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The Use of Vaginal Electrical Resistance to Diagnose Estrus and Early Pregnancy and Its Relation with Size of the Dominant Follicle in Dairy Cattle

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ABSTRACT

The use of vaginal electrical resistance (VER) for estrous detection and early pregnancy diagnosis were evaluated using three experiments in dairy cattle, in Ethiopia. VER was measured: 1) during the natural estrous (n = 60) cycle, 2) during estrus induced by GnRH+ PGF_{2}. (n = 11) and 3) during post insemination in dairy cows synchronized with GnRH+CIDR+PGF_{2}. (n = 25). Results from Experiment 1 indicated that VER (mean ± SD) during pro-estrus, estrus, metestrus, diestrus and anestrous was 89.6 ± 3.1, 82.2 ± 2.2 102.9 ± 3.1, 106.8 ± 1.4 and 119.7 ± 1.7 ohm, respectively. Results from Experiment 2 indicated that VER was highest (102.4 ± 2.0 ohm; $P < 0.001$) at the time of PGF_{2}. injection and significantly declined to 82.3 ± 0.7 ohm at the time of estrus and increased (94.9 ± 2.0 ohm; P < 0.001) 24 h after estrus. The mean diameter of the largest follicle (DLF) was highest (14.3 ± 0.1 mm; $P < 0.001$) at estrus and lowest (10.0 ± 0.1) at the time of PGF_{2}. injection. The VER value was inversely correlated ($r = -0.50$; $P < 0.001$) with DLF. Results from Experiment 3 showed that VER significantly ($P < 0.01$) declined from day 19 to day 21 post insemination in non pregnant cows compared with pregnant cows. The study proved that VER can effectively be used for estrus detection and can be used to diagnose early pregnancy (day 18–21 post insemination) in dairy cows.

Keywords: dairy cattle, early pregnancy, estrus, vaginal electrical resistance

INTRODUCTION

Cattle breeding herds that utilize artificial insemination (AI) in their reproductive management and genetic improvement require accurate and practical methods to detect estrus and pregnant animals (Heersche and Nebel, 1994). The most common estrous detection method is to observe animals in standing estrus. However, not all ovulating animals display standing estrus (van Eerdenburg *et al*., 1996). In addition, identification of estrus in indoor-housed urban and peri-urban dairy cattle is practically difficult due to restricted movement and contact with other animals to observe any expression of standing estrus.

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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; Meena *et al*., 2003). VER has been studied in cattle and in numerous other species as a method for predicting ovarian status without visual estrus detection (Schams *et al*., 1977; Foote *et al*., 1979). A number of studies have reported large within- and between-animal variations in absolute VER measurements at the time of estrus (Elving *et al*., 1983). In Ethiopia, there is no information available on the use of VER for estrus detection and early pregnancy diagnosis in cattle.

The objectives of this study were to determine baseline VER readings, changes in VER around estrus and their correlation with DLF. In addition, the use of VER in predicting early pregnancy (day 18–21) in synchronized dairy cows was determined.

MATERIALS AND METHODS

Location of the study

The experiments were conducted at the Holetta dairy farm located 34 km west of Addis Ababa city in Holetta town, Ethiopia. The area receives bimodal rainfall with two rainy seasons in a year. The short rainy season occurs between March and May and the main rainy season is during June to September, while the dry season is from October to February.

Experimental animals and herd management

Grass hay constituted the major proportion of the feed supply. Whenever there was a short supply of hay, tef *(Erogrostis tef)* straw was substituted. While they were milked, milking cows were supplemented with concentrate composed of wheat by-products or maize, (28–30%), Noug seed cake (*Guizotia abyssinica*, 68–70%) and salt (2%). The amount of concentrate offered depended on the volume of milk from each cow. AI with semen produced from locally recruited bulls from the Ethiopian National Artificial Insemination Centre was used. AI was undertaken based on visual observation of standing heat by the herd attendant three times per day—namely, in the morning, the afternoon and at midnight. Animals were allowed to graze during daytime and were housed at night in a barn. Cows were hand milked twice a day. Animals on the farm were regularly vaccinated against common infectious diseases such as rinderpest, contagious bovine pleuropneumonia (CBPP), anthrax, blackleg, and foot and mouth disease. Regular preventive treatments were administered against prevalent endo- and ecto- parasites.

Experiment 1: Ovarian structure and VER measurement during natural estrous cycle

A total of 60 dairy cows were selected from the Holetta dairy herd to study herd base VER readings and changes in VER at different stages of the natural estrous cycle. Selected animals were examined via rectum and the ovarian structure was identified by rectal palpation and trans-rectal ultrasound scanning. Two examinations were carried out per animal at 12 h intervals and VER was measured and recorded for individual cows. On the basis of the ovarian structures and presence or absence of uterine tone, the stage of the estrous cycle was identified and classified accordingly. The stage of estrous cycle was also validated on the basis of records of estrus. An ovary containing neither corpus luteam (CL) nor follicles with a flaccid uterus was considered as anestrus.

Vaginal electrical resistance measurement

An Ovate device (Heritage Genetics, LLC) was used to determine VER. 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 introduced into the vagina by spreading the vulva to avoid contamination. The probe was rotated and moved back and forth 4–5 times and then held in place until the readings stabilized. After each VER measurement, the surface of the 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 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.

An analysis of variance repeated measurement model was used to evaluate changes in VER during the different stages of the estrous cycle. Testing of least square means and a t-test were applied to determine any significant differences ($P < 0.05$) between the stages of the estrous cycle.

Experiment 2: Change in VER and DLF in cows synchronized with GnRH+$PGF_{2\alpha}$

Mature Holstein Friesian (HF) dairy cows (n = 15) with postpartum period greater than 50 d and body condition scores of 5 and 6 (on a scale from 1 to 9) were selected and synchronized with a GnRH+$PGF_{2\alpha}$ program containing 2 mL GnRH (Busereline acetate-Receptal; Intervet) on Day 0 (day at which treatment was initiated) and 2 mL $PGF_{2\alpha}$ (estrumate; Intervet) were given at day 7 of the synchronization program. After $PGF_{2\alpha}$ injection, animals were observed for sign of estrus for 30 min at 8 h intervals three times per day for 5 d. VER measurements were taken at $PGF_{2\alpha}$, and at 12 intervals twice per day after $PGF_{2\alpha}$ injection for 5 d.

Trans-rectal ultrasonography (Sonosite ultrasound system, VET 180 plus, L52/10-5 MHs Transducer, USA) was used to measure DLF at the time of $PGF_{2\alpha}$ injection and at 24 h intervals until the date of onset of estrus. Follicles greater than 6 mm in diameter were measured, images were frozen on the screen at maximal size, and a single measurement of the shortest diameter was made. All ultrasound examinations were performed by the same operator. The dominant follicle was defined as the follicle that reached the largest diameter (Sirois and Fortune, 1988).

Changes in the VER values around induced estrus were evaluated by analysis of variance using a repeated measurement model. Least square means and standard error were used to describe changes in the VER values within the day (12 h interval) and day-to-day around estrus after $PGF_{2\alpha}$ injection.

Data on DLF were also analyzed using an analysis of variance repeat measurement model and least square means were used to describe the day-to-day changes in VER. Pearson's correlation coefficient was used to determine the correlation between VER and DLF around estrus and any correlation between VER values during estrus.

Experiment 3: Change in VER post insemination in dairy cow synchronized with GnRH+ CIDR+$PGF_{2\alpha}$

Mature Holstein Friesian (HF) dairy cows (n = 40) with postpartum period greater than 50 d and body condition scores of 5 and 6 (on a scale from 1 to 9) were selected and synchronized with GnRH+CIDR+$PGF_{2\alpha}$ program containing 2 mL GnRH (Busereline acetate-Receptal) on Day 0, and a controlled internal drug release unit (CIDR; containing 1.9 g of progesterone; Pfizer Animal Health, New Zealand, EAZI-Breed) was placed 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) was given intramuscularly. After the $PGF_{2\alpha}$ injection, animals were observed for signs of estrus for 30 min at 8 h intervals three times per day for 5–7 d.

Cows (n = 25) observed in estrus were inseminated 12 h after the first sign of heat with frozen semen, after confirmation of estrus by rectal palpation. VER was measured on Days 0, 3, 6, 10, 13, 18, 19, 20 and 21 post-insemination. Pregnancy status was determined at Day 36 after AI, using an ultrasound device.

Statistical analysis: Analysis of variance was used to determine the effect of pregnancy status on VER values using pregnancy status (pregnant and not pregnant) as the independent variable and the VER value as the dependent variable. Least square means were used to compare VER values between pregnant and non pregnant cows on Days 0, 3, 6, 10, 13, 18, 19, 20 and 21 post-insemination. Pearson correlation coefficient analysis was used to determine the correlation between VER values during Days 3, 6, 10, 13, 18, 19, 20 and 21 post-insemination.

RESULTS

Ovarian structure and VER measurement at natural estrous cycle

The various ovarian structures identified and the VER values recorded (Table 1) showed that VER was significantly lowest when a mature graafian follicle was palpable on either of the ovaries (estrus) and highest when a mature CL (diestrus) or no structure (anestrus) was palpable on the ovaries. The mean VER values during pro-estrus, estrus, metestrus, diestrus and anestrus are presented in Table 1. Statistically, all these values were highlysignificantly ($P < 0.01$) different from each other, except for values recorded during metestrus and diestrus which were not significantly different ($P > 0.05$).

Changes in VER and DLF around estrus in animal synchronized with GnRH+$PGF_{2\alpha}$

A total of 15 cows were treated, from which 11 cows responded to synchronization treatment. Of the 11 cows, six showed behavioral estrus within 48 h after $PGF_{2\alpha}$ treatment while the remaining five cows showed behavioral estrus 72 h after $PGF_{2\alpha}$ treatment. The changes in VER after $PGF_{2\alpha}$ injection around estrus (Day 0 = estrus) are presented in Figure 1. The fall in VER was rapid and occurred 1 d after $PGF_{2\alpha}$ injection for all cows. The lowest VER values were recorded at different times (48 and 72 h) after $PGF_{2\alpha}$ injection. Mean VER was highest at $PGF_{2\alpha}$ injection and highly significantly declined ($P < 0.001$) at estrus and highly significantly ($P < 0.001$) increased 24 h after estrus. Mean VER values were not significant ($P > 0.05$) one day before and after estrus. Within-day differences (12 h interval) in VER were not significant ($P > 0.05$).

The correlation coefficients between VER values at different times during estrus are presented in Table 2. The highest correlation ($r = 0.97$; $P < 0.001$) was observed between 24 h and 48 h after $PGF_{2\alpha}$ injection

Results from analysis of variance on DLF indicated that the overall mean DLF was estimated to be 12.4 ± 0.4 mm and significantly increased

Table 1 Least square mean VER values according to stage of estrous cycle (natural).

Stage of estrous cycle	n[1]	Mean ± SE (ohm)
Anestrus	26	119.7 ± 1.7[a]
Diestrus	44	106.8 ± 1.4[b]
Estrus	10	82.2 ± 2.2[e]
Metestrus	8	102.9 ± 3.1[bc]
Proestrus	8	89.6 ± 3.1[d]

Within column means followed by different letter are highly significantly different ($P < 0.001$).

[1] = number of records; SE = standard error of the mean.

from day of PGF_{2}. injection to estrus. Mean DLF was 10.0 ± 0.1, 12.9 ± 0.1 and 14.3 ± 0.1 mm at PGF_{2}. injection, one day before estrus and at estrus, respectively and highly significantly (P < 0.001) highest at estrus when the VER value was lowest. The VER value in the current study was inversely associated ($r = -0.50$; $P < 0.001$) with DLF.

Change in VER post insemination in dairy cow synchronized with GnRH+CIDR+$PGF_{2\alpha}$

Vaginal electrical resistance values during the sexual cycle of cows (post insemination) are presented in Figure 2. Mean VER values determined on Days 0, 3, 6, 10, 13 and 18 post-insemination were not significantly different ($P > 0.05$) in both pregnant and non pregnant animals. However, mean VER declined from day 18 to 21 post insemination for non pregnant cows compared with VER values for pregnant cows which increased during the same time. VER measured at Day 19, 20 and 21 were significantly higher ($P < 0.01$) for pregnant cows compared to non-pregnant cows (Figure 2).

The correlation between VER on different days (Days 3 to 21) post AI is presented in Table 3. The highest correlation coefficients

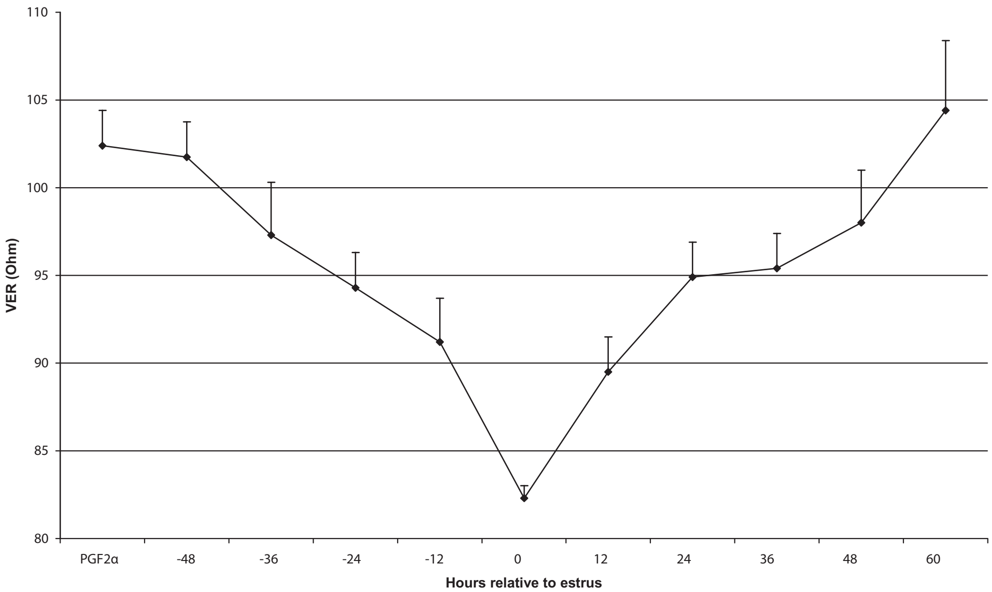

Figure 1 Change in VER around estrus after $PGF_{2\alpha}$ injection in dairy cows synchronized with GnRH+$PGF_{2\alpha}$ (0 = day of estrus). The vertical bars indicate the standard error.

Table 2 Correlation coefficients between VER values during estrus induced by GnRH+$PGF_{2\alpha}$ in dairy cattle.

Time after $PGF_{2\alpha}$ injection (h)	Time after $PGF_{2\alpha}$ injection (h)				
	0	24	48	72	96
0		0.43	0.44	0.10	0.02
24			0.97*	-0.35	0.01
48				-0.26	0.02
72					0.28

* = Significant difference ($P < 0.05$).

were observed between Days 10 and 13 (r = 84; $P < 0.05$). There was also a significant ($P < 0.05$) correlation between VER during the period Days 19–21.

DISCUSSION

The overall VER values observed in the present study were relatively greater than the VER values reported previously by Wehner *et al*. (1997) and Scipioni and Foote (1999), and similar to the VER values reported more recently (Zuluaga *et al*., 2008; Hockey *et al*., 2009). These differences may have been the result of changes in intra-vaginal probe designs since the earlier studies. Other factors influencing the results could include: the depth of probe insertion in the vagina (Aboul-Ela *et al*., 1983); the position of the probe within the vagina (Foote *et al*., 1979 and Heckman *et al*., 1979); pressure against the mucus membrane and pathological conditions of the reproductive tract (Leidl and Stolla, 1976).

The changes in VER recorded in relation to various ovarian structures or estrous cycle stages in the present study followed similar trends in earlier reports (Schams *et al*., 1977; Aboul-Ela *et*

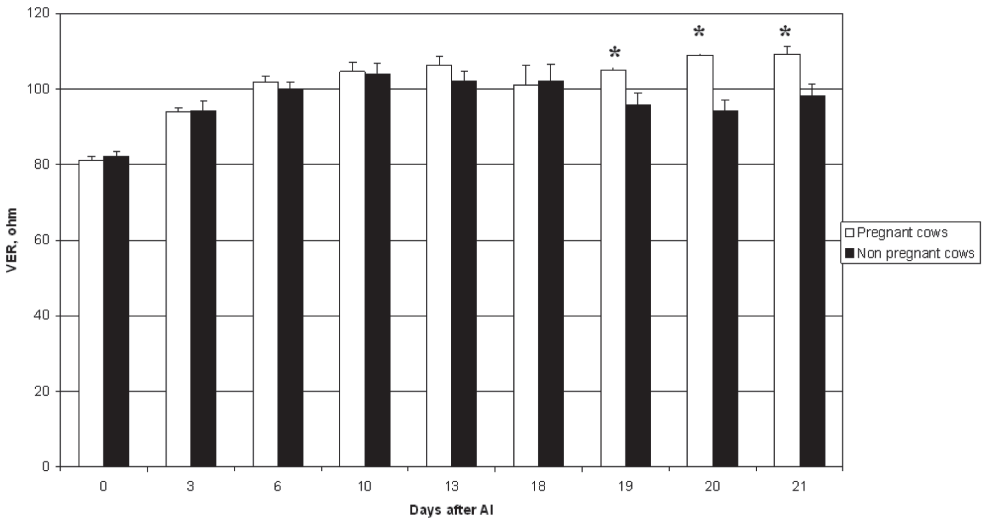

Figure 2 Variation in vaginal electrical resistance (VER) between pregnant and non pregnant cows post AI; * = Significantly different ($P < 0.05$). The vertical bars indicate the standard error.

Table 3 Correlation coefficients between VER values measured during Days 3, 6, 10, 13, 18, 19, 20 and 21 post AI in dairy cattle.

	Day 3	Day 6	Day 10	Day13	Day 18	Day 19	Day 20	Day 21
Day 3		0.60*	0.37	0.26	-0.18	-0.13	0.06	0.36
Day 6			0.58*	0.44*	-0.49*	-0.02	0.18	0.65*
Day 10				0.84*	-0.09	-0.06	-0.07	0.36
Day 13					0.18	0.30	0.16	0.45*
Day 18						0.48*	-0.15	-0.20
Day 19							0.55*	0.53*
Day 20								0.60*

* = Significant difference ($P < 0.05$).

al., 1983; Canfield and Butler, 1989) and were in agreement with more recent reports in cows (Meena *et al*., 2003; Zuluaga *et al*., 2008), and in buffaloes (Gupta and Purohit, 2001). A decrease in VER at estrus in cows and buffaloes could be the result of increased hydration and congestion of the vaginal mucus membranes (Gupta and Purohit, 2001) that has been closely associated with the onset of behavioral estrous in sheep Bartlewski *et al*. (1999) and sows Dusza *et al*. (1996) and is apparently due to increasing estradiol and decreasing progesterone concentrations at this time.

The significant correlation between VER and DLF in the present study was similar to that reported in previous studies on buffaloes (Markandeya *et al*., 1993; Gupta and Purohit, 2001). Zuluaga *et al*. (2008) also found a significant correlation between VER and DLF in a Brahman- Hereford cross. The significant increase in DLF from the day of $PGF_{2\alpha}$ injection to the day of onset of behavioral estrus and the significant reduction in VER value during the same period could have been due to an increase in estradiol and a decrease in the progesterone level during this period (Wehner *et al*., 1997).

In the present study, a reduction in VER at estrus and an increase in diestrus during the estrous cycle was also clearly observed in synchronized pregnant and non pregnant cows. Similarly, changes in VER with an increase during estrus (Heckman *et al*., 1979; Scipioni and Foote, 1999) and an increase during diestrus (Lewis *et al*., 1989; Tasal *et al*., 2005) in the sexual cycle have been reported in cattle. The maximum VER value (greater than 100 ohm) obtained in the present study at diestrus for pregnant cows was similar to the mean VER values (greater than 110 ohm) of diestrus and pregnant cows (Tasal *et al*., 2005). Similar peak VER values were also reported for pregnant animals during the diestrus stage of the estrous cycle (Robinson *et al*., 1970; Leidl and Stolla, 1976).

In the present study, the lower VER values on Days 19–21 after insemination in non-pregnant cows compared with pregnant cows are similar to those reported by Nebel *et al*. (1988), Scipioni and Foote (1999) and Tasal *et al*.(2005). However, Hockey *et al*. (2009) using single VER measurements at approximately 3 and 9 weeks following insemination was unable to accurately diagnose the pregnancy status in individual animals. This could have been related to a large variation in individual animals in their return to the estrous interval, meaning that some proportion of the non-pregnant females may have returned to estrus on Days 18 and 19 and were in diestrus on Days 20–21 and therefore had higher VER values which reduced the difference between the observed pregnant and non-pregnant VER values.

In the present study, by measuring VER on Days 18, 19, 20 and 21 post inseminations, it was possible to detect a significance difference in VER values between pregnant and non pregnant cows. These results indicated the benefit of taking multiple measurements (Days 18–21) to capture the variation in the day of return. The ability to accurately identify animals that have returned to service at an early stage from Days 18–21 post insemination would greatly improve the efficiency of artificial insemination programmes. Failure to inseminate return animals increases the number of days to conception and increases the risk of culling because of delayed or non-conception, and inseminating early pregnant animals can lead to iatrogenic abortion (Cavestany and Foote, 1985; Sturman *et al.,* 2000).

CONCLUSION

It can be concluded that VER values of vaginal mucus can be satisfactorily used to predict the ovarian status and stage of the estrus cycle. By measuring and plotting changes in VER, it was possible to estimate the time of onset of estrus and insemination. While results of the current study indicate VER in dairy cows may play a role in farm pregnancy diagnosis (Days 18–21 post insemination), multiple measurements may still be required for increased precision.

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