following the Ovsynch plus CIDR protocol than after estrus with the single $PGF_{2\alpha}$ treatment followed by ET in suckled postpartum recipient beef cows. Pregnancy rates were similar. Also, the increase in blood progesterone concentrations during luteal development following ovulation synchronized by the Ovsynch plus CIDR protocol was similar to that after estrus induced by the $PGF_{2\alpha}$ treatment.

Key words: Beef cattle, Luteal development, Ovsynch plus controlled internal drug release (CIDR), $PGF_{2\alpha}$, Timed embryo transfer

(**J. Reprod. Dev. 53:** 811–817, 2007)

Accepted for publication: March 9, 2007
Published online: April 20, 2007
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Embryo transfer (ET) and related techniques have been used to copy and improve desirable genetic traits in cattle. However, embryonic survival rates after ET varies widely, between 40 and 70% [1, 2]. Mismatch of the estrus cycle of

donors with recipients, arising from failure of estrus detection, has been proposed as a cause of embryo death following transfer [3]. Ovulation synchronization protocols such as Ovsynch [4] allow timed ET without the need for estrus detection in recipients. In a few previous studies, Ovsynch or progesterone plus estradiol protocol followed by timed ET has been applied to lactating dairy cows [5, 6] or to *Bos indicus* X *Bos taurus* heifers [7]. However, there have been few studies of the efficacy of ovulation synchronization protocols followed by timed ET in suckled postpartum beef cows [8].

Ovsynch or its derivatives have been used for timed-AI in postpartum beef cows [9–11]. We have previously found that addition of a CIDR to the Ovsynch protocol (Ovsynch plus CIDR) improves the rate of conception following timed-AI in postpartum Japanese Black beef cows [11–15]. Also, the increase in plasma progesterone concentration after the second GnRH was faster in cows treated with Ovsynch than with Ovsynch plus CIDR protocol, partly due to premature follicular maturation and ovulation occurring in non-pregnant cows treated with Ovsynch [12, 14]. However, it is not known whether the increase in progesterone concentrations during luteal development after ovulation, synchronized using the Ovsynch plus CIDR protocol, is similar to that after spontaneous (non-induced) ovulation.

The present study compares synchronization, transfer and pregnancy rates, and increase of blood progesterone concentrations during luteal development, between Ovsynch plus CIDR protocol followed by timed ET, and the conventional estrus synchronization method by single $PGF_{2\alpha}$ treatment and ET, in suckled postpartum Japanese Black beef cows.

Materials and Methods

Animals

This study was conducted from March to November 2004 on an experimental beef cattle station in the Northern Center of Agriculture Technology of Hyogo Prefecture, Tajima district, Hyogo, Japan. Thirty seven suckled Japanese Black beef cows (age, 4.4 ± 0.3 yr; body weight, 389.0 ± 5.5 kg; height, 127.2 ± 0.5 cm; mean ± SEM) were recipients of embryos between 32 and 65 d postpartum (47.2 ± 1.7 d; mean ± SEM). The cows were kept in feedlot-type pens during the experiment, and were fed hay and concentrate so as to meet or exceed the Japanese Feeding Standard recommendations for beef cows that are nursing calves [16]. At the start of the hormonal treatments, the cows were assigned an index number for their degree of nutrition, according to weight and height; this was positively correlated with BCS [17].

Treatments and sample collection

The cows were randomly assigned into two groups. Cows in the PGF group (n=18) received 500 μg of $PGF_{2\alpha}$ analogue (cloprostenol, Estrumate™, Schering-Plough Animal Health, Tokyo, Japan) im when a corpus luteum (CL) was first palpated per rectum at 10-d interval after 1 to 2 months postpartum. They were observed for estrous (standing) behavior twice daily (1000 and 1700 h) for 5 d after the $PGF_{2\alpha}$ treatment. On Day 7 (day of estrus=Day 0), an embryo was transferred to the uterine horn ipsilateral to the CL of cows in which ovulation was confirmed per rectum on Day 1. Cows without CL did not receive any embryos. Cows in the Ovsynch+CIDR group (n=19) received 100 μg of a GnRH analogue (fertirelin acetate, Conceral™; Schering-Plough Animal Health) im on Day–9 (start of the protocol), and an intravaginal CIDR containing 1.9 g of progesterone (Eazibreed™; Livestock Improvement Association of Japan, Tokyo, Japan) for 7 d, starting on Day–9. The cows then received 500 μg of cloprostenol im on Day–2, and 100 μg fertirelin acetate im on Day 0. In all of the cows treated with the Ovsynch+CIDR protocol, an embryo was transferred to the uterine horn ipsilateral to the CL on Day 7. The frozen embryo was thawed by immersing each straw in a water bath at 37 C for 10 sec. Embryo transfer was completed within 5 min of thawing. All transfers were performed by the same veterinarian. Approximately 30 d after ET, pregnancy was diagnosed by transrectal ultrasonography, using a real-time B-mode scanner with a 7.5 MHz linear array transducer (EUP-033J; Hitachi, Tokyo, Japan).

Blood samples in the PGF group were collected on the day of $PGF_{2\alpha}$ treatment in all cows (n=18), and also on Days 0, 1, 2, 5, 7, 14 and 21 from cows randomly selected (n=7) out of those which showed estrus after the treatment (n=12). Blood samples in the Ovsynch+CIDR group were collected on Days –9, –2, 0, 1, 2, 5, 7, 14 and 21 from randomly selected

cows (n=17). Blood was collected from the jugular vein into heparinized vacutainers and was centrifuged at 800 × g for 20 min. The plasma was separated and stored at –30 C prior to hormone assays.

Transrectal ultrasonography was used to image selected cows (PGF, n=7; Ovsynch+CIDR, n=11), in order to measure the diameter of the largest follicle on Day 0 and the CL on Day 7. Cows were chosen at random for ultrasonography at the time of blood collection. The diameter of the largest follicle and of the CL were calculated as the mean of the vertical and horizontal diameters. For CL with a fluid-filled cavity, the diameter of the cavity was calculated and subtracted from the diameter of the CL.

Superovulation, collection and freezing of embryos

Thirteen Japanese Black beef cows in the same experimental beef cattle station which had normal estrus cycle were used as embryo donors. Superovulation was initiated 9 d after estrus over a 4-d period, using 20 AU of porcine FSH (Antrin R^{TM}; Denka Pharmaceuticals, Kanagawa, Japan) in decreasing doses. Estrus was induced by the $PGF_{2\alpha}$ analogue on the third day of FSH treatment (11 d after estrus). Artificial insemination was performed 12 and 24 h after the start of standing estrus, and embryos were collected non-surgically by flushing the uterus with a Foley catheter. The embryos which were morphologically evaluated as excellent were frozen by the direct method using ethylene glycol [18] and then stored in liquid nitrogen.

Hormone assays

The concentration of progesterone in blood plasma was measured by RIA as described previously, [12, 19] using the processed standard curve. Anti-progesterone-11-BSA serum (GDN no. 337) was used for this assay. The sensitivity of the assay was determined to be 78 pg/ml. The intra- and inter-assay CV for progesterone were respectively 10.7% (n=6) and 16.5% (n=3). Progesterone was measured in the plasma samples on all days.

The concentration of estradiol-17β in the plasma was measured by RIA, as described previously [12, 19]. Before the samples were assayed, the accuracy of the assay was estimated using the processed standard curve. Anti-estradiol-17β-6-BSA serum (GDN no. 244) was used in this assay; the sensitivity was 0.20 pg/ml. The intra-assay CV for estradiol-17β was 17.0% (n=5). Estradiol-17β was measured on Days 0 and 1 within an assay.

Statistical analyses

The estrus synchronization rate was defined as the proportion of treated cows which showed estrus within 5 d of the $PGF_{2\alpha}$ treatment. The ovulation synchronization rate was defined as the proportion of treated cows in which ovulation was detected at 48 h after the second GnRH treatment in the Ovsynch+CIDR protocol. The transfer rate was defined as the proportion of synchronized cows to which an embryo was transferred. The conception and pregnancy rates were defined as the respective proportions of transferred and treated cows diagnosed as pregnant. Differences in those rates between the PGF and Ovsynch+CIDR groups were examined using the Chi-square test (SAS Version 8.2; SAS Institute Japan, Tokyo, Japan). Differences between treatment groups in the means of age, parity, weight-height ratio and postpartum interval were analyzed by ANOVA (SAS Version 8.2 software).

For concentrations of progesterone from Days 0 to 21, and of estradiol-17β, the effects of treatment and day, and the treatment by day interaction, were evaluated by repeated measures ANOVA, using the mixed-model procedure (SAS Version 8.2 software). Differences in mean concentrations between the PGF and Ovsynch+CIDR groups on specific days, and between days in each treatment group, were analyzed by least significant difference. Differences were taken as significant when $P<0.05$. Plasma progesterone concentrations were regarded as high (equivalent to cows with functional luteal tissue; ≥0.5 ng/ml) or low (equivalent to cows without functional luteal tissue; <0.5 ng/ml), as described previously [11, 12, 14].

For the largest follicle on Day 0 and the CL on Days 17, the difference in mean diameters between treatment groups was analyzed by ANOVA and by Fisher's protected least significant difference post-hoc analysis (SAS Version 8.2 software).

Results

No significant differences were found between

Table 1. Synchronization, embryo transfer, pregnancy and conception rates in suckled Japanese Black beef cows which received single $PGF_{2\alpha}$ treatment (PGF) or Ovsynch+CIDR protocol

Treatment	Synchronization rate[a]	Transfer rate[d]	Pregnancy rate[f]	Conception rate[h]
PGF	66.7	61.1	50.0	81.8
	(12/18)[b]	(11/18)[e]	(9/18)[g]	(9/11)[i]
Ovsynch+CIDR	100[c]	100[c]	57.9	57.9
	(19/19)	(19/19)	(11/19)	(11/19)

[a] Proportion of treated cows that were synchronized estrus (PGF group) or synchronized ovulation (Ovsynch+CIDR group). [b] Number of synchronized per treated cows. [c] Difference (P<0.01) compared with PGF group. [d] Proportion of treated cows that an embryo was transferred to. [e] Number of transferred per treated cows. [f] Proportion of treated cows that were diagnosed as pregnant. [g] Number of pregnant per treated cows. [h] Proportion of transferred cows that were diagnosed as pregnant. [i] Number of pregnant per transferred cows.

the PGF and Ovsynch+CIDR groups in the age of the cow, parity, weight-height ratio or postpartum interval at the start of this experiment. The postpartum interval at the first hormonal treatment was shorter (P<0.01) in the Ovsynch+CIDR group (46.1 ± 2.4 d, mean ± SEM) than in the PGF group (70.4 ± 3.7 d); CL was detected at the first time and $PGF_{2\alpha}$ was given. The postpartum interval at ET in the Ovsynch+CIDR group (62.5 ± 2.5 d) was shorter (P<0.01) than in the PGF group (74.9 ± 3.9 d).

The ovulation synchronization rate in the Ovsynch+CIDR group (100%) was greater (P<0.01) than the estrus synchronization rate in the PGF group (66.7%; see Table 1). The embryo transfer rate in the Ovsynch+CIDR group (100%) was greater (P<0.01) than in the PGF group (61.1%). The pregnancy and conception rates did not differ significantly between the PGF and Ovsynch+CIDR groups.

In the PGF group, plasma progesterone concentrations on the day of $PGF_{2\alpha}$ treatment were at least 0.5 ng/ml in 17 out of 18 cows (1.57 ± 0.22 ng/ml, mean ± SEM, n=18). In the Ovsynch+CIDR group, progesterone concentrations on Day–9 were below 0.5 ng/ml in 7 out of 17 cows (1.76 ± 0.70 ng/ml, n=17). Progesterone concentrations on Day–2 in the Ovsynch+CIDR group were greater than 0.5 ng/ml in all cows (2.33 ± 0.27 ng/ml, n=17).

For plasma progesterone concentrations from Days 0 to 21, an effect of day was found (P<0.001), but the effects of treatment and treatment by day were not significant. Progesterone concentrations in all cows (Fig. 1, upper panel) and in pregnant cows (Fig. 1, lower panel) did not differ significantly between the PGF and Ovsynch+CIDR groups on Days 0 to 21. Progesterone concentrations did not change from Day 0 to Day 2, but increased from Day 2 to Day14, in both treatment groups (P<0.05).

For plasma estradiol-17β concentrations, there was an effect of day (P<0.001), but the effects of treatment and treatment by day were not significant. Estradiol-17β concentrations in all cows (Fig. 2, upper panel) and in pregnant cows (Fig. 2, lower panel) did not differ significantly on Days 9 and 10 between the PGF and Ovsynch+CIDR groups. Estradiol-17β concentrations fell from Day 9 to Day10 in both treatment groups (P<0.05).

The largest follicle did not differ significantly in diameter between the treatments on Day 0 (PGF, 10.9 ± 1.0 mm, n=7; Ovsynch+CIDR, 10.9 ± 0.5 mm, n=11). The CL diameter did not differ significantly on Day 7 (PGF, 16.6 ± 1.0 mm, n=7; Ovsynch+CIDR, 16.2 ± 0.9 mm, n=11).

Discussion

In the present study, the Ovsynch plus CIDR ovulation synchronization protocol followed by timed ET was assessed against the conventional estrus synchronization method by single $PGF_{2\alpha}$ treatment in suckled postpartum Japanese Black beef cows. The synchronization and transfer rates were found to be higher for the Ovsynch plus CIDR protocol than for the single $PGF_{2\alpha}$ treatment. The pregnancy and conception rates of the timed ET were more than 50% and comparable to the ET after the conventional estrus synchronization. These results suggest that higher synchronization and transfer rates and shorter postpartum interval to ET

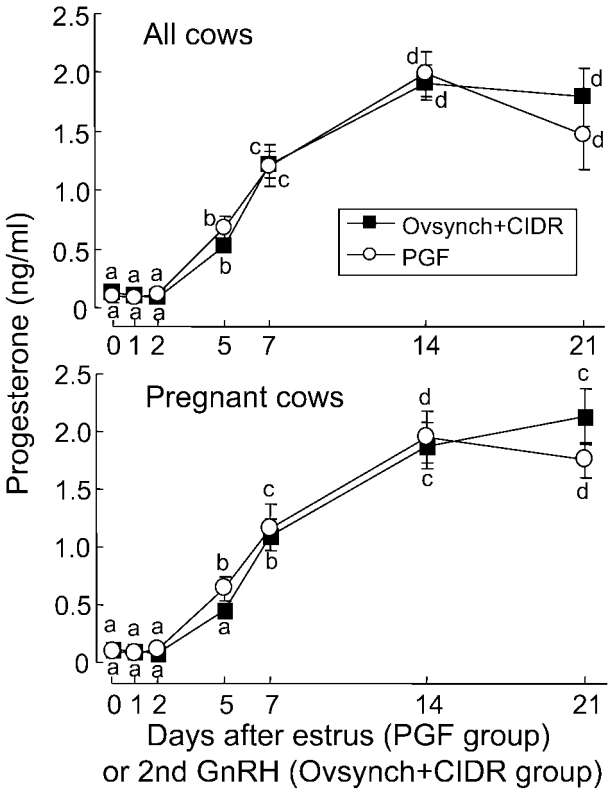

Fig. 1. Changes in plasma progesterone concentrations in postpartum suckled Japanese Black beef cows in the PGF and Ovsynch+CIDR groups. The progesterone concentrations were separately shown in all cows (PGF; n=7, Ovsynch+CIDR; n=17) and pregnant cows (PGF; n=6, Ovsynch+CIDR; n=10). Data are expressed as mean ± SEM. Values with different superscripts differ between days within a group (P<0.05).

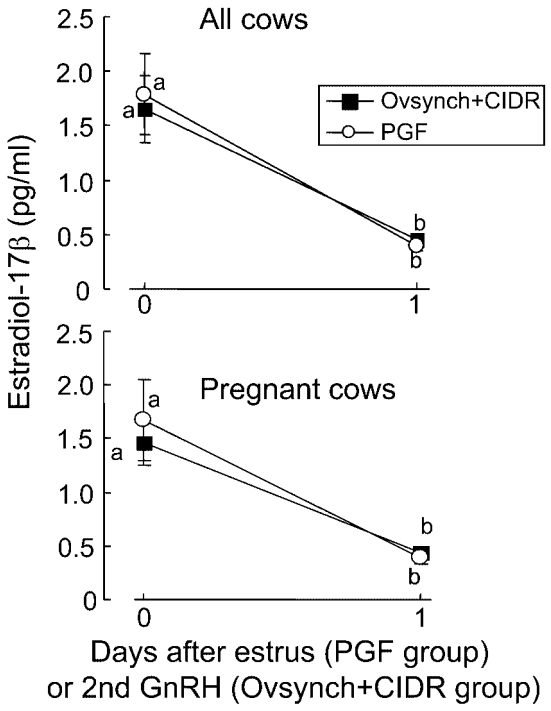

Fig. 2. Changes in plasma estradiol-17β concentrations in postpartum suckled Japanese Black beef cows in the PGF and Ovsynch+CIDR groups. The estradiol-17β concentrations were separately shown in all cows (PGF; n=7, Ovsynch+CIDR; n=17) and pregnant cows (PGF; n=6, Ovsynch+CIDR; n=10). Data are expressed as mean ± SEM. Values with different superscripts differ between days within a group (P<0.05).

(and equivalent pregnancy rates) of timed ET following the Ovsynch plus CIDR protocol can be achieved than with $PGF_{2\alpha}$ treatment in suckled postpartum recipient beef cows; moreover, without the need to detect estrus. However, the hormonal drugs required for the Ovsynch plus CIDR protocol cost more than those for the single $PGF_{2\alpha}$ treatment. A previous study found that the transfer rates were higher in lactating crossbred cows treated with Ovsynch and Ovsynch plus norgestomet (synthetic gestagen) implant than in cows whose estrus was induced by treatments with GnRH followed by $PGF_{2\alpha}$ [20]; the pregnancy rates were comparable. In the present study, the cows in the PGF group appeared to show luteal regression after the $PGF_{2\alpha}$ treatment since almost all of the cows had functional CL on the day of its treatment. Thus, the lower synchronization rate observed after the single $PGF_{2\alpha}$ treatment in the present study was probably due in part to the large variation in the interval between treatment and estrus [21], not due to the absence of CL at the $PGF_{2\alpha}$ treatment. The shorter postpartum intervals to the day of ET in the ovulation-synchronized cows with the Ovsynch plus CIDR protocol than in the estrus-synchronized cows with the single $PGF_{2\alpha}$ treatment is probably due to the following two reasons; 1) the single $PGF_{2\alpha}$ treatment was delayed compared with the Ovsynch plus CIDR protocol, because the CL must be present for the former treatment [22], and 2) the Ovsynch plus CIDR protocol can induce synchronized ovulation and luteal formation with a normal life span in early postpartum beef cows even though they are in non-cycling status [12, 15].

Induction of ovulation in dairy cows with smaller dominant follicles at the second GnRH

treatment with Ovsynch protocol led to reduced fertility, since development of the CL was inadequate and serum progesterone concentrations were reduced in dairy cows [23, 24]. It is not known, however, whether luteal development after ovulation is synchronized with Ovsynch or its derivative protocol is similar to that after spontaneous ovulation. The present study compared the increase in plasma progesterone concentrations during luteal development (1) after ovulation was synchronized with the Ovsynch plus CIDR, and (2) after spontaneous ovulation by the conventional estrus synchronization using the single $PGF_{2\alpha}$ treatment. It was found that the increase in progesterone concentrations during luteal development after the Ovsynch plus CIDR protocol was similar to that after the single PGF2? treatment. Also, the diameter of the CL on Day 7 was not significantly different in the two treatments. Luteal development after synchronized ovulation with the Ovsynch plus CIDR is therefore similar to that after spontaneous ovulation following estrus synchronization with single $PGF_{2\alpha}$ treatment in postpartum beef cows. Furthermore, plasma estradiol-17β concentrations and the diameter of the largest follicle on Day 0 did was not different in the Ovsynch plus CIDR and single $PGF_{2\alpha}$ treatments, suggesting that follicle maturation occurs in a similar way on the day before ovulation in both treatments.

In conclusion, higher synchronization and transfer rates, and shorter postpartum interval to ET, of timed ET following the Ovsynch plus CIDR protocol can be achieved than after estrus with the single $PGF_{2\alpha}$ treatment followed by ET in suckled postpartum recipient beef cows. Pregnancy rates are comparable in the two treatments. The increase in blood progesterone concentrations during luteal development after synchronized ovulation using the Ovsynch plus CIDR protocol was similar to that after synchronized estrus in these beef cows.

Acknowledgments

The authors thank Dr. G. D. Niswender of Colorado State University (Fort Collins, CO, USA) for providing estradiol-17β antiserum (no. 244) and progesterone antiserum (no. 337). We thank Surge Miyawaki Co. and the Livestock Improvement Association of Japan for supplying Eazibreed™ (CIDR). We also thank Schering-Plough Animal Health Co. for supplying Estrumate™ (cloprostenol) and Conceral™ (fertirelin acetate). This study was partly supported by a Research Grant for Meat and Meat Products from Ito Foundation.

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