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Reproductive Characteristics of Holstein Friesian Dairy Breed after Estrous Synchronization with Select-Synch with or without CIDR

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Abstract

The objectives of this study were to determine estrus (ER), conception (CR) and pregnancy rate (PR) of dairy cows after synchronization of estrus and the relationship between synchronization method and vaginal electrical resistance (VER). A total of 52 Holstein Friesian (HF) females were synchronized with Select-Synch or Select-Synch + CIDR protocols. Results showed that the overall ER, CR and PR were 67.30, 55.90 and 37.00 %, respectively. Mean ER was significantly (P < 0.05) higher for Select-Synch + CIDR treatment compared to Select-Synch alone (80.80% versus 53.90 %). Mean PR was higher for Select-Synch + CIDR compared to Select-Synch (46.30% versus 28.00%; P<0.05). The difference in CR between the two treatments was not significant. Cycling females had significantly higher ER (89.70% versus 39.13%; P<0.05) and PR (57.14% versus 13.04%; P < 0.05) compared to non cycling females. The interval from $PGF_{2\alpha}$ injection to onset of estrus was not significantly (P < 0.05) influenced by synchronization methods and ovarian status. Duration of estrus was significantly higher for cycling females compared to non-cycling females. Mean VER was significantly (P < 0.05) higher for females synchronized with Select-Synch compared to Select-Synch + CIDR. Mean VER was significantly highest at Day 0 (106.64±1.90) and lowest (84.20±0.90 ohm) at the time of the onset of estrus in both synchronization methods. Mean Diameter of Largest Follicle (DLF) was significantly higher for cycling females compared to non cycling one (12.4±0.30 versus 11.6±0.50 ohm). The inclusion of CIDR in the Select-Synch estrus synchronization protocol significantly improved ER and PR but CR was not significantly different from Select-Synch protocol. VER may be useful for improving accuracy of estrus detection during artificial insemination program.

Keyword: Holstein Friesian, estrus synchronization, pregnancy rate, vaginal electrical resistance

Introduction

Increased milk production in dairy cattle resulted in declined reproductive performance due to a prolonged inter-calving period (Butler and Smith, 1989). Several factors, such as a longer time period from parturition to first estrus, poor estrus expression or detection, improper timing of artificial insemination (AI), and reduced conception rate at first AI, have contributed to a longer inter-calving period. In doing so, they have affected profitability in dairy farming (Pelissier, 1972; Senger, 1994; Sturman *et al*., 2000). In Ethiopia, the introduction of AI into dairy cattle allowed for genetic improvement of local breed through crossbreeding specially in urban and per-urban areas. However fertility of high grade and pure exotic dairy cattle suffered from poor CR, long postpartum anestrus and calving interval (Kiwuwa *et al*., 1983; Tadesse *et al*., 2010). For a producer to ensure that each cow calves on a yearly basis, cows are required to conceive within 83 days after calving. However, the most critical step during this process is the determination of estrus and subsequent timing of insemination. Inaccurate detection of estrus is claimed to be the single most important problem limiting successful reproductive performance and use of AI in tropical countries (Tadesse *et al*., 2010). In order to improve estrus detection rate, estrus synchronization programs using $PGF_{2\alpha}$ or progesterone that focused on controlling lifespan of corpus luteum (CL) have been used. However estrus was not precisely synchronized with $PGF_{2\alpha}$ as this treatment dose not synchronize growth of follicle but only regulates the lifespan of functional CL. The use of GnRH in combination with $PGF_{2\alpha}$ or progesterone sources to synchronize follicular growth is one of technological advance in reproduction (Pursley *et al.,* 1995; Pursley *et al*., 1997). The Select Synch protocol which is injection of GnRH at day 0 followed by injection of $PGF_{2\alpha}$ at day 7 produced pregnancy rates in dairy females similar to those with two injection of $PGF_{2\alpha}$ (Stevenson *et al*., 2000). One of the drawbacks of Select-Synch protocol is the early expression of estrus before $PGF_{2\alpha}$ in some treated females. The problem of estrus before $PGF_{2\alpha}$ can be solved by addition of CIDR at the time of GnRH and its removal at $PGF_{2\alpha}$ injection (Stevenson *et al*., 2000). Moreover, short-term treatment with CIDR produced tight synchrony of estrus, but fertility was variable across synchronization methods, breed and management level (Xu and Burton, 2000). It is also considered that responses to hormonal treatment in dairy cattle vary among different countries because of climate, nutrition and the nature of management differences. In Ethiopia, HF dairy cattle has been used for milk production however, there is no information about responses to synchronization treatments and estrous characteristics of the breed under Ethiopia condition.

Measuring vaginal electrical resistance value in cattle and in numerous other species can be used as a method for predicting ovarian status and onset of estrus without visual estrus detection (Foote *et al*., 1979). Recent studies in cattle indicated that VER can also be used to estimate stage of follicular maturity and response to synchronization treatment (Zuluaga *et al*., 2008). However a number of studies have reported large within- and between-animal variations in VER measurements at the time of estrus (Elving *et al*., 1983).

The objectives of this study were to determine (i) estrus, conception and pregnancy rate of HF dairy cattle after synchronization of estrus with Select-Synch with or without CIDR, (ii) interval from $PGF_{2\alpha}$ injection to estrus and duration of estrus and (iii) VER and its relation with synchronization method.

Materials and Methods

Location of the Study

The experiment was conducted at Holetta dairy farm (owned by National Artificial Insemination Center) located in central highland of Ethiopia, 34 km west of Addis Ababa in Holetta town. The area receives bimodal rainfall with two rainy seasons in a year. The short rainy season occurs between March and May and main rainy season is during June to September while the dry season from October to February. The average temperature is 15.90ºC with variations between 7ºC and 25ºC. The average monthly rainfall is 90.75 mm. The highest average rainfall is 269 mm occurring in August and the lowest rainfall is 9 mm occurs during month of November. The average relative humidity is about 60.7% and the lowest average relative humidity is 49% which occurs in February; 82% is the highest average monthly relative humidity which occurs in July (long rainy season).

Experimental Animals and Herd Management

Animals were allowed grazing during day time and housed at night in barn. Grass hay constituted the major proportion of the feed supply. Whenever there was a short supply of hay, tef *(Erogrostis tef)* straw was substituted. Milking cows were supplemented with concentrate composed of wheat by-products or maize, (28-30%), Noug seed cake (68-70%) *(Guizotia abyssinica*) and 2% salt while they were milked. Artificial insemination with semen produced from locally recruited bulls from Ethiopian National Artificial Insemination Centre (NAIC) was used. Cows were hand milked twice a day. AI was done based on visual observation of standing heat by herd attendant three times per day; morning time, afternoon and mid night. Animals on the farm were regularly vaccinated against common infectious diseases such as rinderpest, contagious bovine pleuropneumonia, anthrax, blackleg and foot and mouth diseases. Regular preventive treatments were administered against prevalent endo- and ecto-parasites.

A total of 52 HF lactating cows were used for the study. Prior to the start of the experiment, animals were examined via rectal palpation and ultrasonography to confirm their reproductive stage. Based on rectal palpation and ultrasound results, two ovarian status groups were identified; cycling animals with active CL or dominant follicle and non cycling animals with no any visible structures on the ovaries. Animals were assigned randomly (within ovarian status) into one of the two treatments. Treatment: 1. Select-Synch (GnRH + $PGF_{2\alpha}$ (n=26) and Treatment 2. Select-Synch + CIDR (GnRH + CIDR + $PGF_{2\alpha}$ n=26). Animals assigned to Treatment 1 received an intramuscular injection of 2 ml GnRH (Busereline acetate-Receptal;

Intervet) at Day 0 and 2 mL $PGF_{2\alpha}$ (estrumet; Intervet) 7 days later. Animals assigned to Treatment 2 were injected with 2 ml of GnRH at Day 0 and controlled internal drug release (CIDR; containing 1.9 g of progesterone; Pfizer Animal Health, New Zealand, EAZI-Breed) was placed into the vagina at the time of first GnRH injection. After 7 days CIDR was removed and 2 ml $PGF_{2\alpha}$ was given intramuscularly (Figure 1). After $PGF_{2\alpha}$ injection animals were observed for signs of estrus for 30 minutes at 8 h interval three times per day for 5-7 days. Animals observed in estrus were inseminated 12 h after onset of estrus (standing heat).

Estrus was defined as a period of sexual receptivity where two or more mounts received, preceded and followed by a 4 h period of no activity (White *et al*., 2002). Onset of estrus was determined when the first of two mounts were received within a 4 h period. The end of estrus was the last mount received, with a mount 4 h before, and no mounts received during the next 4 h (White *et al*., 2002).

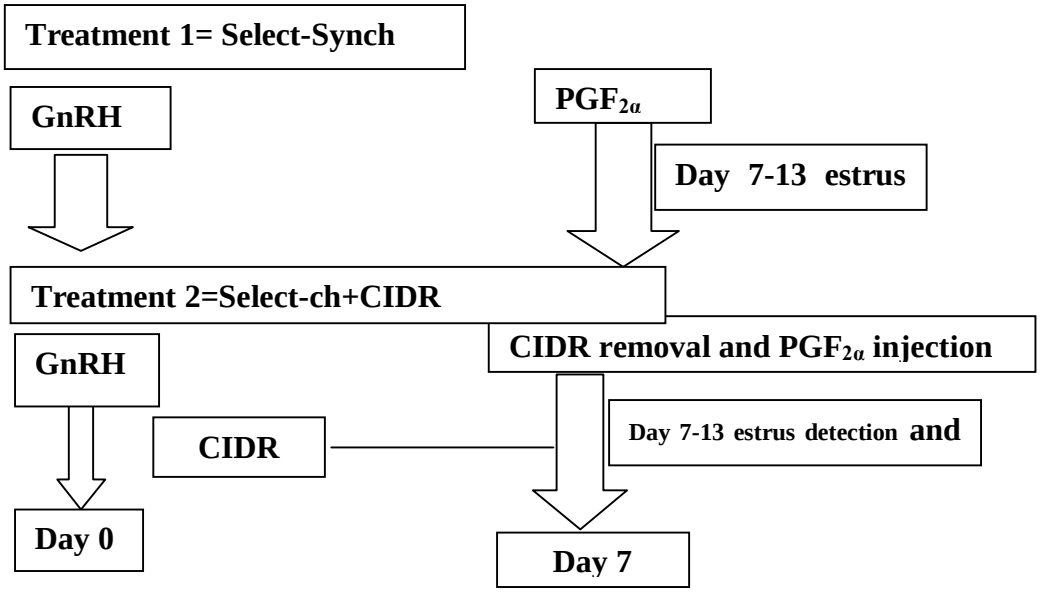

Figure 1 Schematic presentation of estrus synchronization protocol for both treatments

Vaginal electrical resistance was measured using Ovatec unit (Hertage Genetics, USA) at start (Day 0), completion of synchronization treatments (Day 7) and at the time of onset of estrus. The device consists of a battery-operated main unit with a digital display and a stainless steel detachable probe. The probe was disinfected daily before use and tested for calibration. The vulva area of each female was cleaned with a paper towel and the probe was inserted in the vagina by spreading the vulva to avoid contamination. The probe was rotated and moved back and forth 3–4 times and then held in place until the readings on the display stabilized. After each VER determination, the surface of the moistened probe and shaft was rubbed with cleaner largo using scrub pad and rinsed thoroughly. Then the probe was wiped from sensor

end to handle, with undiluted chlorexidine solution using a clean paper towel to remove contamination and then 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.

Diameter of largest follicle (DLF) was measured using trans-rectal ultrasonography (Sonosite ultrasound system, VET, USA) at Day 0, Day 7 and at the time of onset of estrus. A single measurement of the shortest dimension was made. The dominant follicle was defined as the follicle that reached the largest diameter (Sirois and Fortune, 1988).

Pregnancy status was determined by ultrasound examination of uterus at Day 36 post insemination and confirmed by manual rectal palpation (Roberts, 1986) 60 days after insemination.

Data Analysis

- Estrus rate, CR and PR were determined for the effect of estrus synchronization method and ovarian status at the time of GnRH injection using frequency distribution and Chi-square test
- Estrus rate was calculated as proportion of females in estrus divided by total number of treated females
- Conception rate was calculated as total number of cows conceived to first AI divided by total number of females inseminated.
- Pregnancy rate was the total number of pregnant animals divided by total number treated females.
- Data on interval from $PGF_{2\alpha}$ to estrus and duration of estrus were analyzed by General Linear Model procedure. In this model synchronization method and ovarian status at start of treatment were used as class variables while interval from $PGF_{2\alpha}$ to estrus and duration of estrus as dependant variables. Least square means were used to compare means.

Data on VER value and DLF were also analyzed by General Linear Model procedure using VER and DLF as dependant variable, while synchronization method, ovarian status and days of protocol as class variables. Least square means and standard error were used to describe change in VER and DLF within synchronization method, ovarian status and days of protocol.

Results and Discussion

Estrus, Conception and Pregnancy Rate

Results on ER, CR and PR were presented in Table 1 and figure 2. The overall mean ER, CR and PR were 67.30, 55.90 and 37.00%, respectively. ER was significantly ($P < 0.05$) higher for Select-Synch + CIDR treatment compared to Select-Synch alone (80.80% versus 53.90%). Pregnancy rate was higher for cows synchronized with Select-Synch + CIDR compared to those synchronized with Select-Synch (46.30% versus

28.00%; P < 0.05). ER and PR were significantly higher (P < 0.05) for cycling females compared to non cycling females, while the difference in CR between the two groups was not significant.

Table 1. Estrus rate, CR and PR by different sources of variations

Parameter		ER (%)	CR (%)	PR (%)
Treatment				
	Select-Synch	53.9 (14/26)^{B}	53.9(7/13) ^{A}	28 (7/25)^{1A}
	Select-Synch + CIDR	80.8 (21/26)^{A}	57.14(12/21)^{A}	46.3 (12/26) ^{B}
varian status				
	Cycling	89.7(26/29)^{A}	64(16/25) ^{A}	57.1(16/28)^{A}
	Non-cycling	39.13(9/23)^{B}	33(3/9) ^{A}	13(3/23)^{B}
Overall		67.3(35/52)	55.9(19/34)	37(19/51)

[1]One cows in Select-Synch treatment died after insemination;
Values in parenthesis indicated number of animals observed over total number of animals
Within a column mean values followed by different letters are significantly different (P < 0.05)

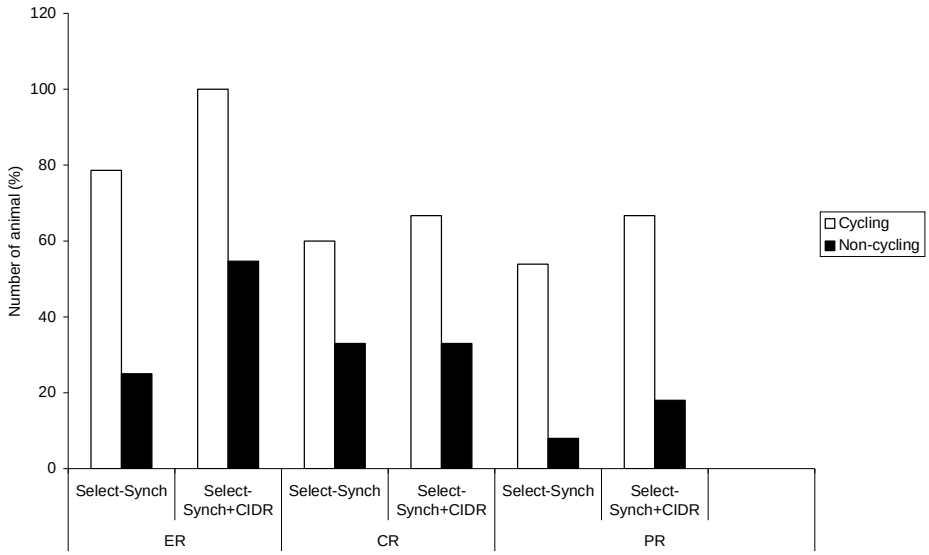

Figure 2. Distribution of estrus, conception and pregnancy by synchronization method and ovarian status at GnRH injection (P < 0.05)

The use of CIDR in Select-Synch protocol produced estrus detection rate that was similar to those observed in lactating dairy cows (76-89%; Ryan *et al*., 1995) and dairy heifers (94%; Lucy *et al*., 2001) treated with CIDR inserts. Moreover, Richardson *et al.* (2002) also found estrus detection rate of 79 and 73% for dairy cattle treated with

Select-Synch + CIDR and Select-Synch alone, respectively. Twagiramungu *et al*. (1992) and Geary *et al*. (2000) reported ER of 83.3 and 87.5%, respectively in lactating cow treated with Select-Synch which was higher than the result obtained with Select-Synch protocol in present study.

The lower ER observed in cows synchronized with Select-Synch compared to Select-Synch + CIDR treatment in present study could be due to the poor responses of non cycling females in Select-Synch treatment. When the percentage of cows cycling at the start of the breeding was less than 50%, Stevenson *et al.* (2000) also observed a substantial decrease in ER of *Bos taurus* cows synchronized with the Select-Synch protocol. In present study the inclusion of CIDR in Select-Synch appeared most effective in cycling cattle compared to non cycling one but it also induced estrous cycle in some anestrous females.

The overall CR and PR in present study were in agreement with CR of 47.5 and 57.8% and PR of 34.9 and 45.7% reported in dairy breed treated with Select-Synch and Select-Synch+CIDR, respectively (Richardson *et al*., 2002). In similar way using Select-Synch protocol Stevenson *et al*. (2000) also found similar CR and PR of 65% and 55%, respectively for cycling females however the CR of 66.00% and PR of 27% reported by the same authors for non cycling females are higher than the results obtained for non cycling females in the present study. The lower PR of non cycling females compared to cycling females in present study was attributed to lower ER of non cycling females.

The interval from $PGF_{2\alpha}$ injection to estrus was not influenced by any of the factors (Table 2). The overall mean interval from $PGF_{2\alpha}$ injection to onset of estrus was 50.24 ± 2.02 h ranging from 21 to 72 h. Distribution of animals across interval to estrus group was presented in Figure 3 for both treatments. The majority of females in both treatments were observed in estrus between 24 to 48 h after $PGF_{2\alpha}$ injection. Seven percent of females in Select-Synch treatment showed estrus between 0 to 24 h after $PGF_{2\alpha}$ injection (Figure 3). The distribution of number of cycling and non cycling females across interval to estrus was presented in Figure 4. The majority of animals in both groups were in estrus within 24-48 h after $PGF_{2\alpha}$ injection. Four percent of cycling females were observed early in estrus within 0-24 h after $PGF_{2\alpha}$ injection

Table 2. Mean Interval from $PGF_{2\alpha}$ injection to estrus and duration of estrus by treatment and ovarian status

			$PGF_{2\alpha}$-estrus interval (h)	Duration of estrus (h)
Treatment		n	Means	Means
	Select-Synch	13	52.34 ± 4.14^{A}	18.90 ± 1.10^{A}
	Select-Synch + CIDR	20	48.85 ± 2.02^{A}	20.33 ± 1.00^{A}
Ovarian status				
	Cycling	24	51.24 ± 2.50^{A}	21.03 ± 1.00^{A}
	Non-cycling	9	48.22 ± 3.40^{A}	18.21 ± 1.20^{B}
Overall mean		33	50.24 ± 2.02	20.41 ± 1.00

Within column means followed by different letter significantly different ($P < 0.05$)

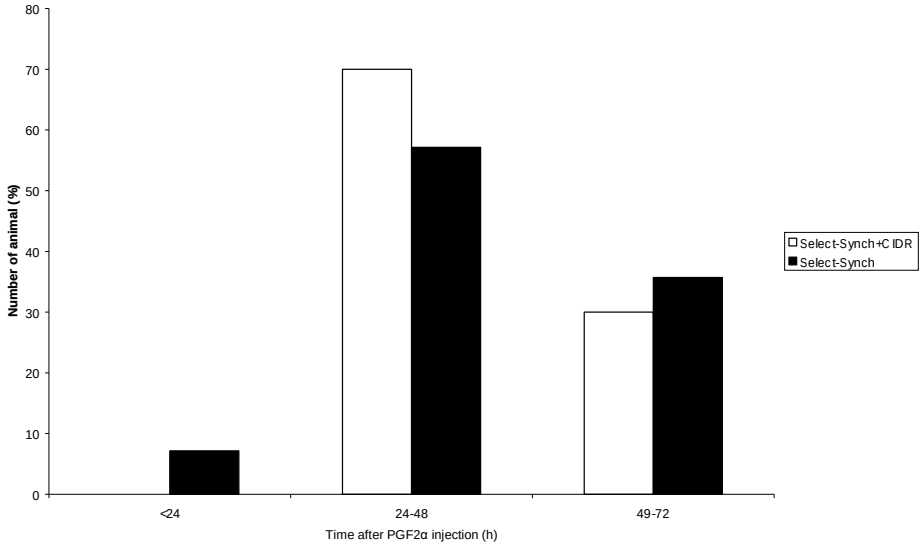

Figure 3 Distributions of animals across interval to estrus group by synchronization method

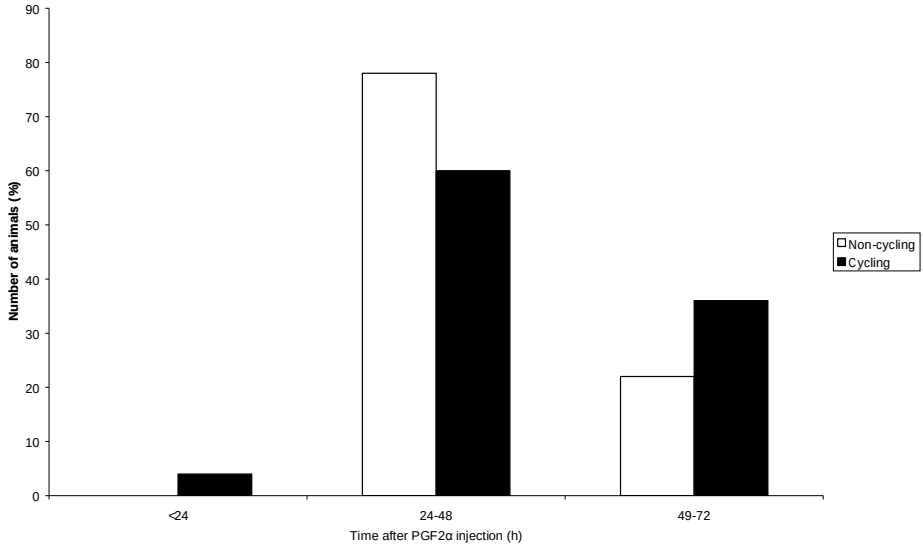

Figure 4. Distributions of animals across interval to estrus groups by ovarian status at GnRH injection

Mean interval from $PGF_{2\alpha}$ injection to estrus in present study was shorter than the interval of 66.00 ± □3.00 h reported for dairy cattle treated with Select-Synch + CIDR (Richardson *et al.,* 2002). Moreover, the present result on interval to estrus was

consistent with 56% of lactating dairy cows detected in estrus within 2 days after CIDR removal (Xu and Burton, 2000). These indicated the opportunity for fixed-time AI if onset of ovulation is further synchronized with an injection of estradiol or GnRH 54-66 h after $PGF_{2\alpha}$.

The overall mean duration of estrus was 20.41 ± 1.00 h ranging from 13.00 to 28.00 h. Mean duration of estrus was not influenced by synchronization methods however, significantly ($p < 0.05$) longer for cycling females compared to non cycling one (Table 2). The mean duration of estrus that was obtained in present study was of similar duration as reported in Holstein cows (20.30 ± 10.4 h; Lyimo *et al*., 2000) but longer than the duration of 11 ± 0.7 h; for Select-Synch and 12 ± 0.6 h for Select-Synch + CIDR protocol reported in dairy cattle (Richardson *et al*., 2002). In similar way duration of estrus were reported to be shorter 8.9-15.4 h (Walker *et al*., 1996; Xu *et al*., 1998). These differences could be related to difference in detection method, frequency and the type of behavioral estrus considered and milk production level. The short duration of estrus reported in dairy cattle was not evidenced in the herd under present study probably due to lack of genetic selection for increased milk production.

Vaginal Electrical Resistance (VER), DLF and Synchronization Methods

Least square means VER and DLF are presented in Table 3. Synchronization method and day of protocol were significantly influenced VER ($P < 0.05$) while, the effect of ovarian status at the time of GnRH injection was not significant. Mean VER was significantly higher for Select-Synch (102.18 ± 2.50 ohm; $P < 0.05$) compared with Select-Synch + CIDR (94.75 ± 1.60 ohm). Mean VER was significantly highest ($P < 0.05$) at Day 0 and Day 7, and lowest (84.01 ± 0.90 ohm) at the time of estrus. At Day 7 of synchronization protocol, mean VER was significantly ($P < 0.05$) higher for cows synchronized with Select-Synch compared to those synchronized with Select-Synch + CIDR. However, at the time of GnRH injection and at the time of estrus the difference between the two synchronization methods was not significant (Figure 5).

Diameter of largest follicle was significantly influenced by day of protocol ($P < 0.05$), while the effect of synchronization method and ovarian status were not significant. Mean DLF was significantly ($P < 0.05$) highest at the time of estrus (14.30 ± 0.20 mm). The difference between DLF at Day 0 and Day 7 of synchronization protocol was not significant (Table 3).

Table 3. Results on mean VER and DLF by treatment, day of protocol and ovarian status

Parameter		VER (ohm) N	VER (ohm) Mean±SE	DLF (mm) N	DLF (mm) Mean±SE
Treatment					
	Select-Synch	35	102.18 ± 2.50^{A}	41	11.80 ± 0.40^{A}
	Select-Synch + CIDR	62	94.75 ± 1.60^{B}	53	12.20 ± 0.30^{A}
Day of protocol					
	Day 0	33	106.64 ± 1.90^{A}	20	11.30 ± 0.20^{B}
	Day 7	31	104.54 ± 1.80^{AB}	43	10.50 ± 0.30^{B}
	Day of estrus	33	84.20 ± 0.90^{C}	31	14.3 ± 0.20^{A}
Ovarian status					
	Cycling	73	96.37 ± 1.70 ^{A}	68	12.40 ± 0.30^{A}
	Non-cycling	24	100.56 ± 2.00 ^{A}	26	11.60 ± 0.50^{A}
Overall mean		97	96.24 ± 1.30	94	12.21 ± 0.24

Within a column mean values followed by different letters are significantly different (P < 0.05)

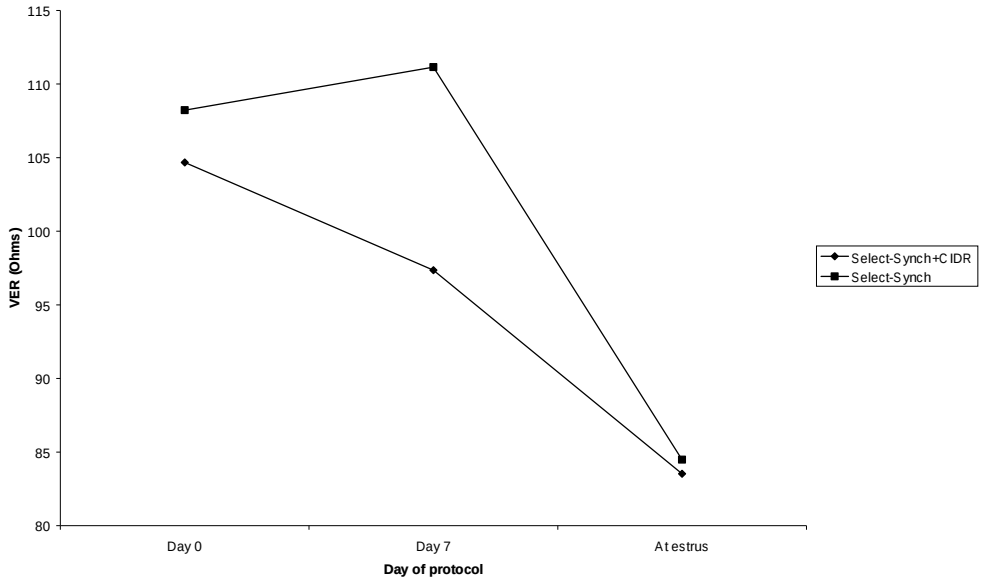

Figure 5 Chang in VER at different times of estrus synchronization protocols by synchronization methods

The significantly lower VER at estrus compared to the value at $PGF_{2\alpha}$ injection is in agreement with previous finding who have reported higher VER at diestrus and lower one at estrus (Heckman *et al*., 1979; Wehner *et al*., 1997; Meena *et al*., 2003). In agreement with our result, recent studies also found significantly lower VER at estrus than at $PGF_{2\alpha}$ injection (Zuluaga *et al*., 2008; Hockey *et al*., 2009). This indicated that VER can be used to predict time of onset of estrus and insemination. However, the lower VER for females synchronized using Select-Synch + CIDR method at Day 7 (when progesterone could be high) in the absence of significance difference in DLF

could be attributed to presence of a CIDR during 7 days period might generate inflammation of the vaginal mucosa, increasing the amount of edema that eventually could reduce VER of vaginal mucosa (Lewis *et al*., 1989; Zuluaga *et al*., 2008).

Conclusions

The present study showed that the inclusion of CIDR in Select-Synch protocol significantly improved ER and PR however interval to estrus, duration of estrus, CR was not significantly different from Select-Synch protocol. Ovarian status at the beginning of the treatment significantly influenced ER and PR. The higher ER and PR observed for cycling female in both treatments indicated that the need for adjusting estrous synchronization program in a manner to ensure that physiologically mature follicles or functional CL are present in the ovary when treatments are administered to synchronize estrus.

The significantly lower VER at the time of estrus and higher value at $PGF_{2\alpha}$ injection indicated the potential for estrus detection in dairy cattle.

Acknowledgments

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