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—Original Article—

Validation of a novel timed artificial insemination protocol in beef cows with a functional corpus luteum detected by ultrasonography

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Abstract. This study aimed to clarify the feasibility of a novel timed artificial insemination (TAI) protocol using ultrasonography, and to determine the associations between the ovarian component and fertility. In Experiment 1, 272 Japanese Black cows with a corpus luteum (CL) ≥ 18 mm in diameter were divided randomly into either the TRT group (134 cows that were administered gonadotropin-releasing hormone [GnRH] 56 h [day 2] after prostaglandin $F_{2\alpha}$ [PGF] administration [day 0], followed by TAI 16–20 h later) or the CN-1 group (138 cows that were administered PGF followed by AI after estrus detection). In addition, the CN-2 group was designated for 306 cows given PGF and inseminated after estrus detection in the past two years at the same farms. In Experiment 2, 38 cows had the same treatment as the TRT group, and the sizes of follicles and CL were video-recorded on days 0 and 2. In Experiment 1, the AI and ovulation synchronization rates were higher in the TRT group than those in the CN-1 group (100 *vs*. 87.0% and 89.2 *vs*. 33.3%, respectively) ($P < 0.01$). The pregnancy rate in the TRT group (60.4%) was higher than that in the CN-2 group (45.1%) ($P < 0.05$). In Experiment 2, cows with a larger CL diameter and greater CL volume on day 0 had a higher pregnancy outcome ($P < 0.05$). In conclusion, this protocol was effective for improving pregnancy rates in beef herds, and fertility was associated with the CL size at the time of PGF administration.

Key words: Beef cows, Corpus luteum, ShortSynch, Timed AI protocol, Ultrasonography

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"Anestrus" beef cows are often not actually anestrus, but exhibit repeated normal-length estrous cycles with silent estrus, or exhibit weak estrous signs that are overlooked. One of the measures to induce estrus in open cows with a corpus luteum (CL) is to administer a single dose of prostaglandin $F_{2\alpha}$ (PGF), and then perform artificial insemination (AI) once estrus has returned. However, the problems with this method are that the estrus observations must be made carefully, as the time for estrus varies widely depending on the size of the dominant follicle, and estrus may be overlooked in some cases. Moreover, the limited value of transrectal palpation in assessing the CL indicates that PGF may be administered to animals without a functional CL, and estrus will not be induced [1]. In addition, PGF-treated cows may display estrous behaviors, such as performing or allowing mounting, at different times. This makes it difficult to determine the appropriate timing for insemination by visual observation of estrus alone, and insemination may not be performed at the appropriate time, thereby reducing the conception rate.

Synchronizing ovulation with a fixed-time AI program is an important method to resolve the aforementioned problems in reproductive management, as they eliminate the need to identify estrus [2]. Development of the Ovsynch technique, which increases the pregnancy rate compared to the common practice of AI after detecting natural estrus, has helped reduced the amount of effort spent trying to detect estrus in dairy cattle [3, 4]. However, if the Ovsynch technique is initiated regardless of the estrous cycle stage, ovulation is not synchronized in about 15% of cows after timed artificial insemination (TAI) (i.e., cows ovulate before TAI) [5, 6]. This is because the CL undergoes regression within seven days of initiating treatment owing to the effect of endogenous PGF, thereby inducing ovulation of the dominant follicle before the second gonadotropin-releasing hormone (GnRH) administration if the Ovsynch technique is initiated in cows around 13 days after ovulation. Two conceivable methods are available to resolve this problem. The first method is to start presynchronization or Ovsynch prior to the Ovsynch-TAI protocol (known as Presynch or Double-Ovsynch) [7, 8]. The second method is to suppress ovulation before TAI using an intravaginal progesterone (P_4) insert (controlled internal drug release [CIDR]) [9]. However, both of these methods involve multiple drug administrations or the use of intravaginal devices, increasing the amount of labor and cost involved, as well as the duration of the procedure.

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Therefore, it would be simpler and more practical to start with PGF administration for cows with a functional CL, and synchronize ovulation induced by GnRH, which would be administered after PGF. Cirit *et al.* administered PGF to dairy cows with a CL, followed by estradiol-17β and GnRH 48 and 60 h later, respectively. As a result, TAI improved the pregnancy rate compared to the rate achieved by AI after detecting natural estrus [10]. However, no study has compared the estrus detection rate (AI rate), conception rate, and pregnancy rate (AI rate multiplied by conception rate) obtained from a TAI protocol after PGF and GnRH treatment with those achieved by AI after detecting estrus following PGF administration alone. In addition, no study has assessed the effectiveness of an ovulation synchronization protocol using PGF–GnRH in beef cattle.

If a cow is assessed as having a functional CL that responds to PGF, an ovulation synchronization protocol initiated with PGF seems to be a logical option. Such a protocol is yet to be practically used and its effectiveness remains unknown, because an accurate assessment of a functional CL and a coexisting dominant follicle had not been possible until recently [11, 12]. Portable ultrasound scanners have come into widespread use [13] enabling assessments of the CL and status of coexisting follicles with much greater accuracy than rectal palpation.

The objective of this study was to elucidate the effectiveness of a novel timed AI protocol using PGF–GnRH in beef cows with a CL. Our specific aims were to clarify the feasibility of this method using a portable ultrasound device to objectively evaluate CL functionality by measuring its diameter, to determine the associations between the ovarian component at the time of hormone administration and pregnancy outcome, and to ascertain whether this protocol would achieve a higher pregnancy rate than the conventional AI method based on estrus detection after administration of PGF alone.

Materials and Methods

Animals

In Experiment 1, 272 Japanese Black cows kept at three farms in Ishigaki City, Okinawa Prefecture, and five farms in Miyazaki City, Miyazaki Prefecture, were used. All cows had CL diameters ≥ 18 mm at least 40 days after calving by ultrasound examination. The mean ± standard deviation (SD) values were as follows: age, 5.5 ± 3.1 years; parity number, 4.3 ± 3.0; days postpartum, 84.0 ± 48.3 days; and body condition score [14], 3.15 ± 0.4. The experiment was carried out from May 2013 to August 2014. The cows were housed in freestalls and fed twice a day with hay and a concentrate diet, and had free access to water.

In Experiment 2, 38 postpartum Japanese Black cows with CL diameters ≥ 18 mm at the beginning of the protocol were used. The cows were kept in four of the five farms in Miyazaki City used in Experiment 1. The mean ± SD values were as follows: age, 6.7 ± 3.4 years; parity number, 4.9 ± 3.1; days postpartum, 73.3 ± 39.2 days; and body condition score, 2.9 ± 0.3. The experiment was carried out between February and September 2015. The cows were housed in freestalls and fed twice a day with hay and a concentrate diet, and had free access to water.

Ovulation-synchronizing treatment and observation of ovarian follicle dynamics

In Experiment 1, the test cows were randomly divided into two groups as follows: cows administered PGF followed by GnRH after 56 h and subjected to TAI 16–20 h later (TRT group, n = 134), and cows administered PGF that underwent AI after estrus was detected using the AM–PM rule (animals in estrus in the morning were inseminated in the afternoon of the same day, and animals in estrus in the afternoon were bred the next morning) (CN-1 group, n = 138). Because the TRT and CN-1 groups were kept together, there was a risk of bias arising from a higher likelihood of CN-1 group cows being inseminated because they were kept together with the test cows to be inseminated at a fixed time. To avoid an overestimation of the AI rate in the control group, a second control group (CN-2 group) composed of 306 cows kept for the previous two years at the same five farms as those used for the the TRT and CN-1 groups was established. These cows had a CL detected by ultrasound, were administered PGF alone, and were inseminated using the AM–PM rule. Farm staff detected estrus by visual inspection twice a day. In the CN-2 group, an ultrasound scan was not used to measure maximum follicular diameter, nor for the ovulation examination. The average numbers of cows allocated to the TRT, CN-1, and CN-2 groups at the same time in the same paddock were the same (n = 3) among the three groups.

The cows in the TRT group were administered an intramuscular dose of PGF (0.5 mg cloprostenol, Resipron®-C; ASKA Pharmaceuticals, Tokyo, Japan) when a CL diameter of 18 mm or more was observed by ultrasonography (day 0), and an intramuscular dose of GnRH (100 μg fertirelin acetate, Consultan®; Aska Pharmaceuticals) 56 h later (day 2). TAI was performed 16–20 h after GnRH was administered (day 3), and 74 of those cows were examined 24 h later to confirm ovulation. The ovulation examinations were carried out 96–100 h after PGF administration in 72 of the 138 cows in the CN-1 group, which were used in Ishigaki City, regardless of whether estrus had occurred or AI was performed (Fig. 1). Timing of the ovulation examinations was determined in order to compare the ovulation synchronization rate with the TRT group, in which ovulation was defined as the disappearance of a large follicle from an ovary within two days after GnRH administration [24], that is, within 96–100 h after PGF administration.

Six different portable ultrasound scanners were used to record ovarian follicle dynamics (maximum CL and largest follicle diameters) when hormones were administered in both experimental groups, as well as to detect evidence of ovulation and diagnose pregnancy 30–50 days after insemination. The following ultrasound scanners were used: SonoSite® 180 Plus (Fujifilm SonoSite Japan, Tokyo, Japan); MicroMaxx® (Fujifilm SonoSite Japan); Tringa Linear VET (Esaote Europe, Maastricht, The Netherlands); HS-1600V (Honda Electronics, Toyohashi, Japan); AGROSCAN Linear (ECM, Angoulême, France); and MyLab One VET (Esaote Europe). All scanners were fitted with a 7.5 MHz linear probe, and no measurement errors were observed between the different types of devices. Records of follicular diameter were grouped into size classes at 2 mm intervals.

In Experiment 2, 38 animals were administered PGF followed by GnRH 56 h later, and subsequently TAI 16–20 h later (same treatment as TRT group in Experiment 1). The same individual (YT) examined the genital tract via the rectum and video-recorded the

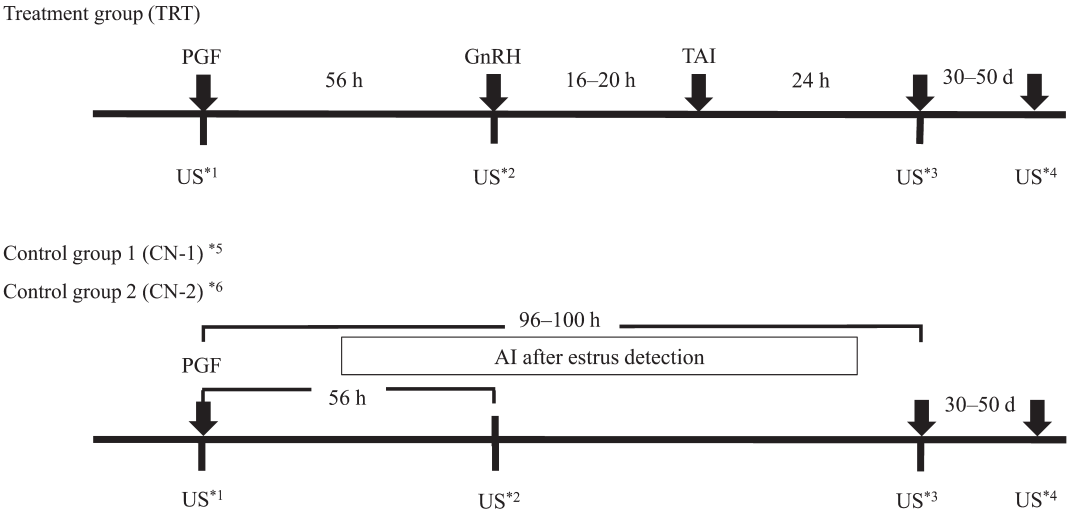

Fig. 1. Experimental design. *1: Ultrasound scan (corpus luteum and maximum follicle diameters) and blood collection to measure progesterone (P_4) concentration. *2: Ultrasound scan (maximum follicular diameter) and blood collection to measure P_4 concentration. *3: Ultrasound scan (examination for ovulation). *4: Ultrasound scan (pregnancy diagnosis). *5: Cows that underwent artificial insemination (AI) by the AM–PM method after detection of estrus induced by prostaglandin-$F_{2\alpha}$ (PGF) administration. *6: Cows that underwent artificial insemination by the AM–PM method after the detection of estrus induced by prostaglandin-$F_{2\alpha}$ (PGF) administration (previous record). An ultrasound scan was not used to measure maximum follicular diameter, nor for the ovulation examination. No blood samples were collected.

ovarian structures, such as follicle and CL diameters, as well as CL volume, on day 0 (time of PGF treatment) and day 2 (time of GnRH treatment), respectively, using an ultrasound scanner (MyLab One VET) fitted with a 10.0 MHz linear probe. After returning to the laboratory, the video was played, and the morphological evaluation of the ovaries, such as determining diameters of the follicles and CLs, as well as the CL volume, was performed with dynamic moving image software (Movie Maker; Microsoft, Redmond, WA, USA). The maximum length (A) and transverse diameter (B) of the CL were measured, and volume was estimated using the equation for a modified prolate ellipsoid, $V = 0.523 \times A \times B \times B$ [15]. CL length and diameter were measured at the outermost limit in the two planes.

Progesterone assay

In Experiment 1, blood samples (10 ml) were taken from the median coccygeal vein of all of the animals in the TRT (n = 134) and CN-1 (n = 138) groups at the time of PGF administration using evacuated blood collection tubes with heparin (Venoject; Terumo, Tokyo, Japan) at the time of PGF administration. No blood samples were collected in the CN-2 group. The plasma was separated by centrifugation and stored at –30°C until hormone levels were measured. Plasma progesterone (P_4) concentrations were measured by enzyme-linked fluorescence assay using the mini-VIDAS automated immunofluorescence assay system (Sysmex; bioMérieux, Marcy l'étoile, Lyon, France). Sensitivity and intra- and interassay coefficients of variation for this assay were 0.2 ng/ml and < 4% and < 8.1%, respectively [16]. A P_4 concentration ≥ 1.0 ng/ml was considered to indicate the presence of a functional CL [17–19].

Statistical analyses

In Experiment 1, the AI rate, conception rate, and pregnancy rate were compared among the three animal groups using a chi-squared analysis. The conception rate among different follicular diameter groups was compared using the chi-squared test, or Fisher's exact test if the cells contained fewer than five observations. An *F*-test was used to compare mean blood P_4 concentrations between the TRT and CN-1 groups at the same blood collection time point, to test whether variance was equal in both groups. If the variance was equal, Student's *t*-test was used, and Welch's *t*-test was performed if the variance was unequal. Correlation analysis using Spearman's rank correlation coefficient was performed for maximum follicle diameter and conception rate data.

In Experiment 2, diameters of the follicles and CLs and CL volume between the pregnant group and non-pregnant group were compared using Student's *t*-test. The optimal cut-off point and receiver operating characteristic (ROC) curve analyses were conducted to determine the diagnostic validity of follicular and CL sizes for subsequent fertility. Conception rates between cows above and below the cut-off point were compared using Fisher's exact test. We performed these analyses using BellCurve for Excel (Social Survey Research Information, Tokyo, Japan). A P-value ≤ 0.05 was considered significant, and probabilities between 0.05 and 0.1 were considered to tend toward significant.

Results

Experiment 1: Association between diameter and functionality of the CL

The plasma P_4 concentration was ≥ 1.0 ng/ml in almost all (98.9%, 269/272) cows with a CL ≥ 18 mm in diameter. Plasma P_4 concentrations at the time of PGF administration in the TRT and CN-1 groups were 8.34 ± 4.21 and 7.34 ± 4.20 ng/ml, respectively. No difference in P_4 concentration was observed between the two groups.

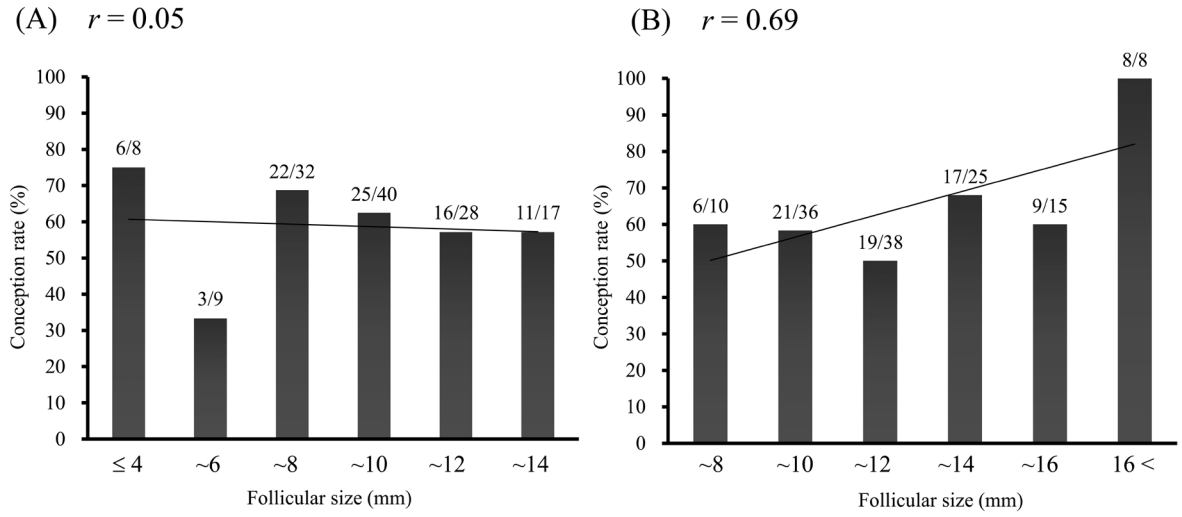

Fig. 2. Conception rates in treatment group (n = 134; Japanese Black cows treated with prostaglandin-$F_{2\alpha}$ [PGF] and gonadotropin releasing hormone [GnRH]) with different maximum follicular diameters. (A) Comparison of conception rates in Japanese Black cows with different maximum follicular diameters at the time of PGF administration. (B) Comparison of conception rates in Japanese Black cows with different maximum follicular diameters at the time of GnRH administration.

Experiment 1: Association between maximum follicle diameter and conception rate

Figure 2(A) shows the association between maximum follicle diameter at the time of PGF administration and conception rate in the TRT group. When the follicle diameters were classified into groups of 2 mm increments and the conception outcomes were compared, no significant differences were observed between the groups.

About 92% (124/134) of the TRT and 87.7% (121/138) of the CN-1 groups had a largest follicle ≥ 9.0 mm in diameter at the time of GnRH administration. No difference was observed in proportion between the two groups.

Figure 2(B) shows the association between maximum follicle diameter at the time of GnRH administration and conception rate in the TRT group. When the follicle diameters were classified into groups of 2 mm increments and conception rates were compared, no significant differences were observed between the groups. A positive correlation ($\rho = 0.609$) was observed between them. Conception rate tended to be higher for follicles ≥ 13 mm in diameter than for those ≤ 12 mm in diameter (P = 0.06).

Experiment 1: Comparison of ovulation synchronization rates, artificial insemination rates, and reproductive performance

Table 1 shows the ovulation synchronization and artificial insemination rates for the TRT and CN-1 groups, and a comparison of the reproductive performance among the three groups. The ovulation synchronization rate in the TRT group (89.2% [66/74]) was higher than that in the CN-1 group (33.3% [24/72]) (P < 0.01). The AI rate in the TRT group was higher (100% [134/134]) than that in the CN-1 (87.0% [120/138]) and CN-2 groups (64.4% [197/306]) (P < 0.01), and the AI rate in the CN-1 group was higher than that in the CN-2 group (P < 0.01). The interval between the time of PGF administration and time of AI in CN-1 group was 88.2 ± 20.6 h.

The conception rates in the TRT, CN-1, and CN-2 groups were 60.4 (81/134), 59.2 (71/120), and 70.1% (138/197), respectively (P > 0.05). In the CN-1 group, the pregnancy rate in the three farms in Ishigaki City (50%, 36/72) was similar to that in the five farms in

Table 1. Effects of GnRH treatment on ovulation synchronization rates, artificial insemination rates, and reproductive outcomes in Japanese Black cows administered with $PGF_{2\alpha}$ after being identified as having a corpus luteum

	Treatment group [1] (TRT)	Control group 1 [2] (CN-1)	Control group 2 [3] (CN-2)
Number of test animals	134	138	306
Ovulation synchronization rate (%)	89.2 [a] (66/74)	33.3 [b] (24/72)	NA
Artificial insemination rate (%)	100 [a] (134/134)	87.0 [b] (120/138)	64.41 [c] (197/306)
Conception rate (number of animals conceived/number of animals inseminated) (%)	60.4 (81/134)	59.2 (71/120)	70.1 (138/197)
Pregnancy rate (number of animals conceived/number of animals examined) (%)	60.4 [d] (81/134)	51.4 (71/138)	45.1 [e] (138/306)

[1] Cows treated with GnRH 56 h after $PGF_{2\alpha}$ administration and inseminated at a fixed time 16–20 h later. [2] Cows administered with $PGF_{2\alpha}$ and inseminated after estrus detection according to the AM–PM role (utilized concurrently with TRT group). [3] Cows administered with $PGF_{2\alpha}$ and inseminated after estrus detection according to the AM–PM role (utilized during the previous two years). Significant differences between different letters within the same row: a–b–c, P < 0.01; d–e: P < 0.05.

Miyazaki City (53%, 35/66). The pregnancy rate of the TRT group (60.4%, 81/134) tended to be higher than that of the CN-1 group (51.4%, 71/138) (P = 0.08), and was higher than that of the CN-2 group (41.5%, 138/306) (P < 0.05).

Experiment 2: Comparison of follicle and CL diameters and CL volume at the time of hormone treatment in pregnant and non-pregnant cows

The mean largest follicle diameter at the time of PGF treatment (day 0) in pregnant cows (10.0 ± 0.05 mm) was similar (P = 0.21) to that in non-pregnant cows (9.11 ± 0.04 mm). The mean largest follicle diameter at the time of GnRH treatment (day 2) in pregnant cows (12.4 ± 0.04 mm) was greater (P < 0.05) than that of non-pregnant cows (11.1 ± 0.05 mm).

Mean CL diameter on day 0 of pregnant cows (20.9 ± 0.30 mm) was greater (P < 0.05) than that of non-pregnant cows (19.8 ± 0.42 mm). In addition, mean CL volume on day 0 of pregnant cows (4.11 ± 0.77 cm^3) was greater (P < 0.01) than that of non-pregnant cows (3.24 ± 0.80 cm^3).

Experiment 2: ROC curve analysis for follicle and CL size at the time of hormone treatment during pregnancy

Areas under the ROC curve for follicle diameter on days 0 and 2 were 0.629 (P = 0.161) and 0.680 (P = 0.059), respectively (Fig. 3). Areas under the ROC curve for CL diameter and CL volume on day 0 were 0.716 (P = 0.026) and 0.806 (P = 0.0003), respectively. Cut-off points for follicle diameter on days 0 and 2 were 10.34 and 12.14 mm, respectively. Those for CL diameter and volume on day 0 were 19.8 mm and 3.61 cm^3, respectively. The pregnancy rate in cows with a follicle equal to or greater than the cut-off point in diameter on day 2 tended to be higher (P = 0.052) than those with a follicle less than the cut-off point in diameter on day 2. Pregnancy rates in cows with a CL equal to or greater than the cut-off points in diameter and volume on day 0 were higher than those in cows with a CL less than the cut-off points in diameter (P = 0.008) and volume (P < 0.0001), respectively (Table 2).

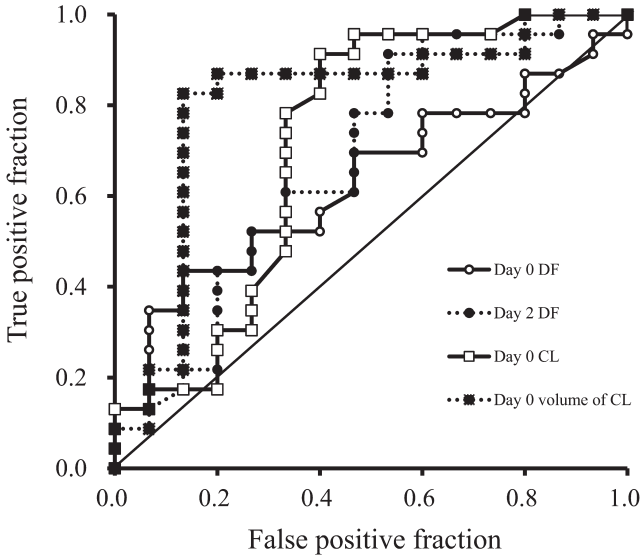

Fig. 3. Receiver operating characteristic curve during pregnancy after timed artificial insemination using the dominant follicle and corpus luteum in 38 prostaglandin $F_{2\alpha}$-gonadotropin releasing hormone (GnRH)-treated Japanese Black cows. White circles and solid line, diameter of largest follicle on day 0 (injected with prostaglandin $F_{2\alpha}$); black circles and solid line, diameter of largest follicle on day 2 (injected with GnRH); white squares and broken line, diameter of corpus luteum on day 0; black squares and broken line, volume of corpus luteum on day 0.

Discussion

When ultrasound scanning was used to diagnose functionality of the CL, with a CL diameter of 18 mm used as the threshold (lower limit), 98.5% (268/272) of the Japanese Black cows had a functional CL at the time of PGF administration, with a blood P_4 concentration ≥ 1.0 ng/ml. We set the diameter of a functional CL as ≥ 18 mm in our study, because a CL ≥ 20 mm in diameter is believed to be capable of secreting a functional amount of P_4 in Holstein cows [17–19]. Setting the value 2 mm less would be reasonable considering our previous observation that the diameter of ovulatory follicles is approximately 2 mm smaller than that in Holstein cows (unpublished observation) and smaller ovulatory follicles are associated with smaller CLs after ovulation [20]. No previous study has reported using ultrasound to measure CL diameter in Japanese Black cows with a simultaneous measurement of P_4 concentration. Our results indicate that a CL ≥ 18 mm in diameter is an indicator of its functionality in this breed. The observation that 99% of cows had a functional CL suggests that ultrasound scanning provided a sufficiently objective method for evaluating CL functionality.

In this study, we did not find a significant association between maximum follicle diameter at the time of PGF administration and conception rate. Dominant follicle diameter was previously believed to affect fertility in an ovulation synchronization program [21]. On the other hand, a recent study reported that when PGF was administered five (D5) or nine days (D9) after the follicular wave was reset in beef cattle, no subsequent difference in the conception rate by AI after estrus detection was observed between the D5 and D9 groups [22], and this finding may be supported by the results of the present study. However, we must be careful in interpreting the results, as AI was not performed under the same conditions. In addition, it is inferred that the maximum follicle diameter recorded at the time of PGF administration was not always the ovulatory follicle. It should be challenging to estimate subsequent fertility by only measuring follicular size at the time of PGF administration.

We found a tendency for the conception rate to rise when the dominant follicle diameter at the time of GnRH administration was larger. Inducing ovulation with GnRH in beef cattle with follicles ≤ 11 mm in diameter reduces the subsequent pregnancy success rate and increases embryonic and fetal death [23]. These findings are in agreement with the reproductive outcomes in our study. However, further studies are required to clarify this point.

The cows in the TRT group that were administered PGF followed by GnRH 56 h later before undergoing TAI exhibited a significantly higher ovulation synchronization rate compared with cows treated with PGF alone when a functional CL was confirmed. GnRH administration

Table 2. Pregnancy rates based on the cut-off points for the size of the follicle and corpus luteum at the time of hormone treatment

Category		Cut-off point	Pregnancy rate (%)		P-value
			≥ Cut-off point	< Cut-off point	
Diameter of follicle	Day 0 [2)]	10.34 mm	75.0 (12/16)	50.0 (11/22)	0.182
	Day 2 [2)]	12.14 mm	77.8 (14/18)	45.0 (9/20)	0.052
CL [1)] on Day 0	Diameter	19.83 mm	78.3 (18/23)	33.3 (5/15)	0.008
	Volume	3.61 cm^3	90.5 (19/21)	23.5 (4/17)	< 0.0001

[1)] CL: corpus luteum. [2)] Day 0: at the time of prostaglandin $F_{2\alpha}$ administration; Day 2: at the time of GnRH administration.

induces ovulation in follicles ≥ 9.0 mm in diameter in a mean time of 28 h [24, 25]. In the present study, 92.5% of the cows had a largest follicle diameter ≥ 9.0 mm at the time of GnRH administration. The ovulation synchronization rate was 89% within 24 h after TAI. The reason for the failure to ovulate may be a diminished response of the pituitary to GnRH, leading to problems in the release of LH [26]. The ovum becomes senescent when ovulation is delayed, causing later problems in fertilization, which can prevent conception [27].

The finding that high ovulation synchronization and 100% AI rates in the TRT group raised the pregnancy rate compared to that in the CN group suggested that the normal practice of detecting estrous signs by visual inspection alone is not accurate, with a certain percentage of animals in estrus being silent or overlooked [28, 29]. This result shows that the protocol used in the present study is effective in such herds, as it does not require the detection of estrus.

The pregnancy rate in the TRT group in the present study was 60.4%, which was higher than that reported for Japanese Black cows treated with the Ovsynch protocol (48–50%) [30–32]. One of the reasons for this is probably due to the fact that the cows subjected to our protocol had a CL, that is, had resumed ovarian cyclicity, whereas those subjected to Ovsynch included animals without a CL as well. Nevertheless, our protocol in this study (termed ShortSynch [SS]) is clinically applicable and highly practicable as a novel ovulation-synchronized TAI protocol. Although the use of ultrasound to measure the CL results in additional treatment time for each cow, it helps to confirm the presence or absence of a functional CL, and increases the likelihood of the CL responding to PGF.

In the SS protocol, PGF is injected intramuscularly as soon as a functional CL is confirmed by ultrasound, followed by a GnRH injected intramuscularly 56 h later, and TAI is performed 16–20 h after the GnRH administration. This protocol has an advantage over conventional ovulation synchronization protocols, as it requires less time for synchronization compared with other conventional protocols (enables AI to be performed one week earlier than the Ovsynch protocol). In particular, for herds where the estrus detection rate is < 85%, the expected pregnancy rate (= estrus detection rate × conception rate = 85% × 70%) would be < 60%, on the presumption that conception rate at AI after detecting estrus is 70% based on the results obtained in the present study. If this method is used for animals that have failed to conceive in reproductive tests after AI or in those exhibiting silent estrus, it could efficiently reduce the mean interval between calving for the herd as a whole.

In the present study, the interval between the PGF and GnRH administrations was set to 56 h to achieve a higher conception rate by extending the time for the dominant follicle to mature [33]. The association between the interval between the PGF and GnRH administrations and fertility should be investigated in future studies to determine a better interval for reproductive performance. In addition, future studies should compare the efficacy of the SS and Ovsynch protocols in cows with a functional CL.

No difference was observed in the largest follicle diameter on day 0 between pregnant and non-pregnant cows, and the largest follicle diameter on day 2 in pregnant cows was greater than that in non-pregnant cows. Administering GnRH to cows with a follicle ≤ 11 mm in diameter to induce ovulation results in a lower conception rate and a higher incidence of late embryonic death [24]. A possible reason is a decrease in P_4 secretion from the CL formed after AI, suggesting that the largest follicle in some of the non-pregnant cows was immature on day 2.

Significant differences in CL diameter and CL volume were observed between pregnant and non-pregnant cows on day 0. A previous study showed that the conception rate in Nelore cows administered P_4 for nine days was significantly higher than that in cows administered P_4 for seven days, suggesting that persistent exposure to P_4 prior to AI may be crucial for a successful pregnancy [34]. The result of the present study, indicating that cows with a larger CL diameter and greater CL volume had a higher conception rate, supports these previous studies.

The results of the present study indicate that conception rate increased by inseminating cows with a CL greater than the diameter (19.8 mm) or volume (3.61 cm^3) cut-off point, and the analysis of ROC showed that the cut-off point of the CL volume gave the highest accuracy among the measured values for fertility after TAI in SS protocol. Estimation of CL volume is calculated by two indices (maximum length and transverse diameter) and these indices can be depicted easily by a mobile ultrasound scanner under field conditions. In this regard, selecting cows for SS protocol by the size of CL volume should increase the conception rate of a herd. However, using the more severe criteria to select cows for TAI would lead to a lower insemination rate, which, in turn, would lower the pregnancy rate. Therefore, a suitable breeding program for the cows left out of selection for PGF administration should be established. The Ovsynch protocol might be an option for cows with a CL that is early in formation but smaller than the cut-off point, because the early luteal phase is an ideal time to start Ovsynch to obtain a high conception rate [35]. In contrast, careful observation and detection of estrus or a P_4-based TAI protocol

might be an option for cows with a regressing CL. Nevertheless, the important thing is to increase the pregnancy rate of the herd, and the SS protocol is a feasible option for cows with a functional CL.

In conclusion, a portable ultrasound scanner is a practical tool for evaluating the presence or absence of a functional CL. CL size at the time of PGF administration was related to the subsequent conception rate, and the SS protocol was effective for improving the pregnancy rate and, subsequently, the calf production rate in beef herds, where the estrus detection rate is low.

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