Electrical Conductivity of Reproductive Tissue for Detection of Estrus in Dairy Cows

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ABSTRACT

The pattern of temporal measurements of electrical conductivity obtained from electrodes that had been surgically implanted in the mucosa of the vagina or in the submucosa of the vulva in each of five dairy cows was evaluated for changes associated with the occurrence of estrus. Tissue conductivity was monitored at 6-h intervals during and around estrus by hard-ware devices operating at either 16 or 100 kHz. Blood samples were taken at time of conductivity measurement for progesterone determination and at 2-h intervals during estrus for LH determination. Vaginal and vulvar biopsies were performed during diestrus and estrus to measure tissue hydration.

Conductivity increased significantly at estrus relative to a nonestrus base period in both vaginal and vulvar tissue. Both electrical frequencies were found to be satisfactory for characterizing changes in tissue conductivity associated with estrus. Peak concentrations of luteinizing hormone, increases in tissue hydration, and patterns of blood progesterone were consistent with the occurrence of estrus during the time of elevated tissue conductivity.

INTRODUCTION

Reproductive inefficiency is the second most important reason for premature culling of dairy cows (5). Such culling is exerted in an effort to attain an ideal calving interval of 12 mo (10, 17), a goal that has remained elusive to all but a small minority of dairy producers. Inefficient detection of estrus is the principal cause of delayed conception and concomitant lengthened calving intervals (4, 18, 20). In addition to direct economic effects, long calving intervals impact adversely on the rate of genetic progress.

Observation by humans is the most frequently used method of detection of estrus (9, 13, 23). When routinely employing this method, between 20 and 40% of estrous periods are missed in many herds and as many as 15 to 20% of the cows are judged to be in estrus when they are not. Additionally, this method has a high labor requirement.

Most methods used currently for detection of estrus are based on changes in overt behavior of the cow; however, between 4 and 26% of all dairy cows experience indistinct or silent estrus (11, 19). Such cows will not be readily detected in estrus by visual observation.

Research efforts to correct deficiencies of existing methods for detection of estrus also must address current realities of larger average herd size and expensive farm labor. One estrous detection aid, the vaginal probe for monitoring intravaginal conductance, has been constrained by its labor-intensive nature (8) and by the variability and instability of the measurement data (6). Such factors as depth of insertion, instability of contact between the vagina mucosa and the probe, posture of the cow, or unequal pressure on the probe influence the measurement data and hence the reliability of the method for detecting estrus.

Recent studies with cattle (14) and with sheep (3) indicate that reproductive tissue impedometry is promising for detecting estrus when sensors are implanted in genital tissue. This approach, combined with telemetry, could result in automatically recorded data suitable for computerized analysis and automatic flagging. However, there is need to explore further methodological approaches to

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obtaining the requisite data and to study biological variation that may influence the conductance of genital tissue.

The objectives of this experiment were 1) to explore two methodologies and electrical frequencies of 16 and 100 kHz for collecting vaginal and vulvar tissue conductivity, 2) to characterize the data in relation to estrus, 3) to evaluate two probe location responses, and 4) to compare hydration of vaginal and vulvar tissue biopsies obtained during diestrus with those obtained during estrus.

MATERIALS AND METHODS

Bipolar electronic probes were surgically implanted in the submucosa of the vulva and in the mucosa of the vagina in five lactating cows. Electrical conductivity of the tissue surrounding each probe was measured at 16 and 100 kHz. Treatment designations were: 1 = 16 kHz, vagina; 2 = 16 kHz, vulva; 3 = 100 kHz, vagina; 4 = 100 kHz, vulva. The lead wire to the vaginal probe broke on cow 4, resulting in only values from the vulva probe being obtained from her. Cows were housed in stanchions except at milking times and stood on rubber mats as a precaution against electrical interference from grounding. Their management was routine and reflected norms of the rest of the herd.

The probes (Figure 1), designed after those used by Lewis et al. (14), were fabricated at the University of Illinois Materials Development Laboratory. They consisted of bipolar electrodes machined from stainless steel type 316, an insulation middle piece of molded Stycast (Pro Pac Corporation, Plainfield, NY), Alpha (Alpha Wire Corporation, Elizabeth, NJ) wire number 1115 as leads and silastic tubing (Dow Corning Corporation, Medical Products, Midland, MI), which enclosed the leads. The probe and the external measuring devices were connected by soldering each unit of a paired lead to one pole of the probe. The lead exited the probe through a hole 4 mm in diameter drilled through one pole. One end of the silastic tubing used to encase the lead was also passed through that hole and terminated in the insulation middle piece. The free end of the lead was used to attach plugs for coupling with measuring devices. Dimensions of the probe were 25-mm long by 8 mm in diameter.

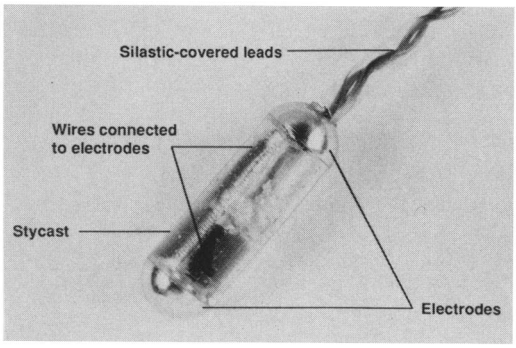

Figure 1. Bipolar electrical probe used for tissue conductivity measurements.

Prior to implanatation, the probes were standardized by sanding their bipolar ends wit with fine sandpaper and polishing with emery cloth.

Physiological saline was used as a standardization solution. The probe with the highest conductivity reading in the standardization solution was used as a standard. The conductivity of other probes was increased by sanding and polishing to increase their surface area and thus decrease their impedance. Each probe was sanded, polished, and tested until measurements in the standardization solution were within ± 5% of the "standard" probe.

Conductivity from the implanted probes was measured at 100 kHz with an on-line continuous monitoring and recording system and at 16 kHz aith a hand-held unit, which was read manually. Conductivity values at 100 kHz were obtained by modifying the in-line electrical conductivity data collection system described by Datta et al. (7) for measuring conductivity of milk. A single interface board, controlled by a CROMEMCO-CS2 computer, and the necessary circuitry for remote installation was positioned in the vicinity of the experimental animals. The conductivity board was modified as follows:

1. The comparison resistor was changed from 100 ohms to 464 ± 1% ohms to produce a voltage across the implanted probe, which was within the range of the amplifier.
2. A pad was installed using a 100K-ohm resister tied to −12 V to raise the offset voltage applied to the amplifier. The facilitated adjustment of zero above or below instrument zero as an offset to incoming voltage.
3. The feedback resistor was changed from 200 to 400 ohms to 100 to 300 ohms. The purpose of this change was to increase the span, or range, of voltage that could be

sensed by the A/D converter.

4. A 4-μF capacitor was added between the comparison resistor and the oscillator to provide a block to the DC current component of the oscillator output; DC current interferes with impedance measurements by causing polarization, thus decreasing the indicated conductivity of the medium being measured.
5. The four inputs originally used to monitor mammary quarters were used to monitor the four probes. The electronic board, thus modified, monitored and recorded values from two locations of each of two cows simultaneously.

Measurements were taken at 0600, 1200, 1800, and 2400 h daily from beginning of the experiment until 6 d after estrus occurred. The computer program used to obtain data from the conductivity board strobed and obtained data every 6 s for 1 min. These 10 digital values, recorded in real time, were subsequently averaged to represent their respective collection period. Because the board was capable of taking measurements from only two cows (two vagina and two vulva probes) simultaneously, the data collection process was repeated three times at each collection period.

Electrical impedance measurements at 16 kHz were made at 6-h intervals with a modified hand-held ESTRON impedance meter (ANIMALTEK Corporation, Boulder, CO). The meter was modified to operate at 16 kHz, a frequency found in preliminary studies to be suitable to characterize changes of reproductive tissue impedance at estrus. The modified unit provided about 120 μA of current to the implanted probes. The digital scale on the meter also was recalibrated to accommodate the range of values obtained with the implanted probe.

The surgical procedures used were a modification of those described by Feldman et al. (12). Anesthesia was provided by a caudal epidural coccygeal block using 2% lidocaine. A standard surgical scrub was used for skin preparation. A 2-cm vertical skin incision was made midway between the dorsal and ventral commissures of the vulva. A trochar-cannula was introduced cranially 12 to 15 cm to a position beneath the vaginal mucosa. After removing the trochar the probe was placed in the distal cannula and held in place by a glass rod while the cannula was removed. The device was palpable beneath the mucosa in the vaginal wall.

From the same skin incision, the vulvar probe was placed in a slab incision extending ventrally. Blunt dissection placed the device in the submucosa position. Leads from the probes were placed subcutaneously to emerge lateral to the tail head by using the trochar-cannula for placement. The skin incisions were closed with sutures and sealed with tissue adhesive. The exteriorized leads with jacks were taped to the tail after allowing some excess for vertical and horizontal movement of the tail. Seven weeks elapsed between implantation and experimental data collection to allow for healing.

Blood samples were obtained from indwelling jugular catheters at corresponding times to conductivity and impedance data collection, except during estrus, when samples were collected at 2-h intervals in an effort to ascertain LH surge. Blood samples were collected in 15 x 175-mm heparinized vacutainer tubes. The samples were spun for 30 min at 300 rpm, the serum was extracted, placed in 25 x 75 mm vials, frozen and stored for subsequent analysis. Radioimmunoassay procedures were used to quantify progesterone (22) and LH (16) in the samples taken. The occurrence of estrus was confirmed by the temporal patterns of progesterone and LH in the blood and by the cows standing to be mounted by other cows during the time that they were loose from their stanchions at milking. Midnight preceding the day during which standing estrus was observed was defined as time 0, and midnight to midnight of the day of observed standing estrus was defined as the day of estrus.

Tissue Biopsies and Morphometrics

Biopsies were performed to obtain vaginal and vulvar tissues during estrus and diestrus. The purpose was to compare the degree of cellular hydration at each location between the two physiological states of estrus and diestrus. Cellular hydration was determined by morphometric analysis, which determined cytoplasmic-to-nuclei ratios per unit area of tissue.

Epidural anesthesia was administered to the cows using 6 ml of 2% lidocaine. Vagina and

vulva tissue biopsies were performed using a Baker skin biopsy instrument. Vulva biopsies were performed approximately 2 cm anterior to the mucocutaneous junction of the vulva lips. Vaginal biopsies were performed approximately 10 cm anterior to the site of vulvar samples. Both vulvar and vaginal samples were taken on the side opposite to the location of the implanted probes. Biopsy samples were fixed in 10% neutral buffered formalin prior to preparation for histological examination.

Following fixing, the tissue samples were embedded in paraffin and sliced at 5 μm using a rotary microtome. The 5-μm pieces were placed on glass slides and stained using hematoxylin nuclear stain and eosin cytoplasmic stains (15). Tissue hydration was determined by calculating cytoplasmic-to-nuclei ratios (21).

Photomicrographs were made from processed and stained tissue sections. These slides were projected at 250 x using an audioviewer projector. A Merz square was imposed and counts made of nuclei and connective tissue intersecting with specific points on the square. The square contained 36 points and five randomly selected areas were counted from each slide. The total area counted per slide represented 18 mm^2. Cytoplasmic points were established by subtracting the number of connective tissue points from nonnuclear points at each throw of the Merz square. Duplicate slides were made of each tissue sample and therefore, duplicate counts were made. These two counts were averaged. Two separate and independent counts of all slides were made by two individuals. Cytoplasmic-to-nuclei ratios were computed from the pooled data representing the two independent counts.

Statistical Treatment

Conductance data over the experimental period were analyzed using the Z statistic (21):

$$Z = \frac{(X - \bar{X})}{SD}$$

where:

X = conductance at a given time,
$\bar{X}$ = a mean of conductance values obtained during a base period, between d −6 and d −2 and between d 2 to d 6,
SD = standard deviation of conductance values obtained during the base period, and
Z = number of standard deviations that an observed conductivity value was above the mean of the base period, between d−6 and d−2 and between d 2 to d 6.

Threshold vaues of Z >1.5, >2.5, and >3.1 were to estimate the magnitude and duration of conductivity changes associated with estrus at both 16 and 100 kHz. These threshold values represented statistical significance of $P<.06$, $P<.006$, and $P<.001$, respectively. Statistical differences between tissue hydration values from vaginal and vulvar biopsies during estrus and during diestrus were determined using the *t* test.

RESULTS AND DISCUSSION

The effect of estrus on the conductivity values obtained at 16 and 100 kHz and from probes implanted in vaginal tissue or vulvar tissue are in Table 1. Substantially higher conductivity values were obtained at estrus at both frequencies and at both locations. Tissue conductivity values increased by 29, 30, 45, and 38% on the day of estrus relative to the baseline values for 16 kHz, vagina; 16 kHz, vulva; 100 kHz vagina; and 100 kHz, vulva. Corresponding peak readings at estrus were increased by 40, 47, 72, and 59%. Intermediate values were present on the day before and the day after estrus reflecting variation among cows in the onset of estrus relative to the time of the day.

There were no significant differences among the combinations of implant sites and electronic frequencies in the values obtained during the baseline period, in the values obtained at estrus, or in the increase in values that occurred at estrus. Within-cow standard deviations during the nonestrus baseline period also were similar among all treatments, although variation was substantially higher for some probes than others (range of standard deviation was .10 to .52).

Temporal relationships for conductivity values and for blood progesterone values relative to h 0 of the day of estrus are in Figure 2 (16 kHz) and Figure 3 (100 kHz). The occurrence of estrus was confirmed by cows standing to be mounted by other cows during the time they were loose from their stanchions at milk-

ing, by the presence of low blood progesterone, and by elevated blood LH concentrations while tissue conductivity was elevated in all cows. The temporal patterns of change in conductivity were consistent in each cow regardless of the frequency used for collection of the data or the site of probe implantation.

The magnitude of the change in tissue conductivity at estrus was expressed either as a percentage of the mean nonestrus baseline (Figures 2 and 3) or as a Z-statistic (Figures 4 and 5). The Z-statistic expressed the values in standard deviation units relative to nonestrus mean. The nonestrus mean that we used was for 10 d (d −6 to d −2 and d 2 to d 6). Conductivity values were relatively stable during this period (Figures 2 to 5). As would be expected from the statistical nature of Z-values, only two Z-values of the 176 observations obtained during this period exceeded 3.0. In contrast, during estrus Z-values exceeded 4.0 in all cases for at least two successive 6-h measurements at both sites and both frequencies. Eighty-nine percent (16 of 18) of the Z-values exceeded 6.0, and 61% of them exceeded 10.0 for two successive measurements.

Thus, a single value from either location at either frequency expressed as a Z-statistic was 100% accurate in discriminating between a cow in estrus (± 1 d) and not being in estrus at any time in the trial by using a threshold of 4.0. The pattern of change in Figures 2 to 5 indicates that the change occurred more rapidly at the onset of estrus than at the end of estrus. The results suggest that a requirement of two consecutive Z-values exceeding the threshold and obtained at 6-h intervals would be likely to almost totally eliminate the possibility of

TABLE 1. Tissue conductivity during estrus and nonestrus.

Treatment	Cow	Baseline[1]		Day of estrus[2]	Peak value on day of estrus
		(mS/cm)			
		$\bar{X}$	SD[3]	$\bar{X}$	
16 kHz, Vagina	1	4.09	.27	5.23	5.32
	2	1.88	.10	2.16	3.11
	3	5.46	.20	6.60	6.96
	5	3.41	.10	5.24	5.43
	Mean	3.71		4.80	5.20
16 kHz, Vulva	1	4.56	.22	5.79	6.84
	2	4.93	.13	5.63	7.34
	3	5.34	.14	6.99	7.45
	4	3.77	.23	5.56	5.85
	5	3.52	.26	4.81	5.06
	Mean	4.42		5.75	6.50
100 kHz, Vagina	1	5.09	.52	8.04	9.10
	2	3.11	.25	3.75	5.95
	3	3.20	.15	4.43	4.83
	5	3.16	.17	5.01	5.28
	Mean	3.64		5.30	6.29
100 kHz, Vulva	1	3.89	.21	5.59	6.11
	2	3.83	.35	4.40	6.37
	3	5.52	.22	8.79	9.63
	4	5.21	.10	7.01	8.00
	5	3.10	.14	4.27	4.47
	Mean	4.34		6.01	6.91

[1] Mean of readings from 3 −6 to −2 and from d 2 to d 6 relative to the occurrence of estrus.

[2] Midnight to midnight on the day cows were observed to be standing while being ridden by other cows.

[3] Within-cow standard deviations during baseline period.

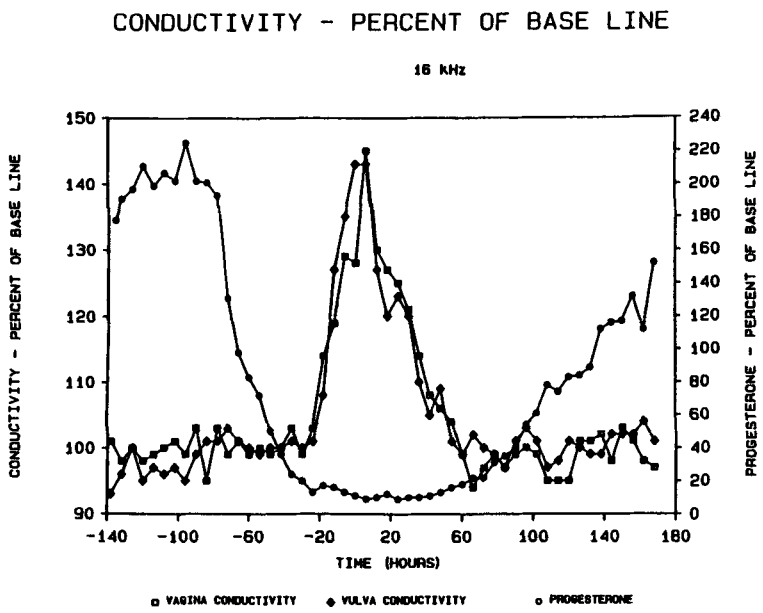

Figure 2. Blood progesterone and tissue conductivity at 16 kHz suring and around the day of estrus. Values are expressed as percent of the mean for d −6 to d −2 and d 2 to d 6 relative to the day of estrus. Time zero is midnight at the beginning of the day during which cows were observed to be in estrus. n = 5 for progesterone and vulva conductivity; n = 4 for vagina conductivity.

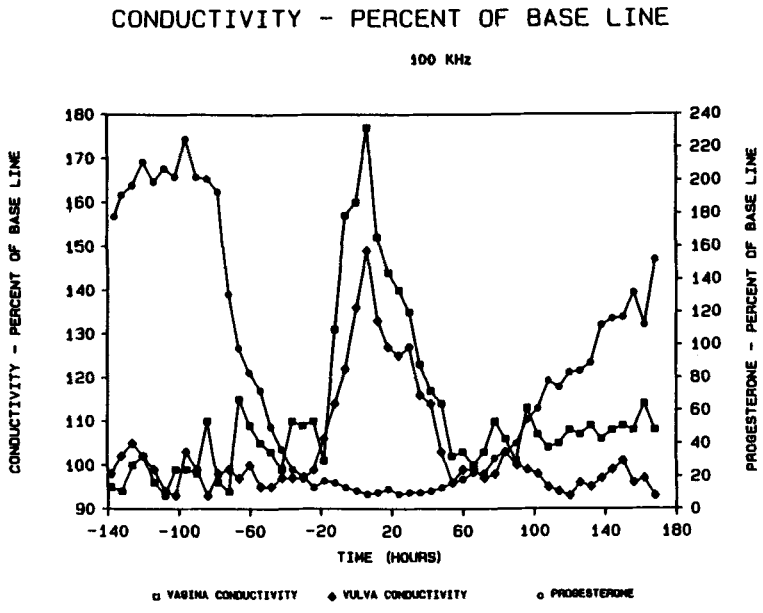

Figure 3. Blood progesterone and tissue conductivity at 100 kHz during and around the day of estrus.Values are expressed as percent of the mean for d −6 to d −2 and d 2 to d 6 relative to the day of estrus. Time zero is midnight at the beginning of the day during which cows were observed to be in estrus. n = 5 for progesterone and vulva conductivity; n = 4 for vagina conductivity.

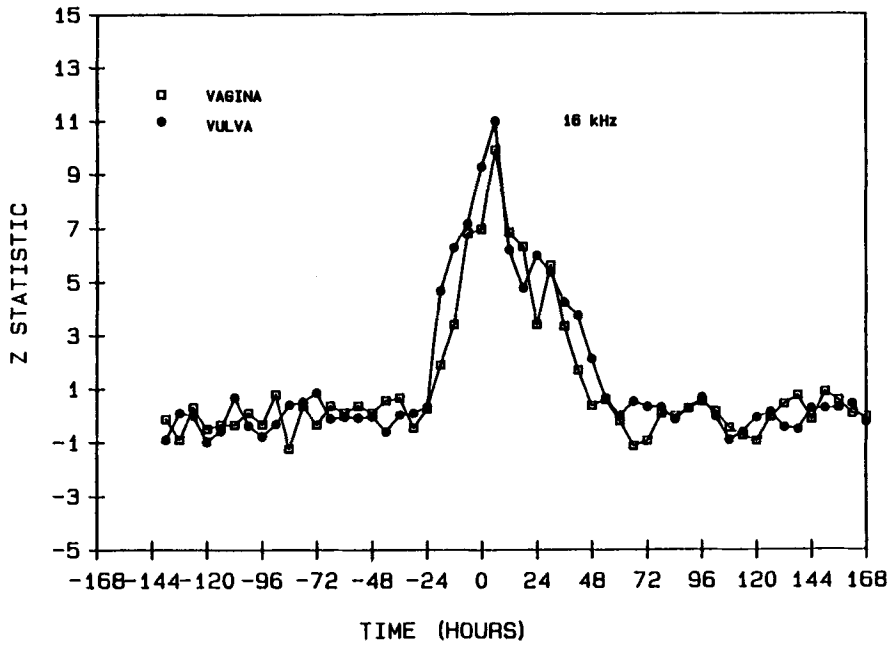

Figure 4. The Z-values for tissue conductivity at 16 kHz during and around estrus. Time zero is midnight at the beginning of the day during which cows were observed to be in estrus. n = 5 for values; n = 4 for vaginal values

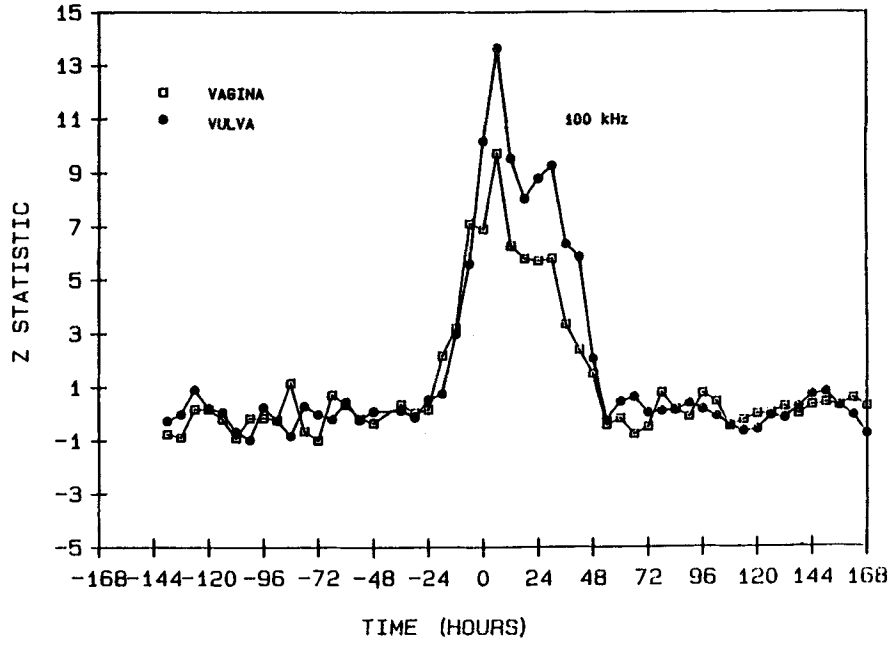

Figure 5. The Z-values for tissue conductivity at 100 kHz during and around estrus. Time zero is midnight at the beginning of the day during which cows were observed to be in estrus. n = 5 for vulvar values; n = 4 for vaginal values

TABLE 2. Durations during which conductivity exceeded Z thresholds.[1]

Treatment	Minimum Z thresholds		
	1.5	2.5	3.1
	(h)		
16 kHz, Vagina	63.0	43.5	40.5
16 kHz, Vulva	56.0	49.2	48.0
100 kHz, Vagina	55.5	48.0	43.5
100 kHz, Vulva	52.8	46.8	45.6

[1]Z = Number of standard deviations that an observed conductivity value was above the mean of the base period, between d−6 and d−2 and between d 2 to d 6.

TABLE 3. Cytoplasmic-to-nuclei ratios during diestrus and estrus.

Location	No. cows	Diestrus	Estrus
Vagina	5	3.66[a]	6.84[b]
Vulva	5	5.16[a]	7.70[b]

[a,b]Means within a row with different superscripts differ ($P<.05$).

an animal more than ± 1 d of estrus if a Z-value greater than 4 was obtained.

Because cows were confined in stanchions except at milking time, behavioral observations were inadequate to determine the precise onset or end of estrus. The LH values were all <5 ng/ml on d 2 to 5 prior to estrus and on d 2 to 5 postestrus. Peak LH values were between 19 and 32 (mean = 26) ng/ml for the five cows. One cow had a peak LH value 6 h before standing estrus was observed; peak LH values followed the observation of the cow standing to be mounted by other cows in the other cows. The interval between the time when a Z value of 2.0 occurred for two consecutive measurements and the peak LH value occurred was 18.0, 22.8, 19.5, and 19.2 h for 16 kHz, vagina; 16 kHz, vulva; 100 kHz, vagina; and 100 kHz, vulva.

The imprecision with which the onset of estrus and the time of ovulation were determined in the trial leaves open the question of whether electrical conductivity of tissue was more closely associated with one than with the other. The possibility of a closer relationship between ovulation and tissue conductivity than between the onset of estrus and tissue conductivity is suggested in this study by the fact that it took several hours after a definite threshold was exceeded before peak conductivity was reached. In addition, conductivity values indicating a high probability of estrus continued for a substantially greater than normal time of standing estrus (Table 2). Additional studies in which the onset of estrus, tissue conductivity, blood concentrations of LH, and timing of ovulation are determined on the same animals need to be conducted.

Results of the morphometric studies are in Table 3. The cytoplasmic-to-nuclei ratios were different ($P<.05$) during estrus and diestrus on every cow in both the vaginal and vulvar biopsies. These results support the hypothesis that differences in tissue conductivity at estrus were due to changes in tissue hydration.

This hypothesis is strengthened by other reports that indicate that blood flow to the reproductive tract increases at estrus in association with increased estrogen (1, 2). Elevation of the blood flow was reported to be associated with increased tissue hydration and thermal conductivity of that tissue.

It is concluded that real time data collection with an implanted telemetric sensor that would monitor conductivity of tissue surrounding the vulva or vagina at 16 or 100 kHz could be a highly effective automated electronic method for detection of estrus in cattle.

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REFERENCES

1 Abrams, R. M., and J. M. Stolwijk. 1972. Heat flow device for vaginal blood flow studies. J. Appl. Physiol. 33:144.

2 Abrams, R. M., W. W. Thatcher, F. W. Brazer, and

C. J. Wilcox. 1973. Effect of estradiol-17 on vaginal thermal conductance in cattle J. Dairy Sci. 56: 1058.
3 Adam, L. E., A. Aizinbud, A. Tadmor, and H. Schindler. 1981. Impedometric properties of the vulvar and vaginal tissue of ewes during the oestrous cycle. J. Reprod. Fertil. 61:11.
4 Barr, H. L. 1975. Influence of estrus detection on days open in dairy herds. J. Dairy Sci. 58:246.
5 Call, E. P. 1978. Economics associated with calving intervals. Page 190 *in* Large dairy herd management. C. J. Wilcox and H. H. Van Horn, ed. Univ. Press Florida, Gainesville.
6 Carter, P. D., and J. H. Dufty. 1980. Assessment of vaginal impedance measurements as an indicator of estrus in cattle. Aust. Vet. J. 56:321.
7 Datta, A. K., H. B. Puckett, S. L. Spahr, and E. D. Rodda. 1984. Real time acquisition and analysis of milk conductivity data. Trans. Am. Soc. Agric. Eng. 27:1204.
8 Davidson, M., E. Aizinbud, E. J. Judmin, and A. R. Lehre. 1982. Intravaginal electrical impedance in postpartum cows and its use in screening for genital disorders. Refu. Vet. 39(1–2):70. (Abstr.)
9 Donaldson, L. E. 1968. The efficiency of several methods for detecting estrus in cattle. Aust. Vet. J. 44:496.
10 Esslemont, R. J. 1974. Economic and husbandry aspects of the manifestation and detection of estrus in cows. Agric. Dev. Advis. Serv. Q. Rev. 12: 175.
11 Esslemont, R. J., and M. J. Bryant. 1974. Economic and husbandry aspects of the manifestation and detection of estrus in cows. Part II. The manifestation of estrus in cattle. Agric. Dev. Advis. Serv. Q. Rev. 13:19.
12 Feldman, R., E. Aizinbud, H. Schindler, and H. Broda. 1978. The electrical conductivity inside the bovine vaginal wall. Anim. Prod. 26:61.
13 Foote, R. H. 1974. Estrus detection and estrus detection aids. J. Dairy Sci. 58:248.
14 Lewis, G. S., E. Aizinbud, and A. R. Lehrer. 1985. Changes in electrical resistance in vulvar and uterine tissue during estrous cycles of Holstein cows. J. Dairy Sci. 68(Suppl. 1):195. (Abstr.)
15 Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. Am. Reg. Pathol.
16 Niswender, D. G., L. E. Reichert, Jr., A. R. Midgley, Jr., and A. V. Nalbandov. 1976. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84:1166.
17 Pellissier, C. L. 1971. Herd breeding problems and their consequences. J. Dairy Sci. 55:385.
18 Pellisser, C. L. 1978. Fertility problems under large herd management. Page 201 *in* Dairy herd management. C. J. Wilcox and H. H. Van Horn, ed. Univ. Press Florida, Gainesville.
19 Rottenstein, K., and R. W. Touchberry. 1957. Observation of the degree of expression of estrus in cattle. J. Dairy Sci. 40:1457.
20 Rounsaville, T. R., P. A. Oltenacu, R. A. Milligan, and R. H. Foote. 1979. Effects of heat detection, conception rate and culling policy on reproductive performance in dairy herds. J. Dairy Sci. 62:1435.
21 Weibel, E. R. 1979. Statistical methods. Academic Press, New York, NY.
22 Weston, P. G., and J. E. Hixon. 1980. Effects of in vivo prostaglandin F administration on in vitro progesterone synthesis by bovine corpus lutea. Biol. Reprod. 22:259.
23 Williams, W. F. 1981. Comparison of estrus detection techniques in dairy heifers. J. Dairy Sci. 64:1738.