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Vaginal Electrical Resistance and Size of Dominant Follicle in Beef Cows Subjected to Synchronization of Ovulation Protocol

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ABSTRACT

The objectives were to determine the efficiency of vaginal electrical resistance (VER) to indicate synchronization success and its correlation with the diameter of the largest follicle (DLF). Crossbred beef cows with more than two lactations from the beef farm at the Kamphaeng Saen campus, Kasetsart University were synchronized with the Co-Synch plus controlled internal drug release (CIDR) protocol that consisted of a CIDR insert and an intramuscular injection of gonadotropin-releasing hormone (GnRH; first GnRH; 2 mL) on Day 0, removal of the CIDR insert and an injection of Prostaglandin F_2 alfa ($PGF_{2\alpha}$; 2 mL) on day 7 and a second injection of GnRH (2 mL) at 48 or 66 h after $PGF_{2\alpha}$ injection. VER was determined with an Ovatec device at Days 0, 7 and at the time of the second GnRH injection. Trans-rectal ultrasonography was performed on the same day to assess ovarian follicular development. The DLF (mean ± SE) was highest (16.3 ± 0.5 mm; $P < 0.05$) at the second GnRH injection and lowest at Day 0 (9.8 ± 1.0 mm). The VER (mean ± SE; measured in ohms Ω) was significantly highest (93.8 ± 1.7 Ω; $P < 0.05$) on Day 0 and lowest (79.1 ± 1.7 Ω) at the time of second GnRH injection. Mean VER was inversely correlated (r = -0.49; $P < 0.05$) with DLF at the second GnRH injection. The significant correlation obtained between VER and DLF indicated that VER can be used to predict the stage of follicular maturity and the response to synchronization treatment.

Keywords: diameters of largest follicles, ovulation synchronization, vaginal electrical resistance.

INTRODUCTION

Cattle breeding herds that utilize artificial insemination (AI) in their reproductive management and genetic improvement require accurate methods to detect estrus (Heersche and Nebel, 1994). The most common estrous detection method is to observe animals in standing heat. However, not all ovulating animals display standing heat (Van Eerdenburg *et al*., 1996). In addition, identification of animals showing estrous behavior in large groups of cattle can be challenging. Recent estrous synchronization research has focused on the use of GnRH in combination with timed artificial insemination (TAI) on a single day without checking heat (Kasimanickam *et al*., 2005). Several protocols have been developed and optimized that combine a progestin, $PGF_{2\alpha}$, and GnRH for synchronization of ovulation and fixed TAI in cattle. Among these,

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the Co-Synch plus controlled internal drug release (CIDR) protocol has resulted in TAI pregnancy rates greater than 50% in *Bos taurus* beef cows (Larson *et al*., 2006) and heifers (Lamb *et al*., 2006). However, in the *Bos indicus* beef breed, an overall TAI pregnancy rate of 39% was reported after treatment with Co-Synch plus CIDR (Saldarriaga *et al*., 2007) which is substantially less than those observed in *Bos taurus* females (Geary and Whittier, 1998; Lamb *et al*., 2001). The lower performance of the *Bos indicus* breed to TAI compared to the *Bos taurus* breed could be related to differences in timing of ovarian events such as follicular wave emergence and preovulatory follicle diameters, environmental stress or both.

In order to incorporate estrous synchronization and TAI effectively and economically in *Bos indicus* and *Bos indicus* × *Bos taurus* crosses, TAI pregnancy rates must be optimized with reduced variability. For this to happen, one approach would be to develop procedures for timely identification of females in whom ovarian follicular development has been inadequately regulated, to ensure an optimal pregnancy rate. Since the follicular diameter at induced ovulation is positively related to fertility (Perry *et al*., 2005), strategies for indirectly assessing the ovarian status, follicular diameter and thus, synchronization success without estrus detection, would be valuable tools. Since the development of AI, a large amount of research has focused on methods for detection of estrus in cattle and a number of physiological and behavioral parameters have been shown to change in relation to the different stages of the reproductive cycle (Nebel *et al*., 2000; Rorie *et al*., 2002). VER has been studied in cattle and in other species as a method for predicting the ovarian status without visual estrus detection (Schams *et al*., 1977; Foote *et al*., 1979). The Ovatec probe (Heritage Genetics, LLC) is one of several electronic devices developed for use in cattle to easily measure VER. The present study hypothesized that VER could be used to estimate the ovarian status of *Bos indicus* × *Bos taurus* females during Co-Synch plus CIDR synchronization. The objectives of this study were to evaluate the change in VER and follicular diameter at different times of the Co-Synch plus CIDR protocol, and their correlation and efficiencies in indicating a response to ovulation synchronization.

MATERIALS AND METHODS

Location and climate of study area

The experiment was conducted at the beef cattle production farm on the Kamphaeng Saen campus, Kasetsart University located at Kamphaeng Saen, Nakhon Pathom province in central Thailand. The climate of Thailand is largely tropical with hot and humid conditions all year round. The temperature ranges from 28 to 35 °C. There are three major seasons in Thailand: a cool season (November–February), a hot season (March–May) and a rainy season (June–October).

Experimental animal and treatment protocol

A total of 31 Kamphaeng Saen beef cows (containing 25% native Thai, 25% Brahman and the 50% Charolais) were utilized for this experiment. Cows with a postpartum period greater than 50 d and a body condition score of 5 and 6 (on a scale of 1 to 9) were selected. Selected cows were maintained on pasture during the daytime and housed at night. Artificial insemination is used as the main breeding system utilizing semen from selected bulls. There are two mating seasons; the first is from May to July and the second is from November to January. The experiment was conducted from June to September 2009.

At the start of the experiment, using a simple randomization technique, the experimental cows (within ovarian status) were randomly assigned to one of the two TAI treatments

(treatment 1 = Co-Synch plus CIDR plus TAI at 48 h; treatment 2 = Co-Synch plus CIDR plus TAI at 66 h after $PGF_{2\alpha}$. All animals received the same Co-Synch program containing 2 mL GnRH (Busereline acetate-Receptal; Intervet) on Day 0 (the day on which treatment was initiated) and CIDR containing 1.9 g of progesterone Pfizer Animal Health, New Zealand EAZI-Breed was inserted into the vagina at the time of the first GnRH injection. After 7 d (Day 7) the CIDR was removed and 2 mL $PGF_{2\alpha}$ (estrumate; Intervet) was given intramuscularly. The second GnRH (2 mL) injection was given in combination with AI 48 h after the $PGF_{2\alpha}$ injection for cows in treatment 1 and 66 h after the $PGF_{2\alpha}$ injection for cows in treatment 2 (Figure 1).

Cows were considered to be either in the luteal phase if active corpus luteum (CL) presented on either of the ovaries, or in the follicular phase when the dominant follicle presented without active CL. If a cow was inspected and no visible structures presented on either of the ovaries at the ultrasound examination and rectal palpation within 7 d immediately before the onset of the study (Day 0), then the cow was considered anestrous.

Vaginal electrical resistance measurement

A commercially available device (Ovatec Heritage Genetics, LLC) was used to determine VER on Days 0, 7 and at the time of the second GnRH injection of the synchronization protocol. The portable device consisted of a battery-operated main unit with a digital display and a stainless steel detachable probe. The probe was disinfected before use and tested for calibration as recommended by the manufacturer. The vulvar area of each female was cleaned with a paper towel and the probe was introduced into the vagina by spreading the vulva to avoid contamination. The probe was rotated and moved back and forth 3–4

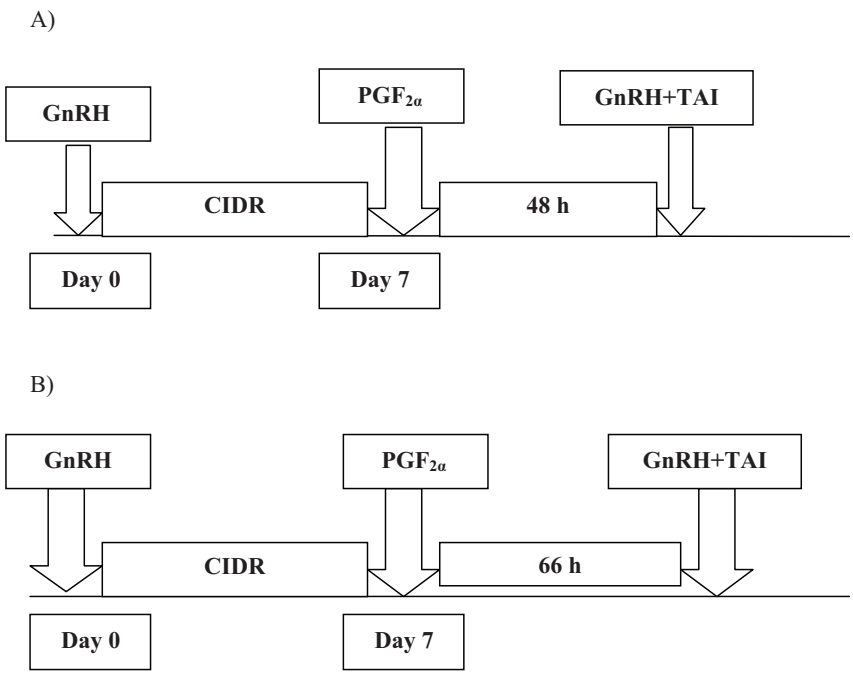

Figure 1 Schematic presentation of Co-Synch plus CIDR protocols: A) Synchronization protocol 1 = Co-Synch plus CIDR plus 48 h TAI; B) Synchronization protocol 2 = Co-Synch plus CIDR plus 66 h TAI.

times and then held in place for 20–30 seconds or until the readings on the display stabilized. After each VER measurement, the surface of the moistened probe and shaft was rubbed with cleaner Largo using a scrub pad and rinsed thoroughly. Then, the probe was wiped from the sensor end to the handle, with undiluted Chlorexidine using a clean paper towel to remove contamination and placed into diluted Chlorexidine solution (0.03%). Before each subsequent measurement, the probe was thoroughly rinsed with water and shaken to remove any excess water.

Ultrasound examination

Trans-rectal ultrasonography was used at Days 0, 7 and at the time of the second GnRH injection of the synchronization protocol to assess the size of the largest follicle. The size of the CL was also measured at Days 0 and 7. All ultrasound examinations were performed by the same operator. Follicles larger than 6 mm in diameter were measured and the largest diameter was recorded. The dominant follicle was defined as the follicle that reached the comparatively largest diameter (Sirois and Fortune, 1988). All palpable CL during rectal palpation with a diameter > 15 mm at the ultrasound examination was considered as active (functional) CL.

Statistical analysis

The frequency distribution was used to determine the ovulation rate to the first GnRH injection (measured by the presence of active CL at Day 7 of the synchronization protocol) and the follicular diameter class (less than 10.7, 10.7–15.7 or greater than 15.7 mm) at the time of $PGF_{2\alpha}$ and the second GnRH injection (Perry *et al.*, 2005).

The experimental design for data (VER and DLF) analysis consisted of days of protocol (first GnRH injection, $PGF_{2\alpha}$ injection and second GnRH injection), time of second GnRH injection plus TAI (48 and 66 h after $PGF_{2\alpha}$) and the ovarian status at the first GnRH injection (luteal phase, follicular phase and anestrous) and their two-way interaction effects. After testing the data for normality and homogeneity of variance, analysis of variance using a GLM procedure was carried out to determine the effects of the day of protocol, time of second GnRH injection plus TAI and the ovarian status at the first GnRH injection and their interaction effects using VER and DLF as dependant variables. Least square means was used for mean separation and differences between means were tested using a t-test.

Pearson correlation analysis was used to evaluate the relationship between DLF and VER at the time of second GnRH plus TAI using data from both treatments.

RESULTS

Ultrasound examination of ovarian structure and VER at first GnRH injection

From the 31 beef cows used in the study, 10 cows (32%) had active CL at Day 0 of the synchronization protocol and were at the diestrous stage of the estrous cycle. Eight cows (26%) had a dominant follicle without CL inside either of the ovaries and the mean diameter (mean ± SD) of these follicles was 11.3 ± 1.2 mm. The remaining 13 cows (42%) had neither CL nor a dominant follicle (greater than 6 mm) in either of their ovaries. The VER records showed that the mean VER (mean ± SE) was lowest (83.2 ± 2.9 Ω) when the dominant follicle presented inside the ovaries and was highest when active CL (97.9 ± 3.0 Ω) or no structure (104 ± 4.5 Ω) presented inside the ovaries.

The overall DLF (mean ± SD) at Day 7 was estimated to be 12.7 ± 3.1. The mean VER at Day 7 was estimated to be 86.7 ± 8.0 Ω (Table 1). About 25 cows had their largest (dominant) follicles at Day 7 and the follicle size was distributed as 35, 46 and 19% for a diameter less than 10.7, 10.7–15.7 and greater than 15.7 mm, respectively (Figure 2).

Table 1 Vaginal electrical resistance and diameter of largest follicle of Co-Synch plus CIDR-treated beef cows.

Protocol	Day of protocol	VER (Ω)	DLF (mm)
2nd GnRH injection + 48 h TAI	1st GnRH injection (Day 0)	93.1±14.1	11.0±2.7
	$PGF_{2\alpha}$ injection (Day 7)	88.9± 9.8	12.6±4.0
	2nd GnRH injection	80.3± 7.1	15.5±4.0
2nd GnRH injection + 66 h TAI	1st GnRH injection (Day 0)	95.3±12.1	9.9±2.5
	$PGF_{2\alpha}$ injection (Day 7)	84.6± 5.4	12.8±2.3
	2nd GnRH injection	78.7± 5.0	16.6±2.7
Both	1st GnRH injection (Day 0)	94.2±12.9	10.5±3.6
	$PGF_{2\alpha}$ injection (Day 7)	86.7± 8.0	12.7±3.1
	2nd GnRH injection	79.4± 6.5	16.1±3.4

Vaginal electrical resistance measured as mean ± SD and diameter of the largest follicle measured as mean ± SD

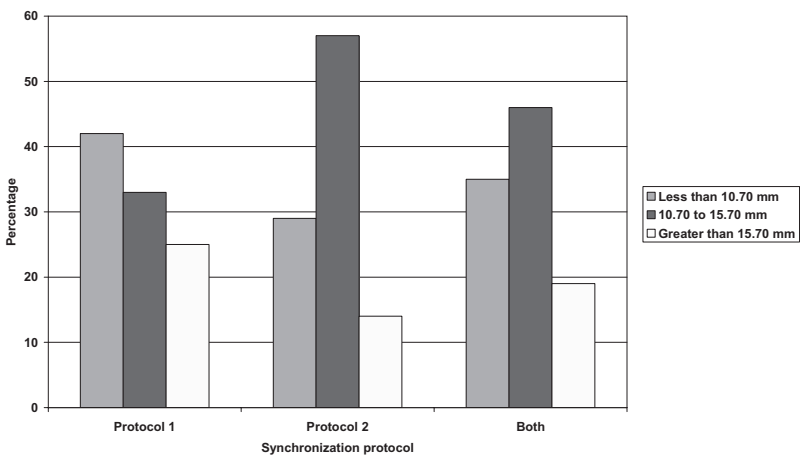

Figure 2 Percentage distribution of diameter of the largest follicle class at the time of $PGF_{2\alpha}$ injection for Co-Synch-treated cows.

The overall mean DLF at second GnRH injection was 16.1 ± 3.4 mm. The corresponding VER was 79.4 ± 6.5 Ω (Table 1). The proportion of cows with the largest follicle less than 10.7, 10.7–15.7 and greater than 15.7 mm in diameter at the second GnRH injection was 13, 32 and 55%, respectively (Figure 3).

The results from analysis of variance using pooled data (Table 2) indicated that the overall mean DLF of 13.7 ± 0.5 mm was significantly affected by the day of the protocol and the ovarian status at the first GnRH injection, while the time of the second GnRH plus TAI was not significantly influenced by DLF. The mean DLF was significantly highest (16.3 ± 0.5 mm; $P < 0.05$) at the second GnRH injection plus TAI and lowest at the time of the first GnRH injection (9.8 ± 1.0). Cows at the follicular phase at the first GnRH injection had the significantly highest DLF (14.9 ± 0.7 mm; $P < 0.05$) compared to cows at the luteal phase (12.3 ± 0.7 mm) or cows with no structure on either of their ovaries (11.8 ± 0.7 mm).

Overall, the mean VER of 86.8 ± 7.8 Ω was significantly influenced by the day of protocol

($P < 0.05$) and the ovarian status at the first GnRH injection ($P < 0.05$), while the effect of the timing of the second GnRH injection plus TAI was not significant (Table 2). The mean VER was significantly ($P < 0.05$) highest at 93.8 ± 1.7 Ω on Day 0 followed by 86.3 ± 1.7 Ω at day 7 (diestrous) and significantly ($P < 0.05$) the lowest at the second GnRH injection plus TAI (79.1 ± 1.7 Ω).

The value of the vaginal electrical resistance in the present study was inversely associated (r = -0.49; $P < 0.005$) with DLF (Figure 4).

DISCUSSION

The changes in the value of VER at different times during ovulation synchronization in the present study followed a similar trend to

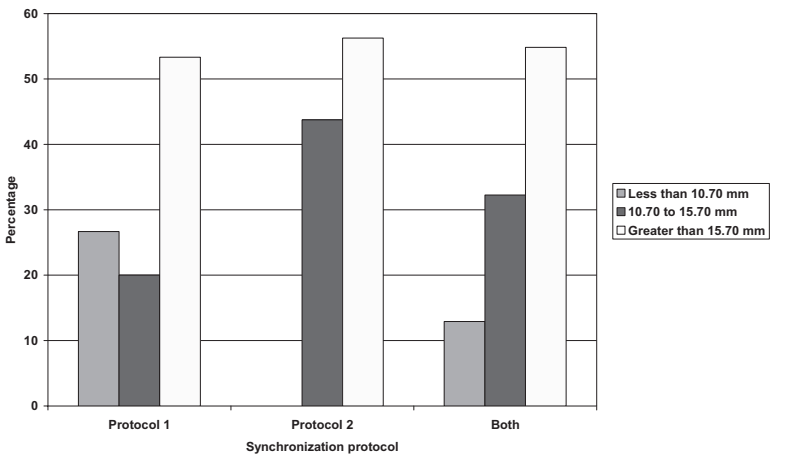

Figure 3 Percentage distribution of diameter of the largest follicle class at the time of second GnRH injection for Co-Synch-treated cows.

Table 2 Vaginal electrical resistance and diameter of the largest follicle of Co-Synch plus CIDR-treated beef cows.

Parameter	Vaginal electrical resistance (Ω)	Diameter of largest follicle (mm)
Protocol		
2nd GnRH injection + 48 h TAI	86.4±1.4[a]	12.7±0.6[a]
2nd GnRH injection + 66 h TAI	85.4±1.4 [a]	13.3±0.6[a]
Day of protocol		
1st GnRH injection (Day 0)	93.8±1.7[a]	9.8±1.0[c]
$PGF_{2\alpha}$ injection (Day 7)	86.3±1.7[d]	12.9±0.6[b]
2nd GnRH injection + TAI	79.1±1.7[c]	16.3±0.5[a]
Ovarian status		
Follicular phase at 1st GnRH injection	82.6±1.9[b]	14.9±0.7[a]
Luteal phase at 1st GnRH injection	88.4±1.8[a]	12.3±0.7[b]
Anestrous at 1st GnRH injection	88.1±1.5[a]	11.8±0.7[b]

Vaginal electrical resistance measured as mean ± SE and diameter of the largest follicle measured as mean ± SE
Different letters in the same column (within a group) denote a significant difference ($P < 0.05$).

previous reports on the estrous cycle of dairy cows (Gartland *et al.,* 1976); heifers (Aboul-Ela *et al.*, 1983); beef cows (Zuluaga *et al.*, 2008) and buffaloes (Gupta and Purohit, 2001). A decrease in VER after $PGF_{2\alpha}$ injection was clearly observed in this study, with the minimum value at the second GnRH injection of the Co-Synch protocol (presumed estrus) when the dominant follicle was present. Similar to the present study, minimum VER values have been reported to be near estrus (Schams *et al.*, 1977; Canfield and Butler, 1989).

The mean diameter of the largest follicle in the present study was similar to the value of 12.2 ± 2 mm reported in beef heifers on day 7 and at the time of the second GnRH injection of the Co-Synch plus CIDR protocol on Brahman × Hereford crossbred cows (Zuluaga *et al.*, 2008). The increased DLF at the second GnRH injection compared to the value at the $PGF_{2\alpha}$ injection for the majority of the cows indicated that luteal regression reduced the progesterone concentration and increased the GnRH pulse allowing the LH pulse frequency to increase, and the dominant follicle present at that time increased its growth. However, the variation in DLF at the second GnRH plus TAI indicated the diverse developmental stage which could be related to the poor synchronization rate in response to the first GnRH injection or the $PGF_{2\alpha}$ injection. A similar diverse developmental status of the dominant follicle at the time of the second GnRH was reported by Vasconcelos *et al.* (1999; 2001) and Peters and Pursley (2003).

The significant correlation between VER and DLF was similar to that reported in previous studies on buffaloes (Markandeya *et al.*, 1993; Gupta and Purohit, 2001). In cattle, a significant correlation between VER and the developmental stage of the largest follicle was reported in Brahman × Hereford crossbred cattle after the ovulation synchronization protocol (Zuluaga *et al.*, 2008). The negative correlation between VER and DLF is the result of increased DLF and decreased VER from the day of the $PGF_{2\alpha}$ injection to the time of the second GnRH injection, with both of them seeming to be controlled by circulating concentrations of estradiol (Wehner *et al.*, 1997; Zuluaga *et al.*, 2008). The dominant follicle produced estradiol and the concentration of this hormone increased with the growth of the dominant follicle during estrus; this hormone regulates the degree of hydration of the vaginal tissue and the electrolytic content of reproductive tract secretions (Lewis *et al.*, 1989). The electrical resistance of the vaginal tissue decreased as the

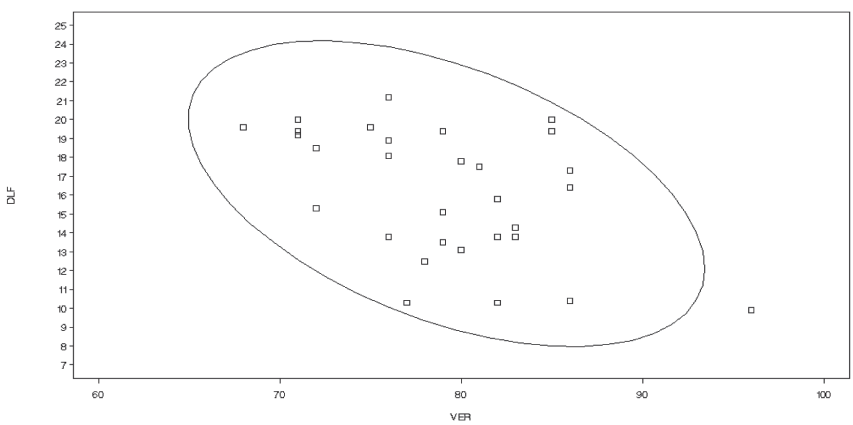

Figure 4 Correlation between diameter of the largest follicle (DLF) and vaginal electrical resistance (VER) at second GnRH injection for Co-Synch plus CIDR-treated cows. The ellipse shows the 95% confidence limits.

estradiol concentration produced by the dominant follicle increased.

CONCLUSION

A single measurement of VER using an Ovatec probe in the present study was able to accurately differentiate between animals in the luteal phase (diestrous) and the follicular phase (estrus) but failed to differentiate between the luteal phase and anestrous beef cows. The significant and declining trend in the VER value during the days of the protocol and the significant differences between the time of CIDR removal and targeted TAI indicated the efficiency of VER for the selection of suitable animals for TAI. The significant correlation between VER and DLF obtained in the synchronized females in this study indicated that VER can be used for the estimation of the stage of follicular maturity and the response to synchronization treatment. However, multiple measurements may be required for increased precision.

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