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Relationships Between Measurements of Vaginal Electrical Impedance, Uterine Involution and Hormonal Profiles in Postpartum Dairy Cows

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Abstract: The objective of this study was to determine whether relative changes in Vaginal Electrical Impedance (VEI) readings would correlate to characteristics associated with involution of the uterus and hormone profiles postpartum in the dairy cow. In experiment I, the use of VEI measurements for monitoring follicular development and ovulation were verified. Jersey cows (n = 16) were administered Prostaglandin $F_2\alpha$ (PG) and sampled at 12 h intervals for 7 d post-PG. At each sampling period, VEI measurements were recorded, ultrasonography was performed to obtain follicular measurements and serum samples collected for the determination of progesterone (P_4) and estradiol (E_2) using RIA. Serum concentrations of P_4 decreased and E_2 increased post-PG, while VEI decreased prior to ovulation; which is consistent with previous studies. In experiment II, Jersey cows (n = 18) were sampled twice weekly from d 1 to 60 postpartum (PP). On these days, VEI measurements were recorded, follicular sizes and cross-sectional area of the left and right uterine horns captured using ultrasonography, uterine tone scores assigned by palpation and serum samples collected for P_4 and E_2 determinations. Uterine horn diameter differences were negatively correlated with uterine tone scores and VEI measurements (i.e., as uterine horn diameter differences decreased, uterine tone and VEI measurements increased). Uterine tone scores were also positively correlated with VEI measurements. The relationship of VEI to early PP uterine involution was further confirmed in Holstein cows (n = 25), with increases in VEI readings occurring from week 2 to week 4 PP. In summary, VEI measurements patterned periovulatory hormonal profiles for cycling Jersey cows and were significantly correlated with changes in uterine size, uterine tone, resumption of estrous cycles and/or day PP in Jersey and Holstein cows post-calving. These data suggest that VEI may be used as an objective tool for quantifying the association of uterine involution with the resumption of reproductive cyclicity (as determined by P_4 and E_2 profiles) in the PP dairy cow.

Key words: Vaginal electrical impedance, uterine involution, dairy cattle

INTRODUCTION

The importance of maintaining a profitable calving interval has been investigated extensively[1-4], yet is still a goal that has remained out of reach for many dairy cattle producers[5]. The principle causes of lengthened calving intervals are often ascribed to an inefficient detection of estrus[6,7] or uterine maladies[8,9], which can lead to the premature culling of dairy cattle. To prevent this, the need for highly effective and automated methods for identifying cows in estrus[10] and assessing other reproductive characteristics is in great demand. Vaginal Electrical Impedance (VEI) has been used previously to detect estrus and to determine the timing of ovulation in cattle, horses, sheep, pigs and buffalo[11-14]. In practice, measurements of VEI remain constant until four days before the onset of ovulation. As a cow nears estrus the electrical resistance of the vaginal mucus begins to decrease, corresponding to a decrease in observed VEI values[4,15]. The principle behind VEI measurements is that a change in the ionic balance of vaginal and cervical mucus, thus altering electrical conductivity, occurs in response to changes in reproductive hormones such as estradiol and progesterone. Several researchers have found that fluids within the vagina have high electrical resistance during the luteal phase and that this resistance decreases during the follicular phase[16,4]. Nevertheless, the use of VEI for monitoring estrus and ovulation has been hampered by a high degree of individual animal variation and to the increased labor involved; given the need for repeated measurements to follow conductivity trends within individual animals over time[16,17].

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Changes in VEI have also been shown to be present during the annual reproductive cycle of the cow[16]. Moreover, Schindler *et al.*[18] have suggested previously that vaginal electrical impedance may be useful for monitoring the rate of repair of genital tissues after calving. However, the relationship between vaginal electrical impedance and uterine characteristics (e.g. tone and diameter) have not been previously described. If VEI could be used to monitor the rate of uterine involution post-calving, this technology could be beneficial for diagnosing uterine maladies or to identify those cows in which uterine involution is occurring more rapidly; which might facilitate the initiation of earlier breeding postpartum preventing extended calving intervals. The objective of this study was to determine whether relative changes in VEI readings would correlate to characteristics associated with involution of the uterus postpartum and associative hormone profiles.

MATERIALS AND METHODS

All animals used in the study were housed at the Mississippi State University (MSU) Bearden Dairy Research Center in a traditional free stall environment. Cows were maintained on a TMR with daily access to improved pastures and were milked twice daily. For experiments I and II, the measurements were obtained with cows restrained in a chute or stanchions that were equipped with locking headgates. Vaginal electrical conductivity in these studies was determined using the Ovatest meter (Animark Inc., Aurora CO). Prior to each use, the probe was disinfected using Nolvason (1% v/v water) and re-disinfected between measurements after each cow. The VEI meter was checked for performance (i.e., repeatability of readings) at the beginning and end of each sampling period in clean water. Moreover, the Ovatest meter was calibrated to verify changes in the ionic content of a standard solution using NaCl. Briefly, dilutions of sodium chloride (NaCl; 1mM to 1M) in water were quantified using the VEI probe, in which three consecutive measurements were recorded. Statistical analysis of these measurements was performed using the Student's T-test for mean separation and Coefficients of Variation (CV%) were calculated to assess repeatability and precision of the Ovatest meter. The results of the calibration indicated that as the concentrations of NaCl increased the conductance measurements (i.e., electrical resistance) decreased ($p<0.05$); from 694.7±1.5 Relative Units (RU) in water (no NaCl) to 24.7±0.3 RU in a 1 M NaCl solution, with CV% of 0.36 and 2.3 %, respectively.

This study was reviewed and approved by the MSU Institutional Animal Care and Use Committee (MSU IACUC 00-004) and followed the General Guidelines for Animal Husbandry as described in the Guide For the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, Savoy, IL).

Experiment I: The objective of this experiment was to verify the relationship between VEI and hormonal profiles in the non-pregnant cow as coupled to changes in follicular development and the timing of ovulation. The relationship between VEI and estrous cycles in cattle has been studied and verified previously[19,18]. The rationale for examining this relationship again as part of this study was to establish the baseline patterns for the VEI meter used in these studies with respect to actual VEI readings and reproductive events in the bovine. Mature Jersey cows (n = 16) were administered prostaglandin $F_2\alpha$ (PG, 25 mg i.m.; Lutalyse, Pharmacia Upjohn, Kalamazoo, MI) and sampled at 12-hour intervals for seven days post-PG. At each sampling period, VEI measurements (relative units; RU) were recorded using the Ovatest meter (Animark Inc., Aurora CO) and ultrasonography (Aloka 500V; 7.5 MHz transrectal probe; Aloka Co., Ltd., Wallingford, CT) was performed to obtain follicular diameter measurements and to determine the timing of ovulation post-PG. In addition, serum samples were collected for the determination of progesterone (P_4) and estradiol (E_2) using RIA procedures established in our laboratory[20,21], respecitvely. The intra-and inter-assay coefficients of variation for E_2 were 1.67 and 5.28%, respectively and for P_4 were 3.81 and 7.7%, respectively. The VEI probe was disinfected after each use and was tested at the beginning and end of each sampling period in tap water as described previously. Of the sixteen animals sampled, only nine (56.3%) were used in the analysis of VEI measurements and the timing of ovulation. The remaining seven animals (43.7%) did not ovulate or exhibited cystic follicular activity (determined by ultrasonography and/or altered E_2 profiles). For the analysis, the largest follicles on each ovary were measured (mm) from the ultrasound pictures using sliding calipers to the degree of 0.01 mm (Mitutoyo, MTI corporation, Aurora IL).

Statistical analysis was performed using Pearson correlation coefficients and Fishers r to z transformations to elucidate the relationships between serum concentrations of P_4 and E_2, size of the ovulatory follicle and VEI measurements. For analysis of these parameters over time prior to ovulation, ANOVA specific for repeated measures followed by mean separation using the Student's T-test was employed and data expressed as the mean±SEM[22].

Experiment II: After obtaining base-line data for the VEI meter in use (Exp. I), the objective of this experiment was to examine the relationship between VEI, postpartum uterine involution and reproductive hormones (P_4 and E_2) in the postpartum dairy cow. Mature Jersey cows (n = 8) and first-calf heifers (n = 10) were sampled twice weekly from day 1 to day 60 postpartum. At each sampling period VEI measurements were recorded and follicular sizes and cross-sectional area of the left and right uterine horns (i.e., diameter of the pregnant vs. non-pregnant horn) were captured by transrectal ultrasonography. In addition, a uterine tone score (0.5 increments; 1 to 5 scale; with 1 = Poor: soft, doughy, caruncles enlarged, 5 = Excellent: both horns pliable and springy-no difference in size) and a uterine position score (0 to 2 scale; 0 = below the pelvic brim, 1= on the pelvic brim, or 2 = above the pelvic brim) were assigned. Serum samples were also collected for analysis of serum concentrations of P_4 using RIA procedures as described previously. As before, the VEI probe was disinfected after each use and was tested at the beginning and end of each sampling period in clean water. The diameter of the pregnant and non-pregnant horn and follicular diameters were measured (mm) using sliding calipers as described previously. In addition to assessments in Jersey cows, Holstein cows (n = 25) were evaluated as well, however VEI measurements were taken only at week 2 (day 10-14) and week 4 (day 24-28) after calving. This additional assessment was conducted to verify that similar VEI relationships existed in Holstein cows for use as an assessment tool of reproductive parameters postpartum and to assess the utility of two single measurement points post-partum versus continuous, repeated measures over time.

Statistical analysis was performed using ANOVA specific for repeated measures to assess the effects of parity (mature vs. first-calf heifer) and time (uterine difference, uterine score and VEI measurements) in Jersey cows. Pearson correlations with Fishers r to z transformations were used to determine the relationship among uterine horn difference, uterine score and VEI measurements. For mean separation (hormonal, uterine, follicular and VEI measurements relative to day postpartum) the Student's T-test was employed and data were expressed as the mean±SEM[22]. Similar analysis were used in Holstein cows for pairwise comparisons of VEI measurements contrasting week 2 versus week 4 postpartum.

RESULTS AND DISCUSSION

Experiment I was conducted to verify the relationship between VEI and hormonal profiles in the non-pregnant cow as coupled to changes in follicular development and the timing of ovulation. Figure 1 and Table 1 depict the relationship between VEI and the timing of ovulation as observed in this study. Serum concentrations of P_4 decreased ($p<0.01$) from 1.9±0.5 to less than 0.5 ng mL within 24 to 36 h post-PG and is depicted in Fig. 1A showing this decrease in serum concentrations of P_4 occuring prior to 72 h before ovulation. This was followed by increasing ($p<0.05$) serum concentrations of E_2, which peaked at 46.6±9.6 h post-PG (i.e., 36 h prior to ovulation ; Figure 1 B) and was followed by ovulation at 76.1±7.4 hrs post-PG. These hormonal parameters in relation to PG administration and ovulation are consistent with other studies[23]. Size of the ovulatory follicle increased ($p<0.05$) an average of 5.4 mm prior to ovulation. In conjunction with these increases in follicle size and serum concentrations of E_2, VEI measurements decreased ($p<0.01$) 17.67±0.52 RU (21%) prior to ovulation. This decrease in VEI values is indicative of decreased electrical resistance and thus greater conductivity. The reduction of electrical resistance or increased conductivity during estrus has been verified and reproduced previously[4,17,24,25]. Smith *et al.*[5] reported that conductivity values increased by 29 to 45% on day of estrus in cattle. In addition, Gupta *et al.*[13] reported a 34% decrease in resistance readings from diestrus to estrus in the buffalo. Serum concentrations of E_2 and P_4 tended ($p<0.10$) to be negatively correlated (r = -0.24), albeit a low correlative value, between -96 and -12 h prior to ovulation. Moreover, serum concentrations of E_2 and size of the ovulatory follicle were negatively correlated ($p<0.05$, r = -0.46). This seems to be contradictory since the size of the follicle and serum concentrations of E_2 increased. However, serum concentrations of E_2 peaked at 46.6±9.6 h and began to decrease prior to ovulation, even though follicle size continued to increase. Thus as estradiol concentrations reached their threshold and the preovulatory LH surge occurred, the thecal cells of the ovulatory follicle most likely began producing P_4 instead of androgens, decreasing substrate for estradiol production by aromatase enzyme activity even prior to when ovulation actually occurs[10].

In the present study serum concentrations of P_4 and VEI measurements were positively correlated ($p<0.01$, r = 0.49). Similarly Schindler *et al.*[18,13,16] also reported a positive relationship between vaginal electrical impedance/resistance and serum concentrations of P_4. In support of this, Lewis *et al.*[19] reported that changes in serum concentrations of P_4 and E_2 seem to regulate changes in the degree of hydration of vulvar tissue and hydration of vulvar tissue appears to govern changes in resistive properties of the tissue. There were no

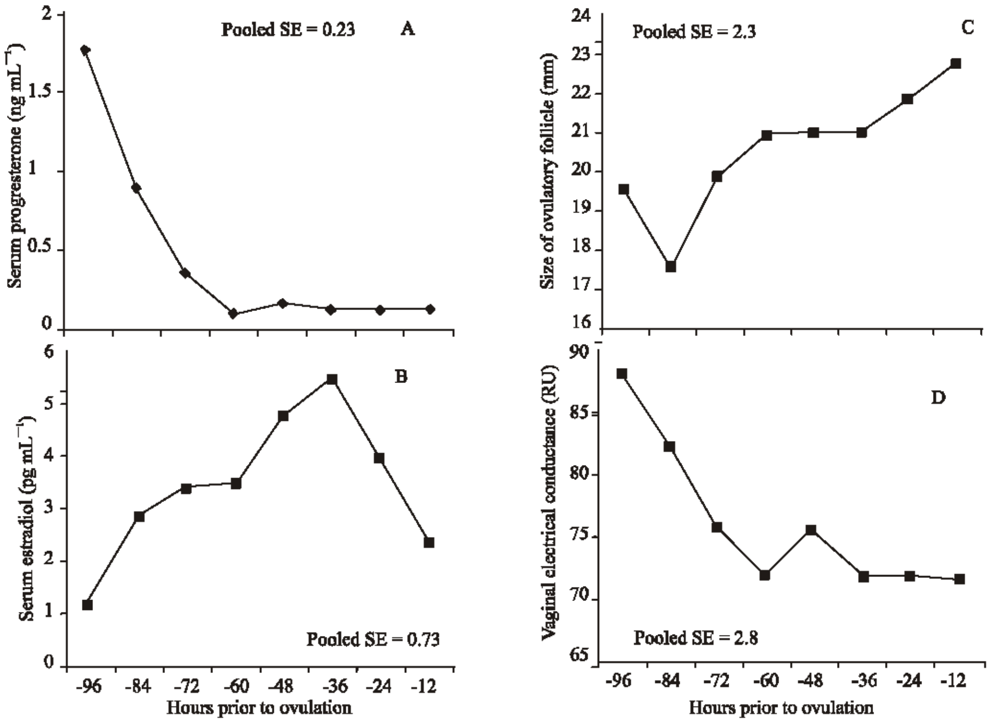

Fig. 1: The relationship between vaginal electrical impedance (VEI; Relative Units; RU) and the timing of ovulation in the bovine in relation to serum concentrations of progesterone (P_4) and estradiol (E_2). In conjunction with increases ($p<0.05$) in follicle size (Panel C) and serum concentrations of E_2 (Panel B), serum concentrations of P_4 (Panel A) and VEI (Panel D) decreased ($p<0.01$) prior to ovulation

Table 1: Mean (±SE) serum concentrations of progesterone (P_4) and estradiol (E_2), follicle size and Vaginal Electrical Impedance (VEI) at administration of Prostaglandin $F_2\alpha$ (PG), timing of peak E_2 post-PG and at 12-h prior to ovulation

	Dominant			
Measurement interval	P_4 (ng mL^{-1})	E_2 (pg mL^{-1})	VEI (RU)	follicle size (mm)
Day of PG administration	1.9 ± 0.5^a	2.5 ± 1.1^a	88.7 ± 4.0^a	17.9 ± 1.8^c
Period of peak estradiol concentration	0.10 ± 0.1^b	6.4 ± 1.2^b	72.9 ± 2.9^b	20.6 ± 1.8^{cd}
12-h prior to ovulation	0.12 ± 0.1^b	$2.5\pm.7^a$	71.0 ± 1.7^b	23.3 ± 2.4^d

Superscripts differ within column: ab $p<0.05$; cd $p<0.10$; RU = Relative Units

Table 2: Hormonal, uterine, follicular and Vaginal Electrical Impedance (VEI) measurements in mature cows and first-calf heifers relative to timing of the first estrous cycle postpartum

Phase of the estrous cycle[1,2,3]	Day PP P_4	(ng mL^{-1})	VEI (RU)	Follicle size (mm)
First-calf heifers (n = 10)				
Late metestrus/Early diestrus	33.0 ± 2.9^a	1.9 ± 0.2^a	92.8 ± 4.6^{ab}	19.1 ± 2.4^{ab}
Mid/Late diestrus	39.6 ± 3.2^{ab}	5.4 ± 0.7^b	95.5 ± 1.9^a	22.3 ± 1.8^a
Proestrus/Estrus	45.2 ± 2.7^b	0.31 ± 0.1^c	86.3 ± 2.4^b	17.9 ± 1.3^b
Mature cows (n = 8)				
Late metestrus/Early diestrus	36.1 ± 3.7^a	1.6 ± 0.2^a	90.4 ± 4.2^{ab}	22.5 ± 1.7
Mid/Late diestrus	42.6 ± 3.6^{ab}	3.7 ± 0.6^b	95.9 ± 4.1^a	20.4 ± 1.5
Proestrus/Estrus	46.8 ± 3.4^b	0.12 ± 0.1^c	87.5 ± 5.2^b	19.2 ± 1.5

Superscripts differ within column within first-calf heifer or mature cow groups: ab, ac, bc $p<.05$; PP = days postpartum, RU = Relative Units. [1]Late Metestrus/Early diestrus was defined as the day that serum concentrations of P_4 reached 1 ng mL^{-1} in two consecutive samples. [2]Mid/Late diestrus was defined as the day with the highest serum concentration of P_4. [3]Proestrus/Estrus was defined as the day that serum concentrations of P_4 were less than 1 ng/mL following Mid/Late diestrus of the first complete estrous cycle (>15d in duration)

significant correlations ($p>0.10$) apparent between VEI measurements and E_2, VEI measurements and size of the ovulatory follicle, or serum concentrations of P_4 and size of the ovulatory follicle. Previous studies have also reported a negative relationship between vaginal electrical impedance and E_2[18,16], though they were not apparent in

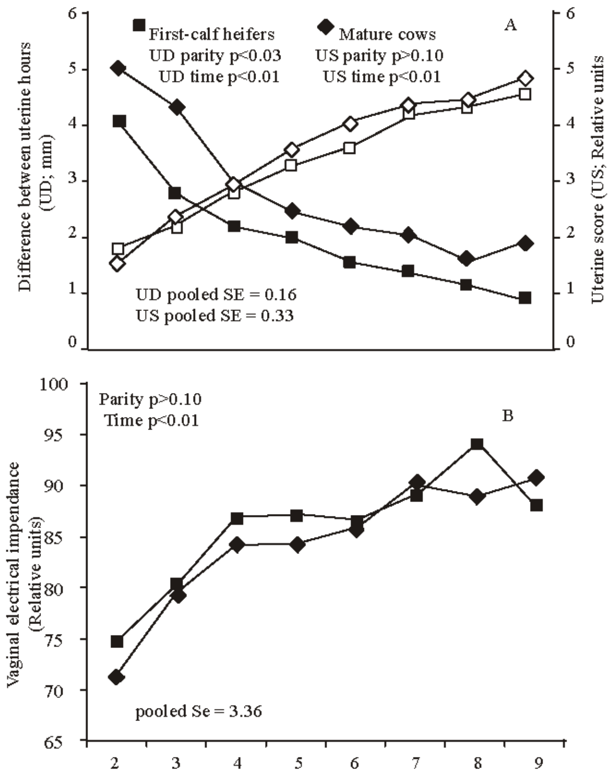

Fig. 2: Uterine measurements and vaginal electrical impedance (VEI) for mature Jersey cows and first-calf Jersey heifers. Uterine score (US; Panel A; □;◇) and VEI (Panel B) increased over time ($p<0.05$), while uterine difference (UD; Panel A;■;♦) decreased, ($p<0.05$). US, UD and VEI did not differ ($p>0.10$) in relation to parity

this study. This could be attributed to individual animal variation in VEI and/or size of the ovulatory follicle. These data illustrate the relationship between VEI values and the hormonal changes that occur prior to ovulation. Specifically, as P_4 decreases and E_2 increases during the onset of estrus, low VEI indicate decreased electrical resistance and thus greater conductivity.

Having established these relationships for the Ovatest (Animark) probe, Experiment II was conducted to examine the relationship between VEI and postpartum uterine involution and associative hormonal profiles in the postpartum dairy cow. Uterine score and horn differences in relation to VEI measurements are depicted in Fig. 2. Uterine tone scores increased ($p<0.01$) for mature cows and first-calf heifers between week 2 (1.53±0.14 and 1.75±0.13, respectively) through week 9 (4.81±0.08 and 4.73±0.12, respectively) postpartum and did not differ ($p>0.10$) between mature cows and first-calf heifers. Difference between the pregnant and non-pregnant horn decreased ($p<0.01$) between week 2 (5.01± 0.64 and 4.78±0.89 mm, respectively) through week 9 (1.89±0.40 and 0.79±0.13 mm, respectively) postpartum for both mature cows and first-calf heifers; although a greater difference between the pregnant and non-pregnant uterine horn was observed in mature cows than first-calf heifers ($p<0.03$). It has been reported previously that parity can affect rate of uterine involution with older Jersey cows having longer intervals to involution of the uterus than younger Jerseys cows[26]. This was not readily apparent in this study as both mature cows and first-calf heifers followed similar trends over time.

Vaginal electrical impedance increased ($p<0.01$) in mature cows and first-calf heifers between week 2 (71.31±3.57 and 75.0±1.52 RU, respectively) through week 9 (90.63±2.83 and 91.55±2.61, respectively) postpartum and did not differ ($p>0.10$) between mature cows and first-calf heifers. Schindler *et al.*[18] have previously noted a tendency for a negative relationship between 13, 14-dihydro-15-keto-PGF$_2\alpha$ (PGFM), an indicator of uterine involution[25] and vaginal electrical impedance from calving to 2 weeks postpartum. In this study, we examined uterine involution using physical measures (via ultrasonography) and concentrated on the period from week 2 to 9 postpartum. As expected, uterine score and uterine difference were negatively correlated for both mature cows and first-calf heifers ($r = -0.66$; $p<0.01$) between weeks 2 through 9 postpartum. Moreover, VEI and uterine difference were negatively correlated ($p<0.01$) in both mature cows ($r = -0.37$) and first-calf heifers ($r = -0.51$), while VEI and uterine score were positively correlated ($P<0.01$) for mature cows ($r = 0.48$) and first-calf heifers ($r = 0.52$). These relationships demonstrate a link between VEI and uterine involution. These relationships are most likely not direct causal effects but rather parallel to the manifestation of changing hormonal environments associated with the resumption of estrous cyclicity postpartum.

Lehrer *et al.*[16] reported an increase in vaginal electrical resistance through 40 days postpartum which coincided with the involution of the uterus and the onset of the estrous cycle. For the present study, hormonal data, VEI and follicle size measurements are depicted in Table 2. There were no differences ($p>0.10$) between mature cows and heifers relative to day postpartum, serum concentrations of P_4, VEI measurements or follicle size. A dramatic rise in VEI is noted from week 2 to week 4 postpartum (Fig. 2). After week 4 postpartum VEI measurements appeared to stabilize until week 7, where they began to rise and fall consistent with early postpartum estrous cycle activity. Vaginal electrical impedance measurements after 28 d postpartum (week 4) appear to coincide with the timing of the first postpartum estrous cycle in mature and first-calf heifers (36.1±3.7 and 33.0±2.9 d, respectively) as defined by serum concentrations of P_4 (Table 2). Lehrer *et al.*[16] reported high correlations between postpartum intervals to vaginal electrical resistance stabilization and resumption of luteal activity in addition to the first postpartum estrus. Moreover, VEI measurements after 28 d postpartum appeared to coincide in this study with the timing of the first complete estrous cycle as defined by serum concentrations of P_4. Similar to the VEI results in this trial for Jersey cattle, VEI measurements in mature Holstein cows increased ($p<0.01$) between week 2 (73.84±1.52 RU) and week 4 (91.56±2.22 RU) postpartum. In a previous study in which an electrical conductivity probe was implanted into the uterus, electrical conductivity was increased immediately after surgery and decreased during the first 2 or 3 weeks[17]. It is thought that this decrease in conductivity coincided with the healing process and might be used as an indicator of that healing process. As a result of the confounding effects of ovarian activity postpartum in conjunction with (and supportive of) uterine healing, whether there is a direct link between VEI and uterine involution, or whether this is solely a function of associative hormonal cues, remains to be determined.

In conclusion these data suggest that VEI may be used to monitor changes in uterine involution and reproductive cyclicity during the postpartum period in the dairy cow. The relationship between VEI and uterine involution is likely coincidental (indirect) to the resumption of estrous cycles, as indicated by hormonal changes postpartum, however undoubtedly one influences the other. We suggest, as has been only alluded to previously[18], that VEI may represent an objective tool to assess postpartum reproductive health and could be used to identify those cows in need of therapeutic intervention (e.g. hormonal therapy to hasten uterine involution and cyclicity) to improve postpartum reproductive efficiency. The use of actual (absolute) VEI values as meaningful information has been hampered previously by individual (cow-to-cow) variability when applied to the monitoring of estrus and ovulation. This has prevented broad use of VEI values as indicators of generalized reproductive changes. The data reported here for postpartum cows would suggest that 'normal' values may be established and applied to assess postpartum reproductive health (e.g. comparisons of week 2 to week 4 PP). While the present study has established firmly the transitional relationships between VEI measurements, hormonal changes and uterine involution in the postpartum dairy cow, additional studies need to be conducted to determine (1) if VEI could be used to monitor the effects of postpartum hormonal therapies on uterine turnover, (2) whether cows with uterine maladies (e.g. pyometria) exhibit altered VEI values at a given stage PP compared to unaffected cows and (3) whether VEI might be useful as a breeding management tool to initiate earlier breeding postpartum.

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