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Electrical Properties of Genital Tissues Relative to their Hydration Throughout Reproductive Events of Cows

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C. ABSTRACT

Electronic methods for automatic detection of estrus and monitoring other reproductive events, should improve the reproductive efficiency of individual cows in the industrialized dairy farms.

Vulvar electrical impedance (VEI) was monitored during reproductive events of cows, and related to hormonal changes. VEI decreased from ~ 150 to $\sim 120 \Omega$ ($P < .001$) between 27 and 10 days before calving - when the plasma estradiol 17β (E_2) to progesterone (P_4) ratio (pg/ml:ng/ml) was 10:1 - and remained that low until calving.

In $PGF_{2\alpha}$ induced calvings the prepartal VEI decrease was non-significant statistically, and the prepartal increase of E_2 lower, compared with spontaneous calvings.

VEI increased sharply and linearly with time postpartum (pp), until stabilization at prepartum levels on 44 days pp. Three to four days of low P_4 plasma concentrations, and 1-2 days of elevated E_2 concentrations, preceded the maximal decrease of VEI at the periestrous periods of spontaneous, and $PGF_{2\alpha}$ induced, luteolyses. The periestrous VEI decrease was clearly manifested at the second and third pp ovarian cycles but not at the first one.

Vulvar cell density declined in periestrus, relative to diestrus mainly due to an increase in extracellular water from 19.8 % to 33.2 % of the tissue weight.

First prototypes of implantable radio-sensors, for automatic VEI monitoring, were developed and used.

d. OBJECTIVES

- 1) To gain a better understanding of the mechanisms that control changes of bovine vulvar electrical impedance (VEI). For this purpose studies were performed in naturally occurring as well in hormonally controlled reproductive events; VEI data were related to (a) plasma concentrations of estradiol 17β , progesterone and in part of experiments $\text{PGF}_{2\alpha}$ metabolite (PGFM) (b) changes in extra- and intracellular water contents and electrolyte concentrations of the vulvar tissue.
- 2) To determine the efficiency of VEI for identifying various reproductive events. For this purpose the VEI changes were studied around spontaneous and induced calvings, at the postpartum involution of genital tissue, and during spontaneous and induced ovarian cycles.
- 3) To improve methods for measuring VEI. For this purpose a bio-impedance analyzer was adopted for hardwire studies and small implantable radio-sensors were developed for automatic telemetric studies.

2. RESEARCH REPORTS

A TELEMETRY SYSTEM FOR DETECTING DAIRY COWS IN ESTRUS

532 A telemetry system for detecting dairy cows in estrus. G.S. Lewis^{a*}, E. Aizinbud^b,
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In dairy cows, at least 50% of estrous periods are not detected. Using bipolar electrodes implanted in vulvar tissue and hard-wired to a multifrequency LCR (inductance, capacitance, resistance) meter, we found previously that changes in electrical impedance can be used to detect cows in estrus. The purpose of the current project was to develop a telemetric system for automatic measurement of electrical impedance of vulvar tissue. Self-contained telemetry devices, with no external attachments, were developed* and surgically implanted in vulvar tissue of eight Holstein cows. These cows were checked twice daily for estrus, and blood samples were collected thrice weekly for progesterone assay. The devices, which are 35 mm long and 15 mm in diameter, transmit the cows' identification and the electrical impedance of the tissue. Data are automatically received and logged onto a computer at 20-min intervals 24 h a day. Results indicate that the telemeters are biologically inert, electronically dependable and can be used to identify cows in estrus. When telemeters were positioned properly in vulvar tissue, telemetered changes in impedance were used to correctly identify 29 of 32 (90.6%)*potentially-detected estruses (24 detected by visual observation and eight by progesterone changes). Results from this project indicate that it may be possible to automate estrous detection in dairy herds.

KEY WORDS: Estrous Detection, Dairy Cows, Telemetry, Automation

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* Automated oestrus detector for farm animals - has two impedance measuring electrodes in tissue to determine period between radio frequency bursts.

Patent Assignee: (DATA-) DATA SCIENCES INC

Author (Inventor): MILLS P A.

Number of Patents: 001

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** A total of 65 potential estruses were monitored in the experiments reported in the above abstract and in later experiments. 92% of these potential estruses were detected by the telemetric system, whereas 67% only were detected by twice daily heat checks.

Changes in electrical impedance of vaginal tissue (upper panel). A telemeter implanted in the vaginal wall on December 27 measured and transmitted data to a computer-driven receiver. Data were logged onto a computer automatically at 30-min intervals 24 h a day. So that we could determine if a measure of impedance deviated from a rolling average, we calculated a Z-statistic for each measure of impedance (lower panel; for explanation, see Lewis et al., 1988a). Z-statistics ≤ -1.65 were considered significant ($P < 0.05$) deviations from the rolling average. Arrows indicate the day of estrus.

I n p r e s s

1 CELL DENSITY, FLUID VOLUME AND ELECTROLYTE CONTENT OF
2 BOVINE VULVAR TISSUE DURING OESTRUS AND DIOESTRUS*

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1 ABSTRACT

2 Ezov, N., Maltz, E., Yarom, R., Lewis, G.S., Schindler, D.,
3 Ron, M., Aizinbud, E. and Lehrer, A.R.

4 Cell Density, Fluid Volume and Electrolyte Content of
5 Bovine Vulvar Tissue During Oestrus and Dioestrus. Anim.
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7 Vulvar tissue hydration of oestrous and dioestrous
8 lactating cows was studied by determining cell density (CD), dry
9 matter (DM), total tissue water (TTW), extracellular water
10 (ECW), and Na^+ and Cl^- concentrations in tissue biopsies. Na^+ ,
11 Cl^- and osmotic concentrations (OC) in blood plasma were also
12 determined. Each cow was biopsied during oestrus (Oe) and 10-13
13 days later (dioestrus, Di). CD (n=4 cows) was determined in
14 quick-frozen microscopic preparations. ECW (n=9 cows) was
15 determined by the ^3H -Inulin space method.

16 During Di and Oe, the mean CDs were 43 and 25 cells/ng of
17 mesenchymal tissue, respectively; the mean DM + TTW/cell (n=17
18 cows) was approximately 74% greater in Oe than in Di. During Di,
19 the mean DM, ECW and calculated intracellular water (ICW) were
20 24.2, 19.8 and 56.0 g/100 g tissue, respectively. During Oe,

1 there was an absolute mean weight increase of 18.4, 38.0 and
 2 17.6 g/100 g tissue in Di, in the DM, ECW and ICW, respectively.
 3 However, on a percentage basis, DM did not differ (24.5 vs
 4 24.2%), ECW increased (33.2 vs 19.8%) and ICW decreased (42.4 vs
 5 56.0%) during Oe vs Di, respectively. Plasma Na^+ , Cl^- and OC
 6 were lower ($P < 0.05$) during Oe than during Di ($\text{Na}^+ = 123.1 \pm$
 7 2.6 vs 130.9 ± 1.5 mEq/l plasma; $\text{Cl}^- = 89.3 \pm 3.1$ vs 97.0 ± 1.4
 8 mEq/l plasma; OC = 281 ± 4.8 vs 300.2 ± 1.8 mOsm/kg plasma,
 9 respectively). In contrast, tissue Na^+ and Cl^- (mEq/100 g
 10 tissue) during Oe and Di did not differ ($\text{Na}^+ = 7.8 \pm 0.8$ vs 7.7
 11 ± 0.9 ; $\text{Cl}^- = 7.6 \pm 0.5$ vs 7.8 ± 0.5 , respectively).

12 The results indicated that the density of vulvar mesenchymal
 13 cells was smaller, and the intra- + extracellular dry matter per
 14 cell was larger, during Oe than during Di; the decreased cell
 15 density was due mainly to an increased extracellular
 16 compartment. The electrolyte and osmotic concentrations of the
 17 extracellular compartment were lower during Oe than during Di.
 18 These results help explain why electrical impedance of vulvar
 19 tissue in cows during Oe is lower than that during Di.

20 Key words: Cow, Vulva, Tissue fluids and electrolytes, Oestrus,
 21 Dioestrus.

1 INTRODUCTION

2 "Hormonal control of water and electrolyte distribution and
3 transport in vertebrate female reproductive organs has received
4 intensive study only in the mammalian uterus, because of use of
5 that organ as a model system in the investigations of hormone
6 action. No attention was paid to the external genitalia"
7 (Spaziani, 1975). This statement also applies to the bovine
8 species, the vulvar tissue in particular.

9 During oestrus (Oe) the bovine vulva becomes oedematous, as
10 determined by clinical and histological observations (Hammond,
11 1927; Cole, 1930; Brown, 1944; Asdell, 1946; Roark and Herman,
12 1950; Smith ^{et al.}, 1989). Vulvar oedema has been suggested as a cause
13 of the drop in electrical impedance of vulvar tissue from
14 approximately 150 ohms during dioestrus (Di) to 105 ohms around
15 the time of Oe (Feldmann et al., 1978; Aizinbud et al., 1984;
16 Lehrer et al., 1985; Lewis et al., 1989; Smith et al., 1989).
17 However, no direct quantitative evidence exists to support the
18 suggestion that vulvar oedema reduces tissue impedance.

19 The present study was undertaken to quantify variables thought
20 to affect vulvar tissue hydration in dioestrous and oestrous
21 cows. Some of the results were reported elsewhere (Ezov et al.,
22 1985).

1 METHODS

2 The experiments were carried out on material obtained in vulvar
3 biopsies from cyclic lactating Israeli-Holstein dairy cows. Two
4 biopsies were taken, with an ophthalmic trephine (10 mm id),
5 from each cow. The first biopsy was taken during Oe and the
6 second, from the contralateral vulvar lip, 10-13 days later,
7 i.e., during Di. Determining cyclical changes in tissue samples
8 from the same cow reduced the effects of individual cow
9 variation on the variable measured.

10 The variables determined in the biopsies were cell density (CD),
11 dry matter (DM), total tissue water (TTW), extracellular water
12 (ECW), intracellular water (ICW), and sodium and chloride ion
13 concentrations (Na^+ and Cl^- , respectively). Na^+ , Cl^- and osmotic
14 concentrations (OC) in blood plasma, collected at the time of
15 biopsy, were also determined.

16 CD was determined in tissue samples from four cows. The samples
17 were quick-frozen by immersion in a vial containing isopentane
18 cooled in liquid nitrogen (Dubowitz and Brooke, 1973). The
19 samples were cryostat-sectioned at -25°C (International-Harris-
20 Cryostat, I.E.C., Needham Heights, Indiana, U.S.A.) at 5-7 μm
21 thickness and stained with hematoxylin-eosin. The use of
22 freezing techniques prevented shrinkage and possible changes in
23 the content and compartmentalization of tissue water (Van
24 Harreveled et al., 1965). Cell nuclei were counted in a number

1 of random high power fields of mesenchymal tissue and their
2 density per unit area was calculated.

3 TTW and DM were determined in 17 cows by weight loss in the
4 biopsies after 24 h of drying at 105°C.

5 ECW was measured in nine cows using the inulin-space method (Hix
6 et al., 1959). Approximately 0.5g of tissue was incubated at
7 25°C for 18-20 min with 3×10^4 dpm of ^3H -inulin (Amersham,
8 England; 357.4 nCi/g) in approximately 1.5 ml plasma under
9 constant shaking. The plasma was weighed by analytical scales
10 beforehand. The tissue and plasma samples were obtained at the
11 same time from the same cow to ensure the isotonicity of the
12 incubation system. The equilibration time and the optimal biopsy
13 sample size and sample:medium volume ratio were determined in
14 preliminary experiments.

15 At the end of the incubation period, the plasma tubes were
16 centrifuged to precipitate blood cells and debris from the
17 tissue samples. Plasma samples were taken before and after
18 incubation (50 ul each time) for counting in a liquid
19 scintillator (Packard-Tricarb 4640). ECW was calculated from the
20 equation $\text{ECW} = W(C_1 - C_2)/C_2$, where C_1 and C_2 are dpm/ml of the
21 plasma medium weight (W), before and after the incubation,
22 respectively.

23 Na^+ and Cl^- were determined in nine cows by boiling the biopsy
24 material in distilled water (tissue:water = 1:3) followed by a

24h diffusion period (Schmidt-Nielsen and O'Dell, 1961; Maltz, 1980).

Na^+ was determined by flame photometry (Corning 480), and Cl^- with a chloride-titrator (CMT-10, Radiometer, Copenhagen). OC was measured by a vapor-pressure osmometer (Osmometer, 5100C, Wescor Inc., Logan, Utah, U.S.A.).

Statistical differences between tissue hydration values from vulvar biopsies taken during Oe and Di were determined using the t-test.

RESULTS

Compared with Di, Oe was characterized (Table 1.A) by a decreased CD and an increased ECW. TTW and consequently DM in 100 g of tissue, were similar in Oe and Di.

Significant differences ($P < 0.01$) in CD, ECW and TTW were found among the cows within Di and Oe. This variation among animals demonstrates the need to compare the variables between Di and Oe within cows.

Tissue Na^+ and Cl^- did not differ between Oe and Di (Table 1.A), but plasma Na^+ and Cl^- and, consequently, OC were lower ($P < 0.05$) during Oe (Table 1.B).

By combining the values of CD, TTW and ECW in Table 1.A, we estimated the mean DM, ECW and ICW of one vulvar mesenchymal cell during Di and Oe. Assuming that the cell-shape is cubic and that the specific gravity of tissue DM and fluids is 1.0, the mean weight (or volume) per cell (WtPC) can be expressed as: $WtPC = \left(\frac{\sqrt{A}}{n} \right)^3$, where: WtPC is the mean DM+ECW+ICW per cell (it may also be defined as tissue weight divided by tissue cell number); A is the area of the examined microscopic field; and n is the mean number of cell nuclei per examined area. Solving this equation for Di and Oe, where n = CD (rounded) = 123 and 85, respectively (see Table 1.A), results in the values in Table 1.C. The ratio of WtPC in Oe to Di is 1.74. Applying this ratio and the values of TTW and ECW in Table 1.A to 100 g of vulvar tissue in Di, results in the values given in Fig. 1. Figure 1 shows graphically the absolute (g) and relative (%) amounts of DM, ECW and ICW in 100 g of vulvar tissue during Di (lower half-circle) and the changes that occur in such 100 g when the cow is in Oe (upper half-circle). Absolute weights are expressed as the areas of each sector forming a half-circle, whereas relative weights are expressed by the size of the central angle of each sector. In Oe, the DM, ECW and ICW increased by 18.4, 38.0 and 17.6 g, respectively, resulting in a total increase of 74.0 g per 100 g of tissue during Di. These absolute weight increases resulted in no change in % DM (24.2 vs 24.5), a decrease in % ICW (56.0 vs 42.3) and an increase in % ECW (19.8 vs 33.2).

1 DISCUSSION

2 The present work shows that a tissue-unit of the bovine vulvar
3 mesenchyma is approximately 74% heavier during Oe than during
4 Di. The additional weight is about 75% water and 25% DM, as
5 evidenced from the absence of change in the percentage of these
6 components in Oe compared with Di (Fig. 1). The additional
7 weight per unit of tissue resulted from an increase in the mean
8 weight per cell. Further increase in total vulvar weight during
9 Oe could result from an increased number of cells as evidenced
10 from the endometrium (Steinez, 1973).

11 CD was approximately 30% lower during Oe than in Di. A
12 similar trend was described by Cole (1930) and Smith et al.,
13 (1989), in formalin-fixed tissue.

14 The decreased CD during Oe could be caused by swelling of cells
15 or by an increase in the extracellular compartment. The absolute
16 increase in ECW and ICW indicated that both changes occurred,
17 but the increase in % ECW compared with the decrease in % ICW
18 indicated that the former was primarily responsible for the
19 increased TTW during Oe. In fact, the amount of ECW per cell
20 increased nearly three fold during Oe, whereas ICW increased
21 only 1.3 fold.

22 The increase in ECW is consistent with data on bovine myometrial
23 tissue (Hawk et al., 1961), but the percent increase was higher
24 in vulvar than in myometrial tissue.

1 The absolute increase of ICW agrees with reports on the
2 response of the uterus to oestrogen (Talbot et al., 1940; Cole,
3 1950; Wrobel, 1971; Spaziani, 1975; Fugimoto and Morrill,
4 1978).

5 The lowered CD in Oe should have reduced the % DM. The lack
6 of change in % DM can be explained by a concomitant increase in
7 the DM content per cell. In fact, natural and experimental
8 oestrogen stimulation of uterine tissue increase the protein
9 content of oedema fluids (Kalman and Loewenstein, 1958; Peterson
10 and Spaziani, 1971; Spaziani, 1975), and the number of
11 mitochondria (Wrobel, 1971) and ribosomes (Teng and Hamilton,
12 1970).

13 Sodium and chloride ions are characteristic of the extracellular
14 compartment (Gamble, 1954). Therefore, an increase in this
15 compartment should be associated with an increased tissue Na^+
16 and Cl^- . Our histological and inulin data showed an increased
17 ECW during Oe, but no differences occurred in tissue Na^+ or Cl^- .
18 The apparent contradiction can be explained by a decreased Na^+
19 and Cl^- in the ECW, as evidenced by the decreased plasma Na^+ and
20 Cl^- ; plasma and ECW ion concentrations are similar to each other
21 (Gamble, 1954).

22 The decreased plasma Na^+ during Oe is in agreement with reports
23 on other species. Oestrogens are known to cause a decrease in
24 plasma Na^+ of rats (Hasan et al., 1982) and menopausal women

1 (Hodgkinson, 1982), and in total body Na^+ of sheep (Michell and
2 Noakes, 1985).

3 The present findings further our understanding of the
4 perioestrous decrease in electrical impedance within the vaginal
5 and vulvar tissues (VEI) (Feldmann et al., 1978; Aizinbud et
6 al., 1984; Lehrer et al., 1985; Lewis et al., 1989; Smith et
7 al., 1989). We conclude that the VEI decrease in Oe is caused
8 mainly by the decline of CD, i.e., the increased TTW and,
9 particularly, ECW. Such a conclusion corroborates our
10 frequency-spectrum analyses of VEI (Lewis et al., 1989) and the
11 decreased electrical impedance of blood with decreased
12 erythrocyte concentrations (Pfuetzner, 1984). Another cause of
13 the reduced VEI could be the tissue swelling, which is analogous
14 to an enlargement of the cross-section of an electrical
15 conductor (Smith, 1987).

16 The results of the present study point to the possibility
17 that changes in VEI are related not only to hydrational changes,
18 but also to variations in tissue contents of soluble proteins
19 (Kalman and Loewenstein, 1958; Peterson and Spaziani, 1971;
20 Spaziani, 1975) and membranous components like mitochondria
21 (Wrobel, 1971) and ribosomes (Teng and Hamilton, 1970).
22 Verification of this assumption should further our understanding
23 of events in the vulvar tissue which cause the cyclical changes
24 in VEI.

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4 Gacitua for technical assistance.

1 List of illustrations

- 2 Fig. 1 The absolute (g, expressed by area) and relative
3 (% , expressed by central angle) amounts of dry matter
4 (DM), extracellular water (ECW) and intracellular
5 water (ICW) in vulvar tissue of dioestrous and
6 and oestrous cows.

Table 1. VARIABLES OF VULVAR TISSUE AND OF BLOOD PLASMA
IN DIOESTROUS AND OESTROUS COWS (MEAN $\pm$ S.D.)

- A. Tissue variables: - Mesenchymal cell density (CD), extracellular water (ECW), total^{tissue} water (TTW), and sodium (Na^+) and chloride (Cl^-) ion concentrations.
- B. Plasma variables: Na^+ , Cl^- and osmotic concentration (OC).
- C. Calculated mean values of tissue variables related to one mesenchymal cell.

<u>Variable</u>	<u>n</u> [*]	<u>Dioestrus</u>	<u>Oestrus</u>	<u>P</u> <
<u>A.</u>				
CD (nuclei/nm ²)	4	122.9 $\pm$ 2.5	84.5 $\pm$ 2.0	0.001
ECW (ml/100g tissue)	8	19.8 $\pm$ 5.9	33.2 $\pm$ 7.1	0.01
TTW (ml/100g tissue)	17	75.8 $\pm$ 1.7	75.5 $\pm$ 1.8	n.s.
Na^+ (meq/100g tissue)	9	7.8 $\pm$ 0.8	7.7 $\pm$ 0.9	n.s.
Cl^- (meq/100g tissue)	9	7.6 $\pm$ 0.5	7.8 $\pm$ 0.5	n.s.
=====				
<u>B.</u>				
Na^+ (meq/100g plasma)	9	13.0 $\pm$ 0.2	12.3 $\pm$ 0.3	0.05
Cl^- (meq/100g plasma)	9	9.8 $\pm$ 0.1	8.9 $\pm$ 0.3	0.05
OC (mOsm/100g plasma)	9	30.0 $\pm$ 0.2	28.2 $\pm$ 0.5	0.01
=====				
<u>C.</u>				
Total weight (pg)		23.2	40.4	
Dry matter (pg)		5.6	9.9	
Total water (pg)		17.6	30.5	
Extracellular water (pg)		4.6	13.4	
Intracellular water (pg)		13.0	17.1	
Number of cells/ng tissue		43	25	
=====				

*n = Number of cows.

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Vulvar Electrical Impedance in Periparturient Cows and its Relation to Plasma Progesterone, Oestradiol-17 β and PGFM

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ABSTRACT

Schindler, D., Lewis, G.S., Rosenberg, M., Tadmor, A., Ezov, N., Ron, M., Aizinbud, E. and Lehrer, A.R. 1989. Vulvar electrical impedance in periparturient cows and its relation to plasma progesterone, oestradiol-17 β and PGFM. Anim. Reprod. Sci.

In previous studies we described the vulvar electrical impedance (VEI) - which refers to tissue hydration - during the oestrous cycle of cows. The objectives of the present study were to: 1) describe VEI changes around the time of calving; and 2) determine the relationship between periparturient changes in VEI and jugular plasma concentrations of oestradiol-17 β (E_2), progesterone (P_4) and 13,14-dihydro-15-keto PGF $_{2\alpha}$ (PGFM). Cylindrical bipolar electrodes were implanted inside the vulvar labiae of seven Israeli-Friesian cows. From day 245 of gestation through 15 d postpartum (pp), VEI was measured, once daily in the morning, by means of a Hewlett-Packard multifrequency impedance analyzer. Jugular blood samples for hormone determination were collected immediately after each VEI measurement. VEI decreased from approximately 150 Ω 27 d before parturition (days of calving = day 0) to 120 Ω on day -10. The % prepartal VEI decrease was greater at current frequencies <10 KHz than at >10 KHz. This indicated the decrease was caused mainly by an increase in the extracellular compartment. During that period P_4 decreased from ~11 ng/ml to 6 ng/ml and E_2 increased from 50 pg/ml to 150 pg/ml. VEI remained low until parturition, while P_4 continued to decrease to <1 ng/ml and E_2 continued to increase to ~600 pg/ml at calving. There was no significant prepartal relationship between PGFM and VEI. VEI increased postpartum from 120 Ω on

day 1 pp to 155 Q on day 11 and remained high thereafter. A negative linear relationship between PGFM and VEI was found during the pp period. The prepartal decrease of VEI occurred when the $E_2:P_4$ (pg/ml:ng/ml) ratio was $\geq 10:1$. Measuring VEI may become a tool for 1) studying the dynamics of hormone action on the hydration of target tissues; 2) determining impending calving; and 3) monitoring the recovery of genital tissue after calving.

INTRODUCTION

Electrical impedance (EI) of biological tissues is negatively associated with the degree of tissue hydration, i.e. increases in tissue hydration reduce tissue EI (Van der Veen et al., 1973; Settle et al., 1980; Segal et al., 1985). Indeed, the EI - measured with surgically implanted electrodes - of bovine and ovine vaginal and vulvar tissues decreased during oestrus, and these decreases in EI at oestrus were associated with increases in hydration of genital tissues (Feldman et al., 1978; Aizinbud et al., 1985a; Ezov et al., 1985; Lewis et al., 1989; Smith et al., 1989).

Before calving, the vulva becomes oedematous, and after calving vulvar oedema dissipates (Gier & Marion, 1968; Ruesse & Grunert, 1978). Until recently, visual and manual examinations were the only in vivo methods for evaluating vulvar oedema. To the best of our knowledge, no objective measurements have been made in vivo of the hydrational changes in vulvar tissues during the periparturient period.

Increased plasma concentration of oestradiol-17 β (E_2) increased blood flow, capillary permeability and retention of water in oestrogen-sensitive organs (Hisaw, 1959; Spaziani & Szego, 1959; Resnik, 1981). In contrast,

progesterone (P_4) appeared to antagonize the hydrational action of E_2 (Walters & Clark, 1979). Because prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can be vasoconstrictive and can alter blood flow, it should influence tissue hydration (Ford, 1982). In cattle, E_2 , P_4 and $PGF_{2\alpha}$ change dramatically and coincidentally with periparturient genital tissue hydration changes (for review, see Bazer & First, 1983). Defining quantitative relationships between changes in vulvar EI (VEI) and changes in E_2 , P_4 and $PGF_{2\alpha}$ during the periparturient period should improve our understanding of the mechanisms controlling the formation and dissipation of vulvar oedema around calving. Therefore, the objectives of this study were to 1) describe changes in bovine VEI before and after calving, and 2) determine the relationships between periparturient changes in VEI and changes in jugular plasma concentrations of E_2 , P_4 and a metabolite of $PGF_{2\alpha}$ (13,14-dihydro-15-keto- $PGF_{2\alpha}$; PGFM). Some of the data in this article were presented elsewhere (Schindler *et al.*, 1985).

MATERIALS AND METHODS

General procedure. On day 220 of gestation, capsular, bipolar electrodes were implanted surgically in the vulvar tissue of seven Israel-Friesian cows from the Agricultural Research Organization's experimental herd. The surgical wounds were allowed to heal for approximately 3 weeks. VEI was measured once daily in the morning until 15 days after calving. On alternate days from at least 20 days before (day -20) until 15 days after (day +15) calving (day 0), jugular blood samples were collected immediately

after VEI was measured, and E_2 , P_4 and PGFM in the plasma were quantified later.

Electrode design, electronic methods for measuring EI and surgical procedures for implanting electrodes have been described previously (Aizinbud *et al.*, 1985b; Lewis *et al.*, 1989). Tissue impedance was measured in ohms (Ω) with a Hewlett-Packard model 4277 multifrequency LCR meter at current frequencies of 0.1, 0.12, 0.3, 0.5, 1, 3, 5, 10, 30, 50 and 100 KHZ. Routine, daily evaluations of changes in EI were based upon measurements made at 50 kHz.

Radioimmunoassays. Plasma concentrations of E_2 were determined with a radioimmunoassay (RIA) described previously (Rosenberg *et al.*, 1987). Prepartum and postpartum samples were assayed in separate batches, in order to adjust the standard curve at each batch to the different expected ranges of plasma concentrations of the assayed hormone. Assay sensitivity was 10 pg/ml for plasma samples collected prepartum and 0.65 pg/ml for those collected postpartum. Intra- and interassay coefficients of variation were, respectively, 11.4% and 16% for prepartum samples, and 14.6% and 27% for postpartum samples.

Progesterone was quantified with commercial RIA kits (Diagnostic Product Corp., Los Angeles, California). Appropriate known concentrations of P_4 , dissolved in plasma of an ovariectomized cow, were used for the standard curves. Intra- and interassay coefficients of variation were 9.8% and 13.2%, respectively.

The RIA for PGFM was the same as that described by Guthrie & Rexroad (1981). Intra- and interassay coefficients of variation were 18% and 15%, respectively.

Statistical analyses. Data analyses were similar to those described previously (Lewis *et al.*, 1989). The General Linear Models (GLM) procedure in the Statistical Analysis System (SAS, 1982) was used to evaluate the effects of days before and after calving on changes in VEI, E_2 , P_4 and PGFM, and the relationships during VEI E_2 , P_4 and PGFM. In models used to analyze VEI data cows were considered as the main effect and day, E_2 , P_4 and PGFM were considered as covariates. Data were analyzed separately for the prepartum and postpartum periods. The models used to predict the dependent variables contained only independent variables that accounted for significant ($P < 0.05$) amounts of variation in the data. Regression equations from GLM analyses were used to describe changes in VEI, E_2 , P_4 and PGFM. Predictions from prepartum and postpartum periods were combined to illustrate overall day trends.

The effects of current frequency on prepartum changes in VEI were evaluated. The change in EI of a given tissue at a given time in response to changes in current frequency is termed the impedance spectrum. For comparing impedance spectra, models included cows and periods as main effects and frequency as an independent continuous variable. Interactions of frequency by cows, frequency by periods and frequency by days within periods were also included in the statistical model.

RESULTS

Prepartum and postpartum changes in VEI are shown in Figure 1, Panel A.

Prepartum, a third-order polynomial regression equation best described changes in VEI. Vulvar EI was 150 Ω before day -27, decreased to 120 Ω on

day -10, and remained at approximately 120 Ω until calving (day 0). Because predicted changes in VEI masked prominent daily changes in individual cows prepartum, changes in VEI in individual cows were evaluated. For each cow, VEI was considered low when it was at least one standard deviation less than the average (baseline) of all her VEI values from -40 days before until 15 days after calving. Vulvar EI was low from day -15 ± 7.5 ($x \pm SD$; range, day -26 to day 6) until day 0.4 ± 2.6 (range, day -4 to day 5). During the period when VEI was low, average VEI was less ($P < 0.001$) than that before or after the low period (115.5 vs 144.6 and 152.7 Ω , respectively; $SD = 16.5$).

The effects of current frequency on VEI before and during periods of low VEI are shown in Figure 2. The impedance spectrum during periods when VEI was low was less ($P < 0.001$) than that during the period before VEI was low. The difference in VEI between periods was greatest at the lowest current frequency and least at the highest current frequency (Fig. 2).

Postpartum, a second-order polynomial regression equation best described changes in VEI (Fig. 1, Panel A). VEI increased from 120 Ω on day 1 to about 155 Ω on day 11 and remained high thereafter.

Prepartum, a third-order polynomial regression equation best described changes in P_4 concentrations (Fig. 1, Panel B). Concentrations of P_4 were approximately 8.5 ng/ml on day -26, 4 ng/ml on day -2 and 0.8 ng/ml on day 0. During the first 15 days postpartum, concentrations of P_4 remained < 0.5 ng/ml of plasma.

Prepartum, a third-order polynomial regression equation best described changes in E_2 concentrations (Fig. 1, Panel C). Concentrations of E_2 increased from 20 pg/ml on day -25 to ~ 600 pg/ml on day 0. Postpartum, a fourth-order polynomial regression equation best described changes in E_2

concentrations (Fig. 1, Panel C). Concentrations of E_2 decreased from ~600 pg/ml on day 0 to <0.1 pg/ml on day 3 and remained low until the end of the sampling period.

Prepartum, a fifth-order polynomial regression equation best described changes in PGFM concentrations (Fig. 1, Panel D). PGFM was 400 pg/ml on day -20, 700 pg/ml on day -5 and 3000 pg/ml on day 0. Postpartum, a first-order regression equation best described PGFM concentrations (Fig. 1, Panel D). Concentrations of PGFM decreased from 1000 pg/ml on day 1 to 500 pg/ml on day 11.

Significant ($P < 0.05$) prepartum relationships between P_4 and VEI and E_2 and VEI are shown in Figure 3. Vulvar EI decreased from ~140 Ω to 120 Ω as P_4 decreased from ~11 ng/ml to 6 ng/ml. Progesterone decreased to <1 ng/ml before calving, but VEI did not decrease to <120 Ω . Vulvar EI decreased from 145 Ω to 120 Ω as E_2 increased from 50 pg/ml to 150 pg/ml. Even though E_2 continued to increase from 150 pg/ml to more than 600 pg/ml at calving, VEI did not continue to decrease. There was no significant prepartum relationship between PGFM and VEI, but VEI tended to have a negative linear relationship with PGFM postpartum.

DISCUSSION

The results of this study indicate that electrical impedance of vulvar tissue was less than baseline for approximately 2 weeks before calving. Vulvar EI before the prepartum decrease averaged 145 Ω , which is similar to diestrous measurements (Lehrer *et al.*, 1985; Lewis *et al.*, 1989). Before calving, as during oestrus, VEI declined ~20% (Lehrer *et al.*, 1985).

However, whereas VEI before calving was low for ~2 weeks, VEI at oestrus was low <24 h (Feldman *et al.*, 1978; Lehrer *et al.*, 1985; Lewis *et al.*, 1989).

Changes in EI of living tissue measured at current frequencies >10 kHz reflect mainly a change in the total fluid volume of the tissues, while changes in EI measured at frequencies <10 kHz reflect mainly a change in extracellular fluid volume (Eyraud *et al.*, 1969; Settle *et al.*, 1980). Therefore, the prepartum decrease in VEI measured at 50 kHz was probably due to an increase in total fluid volume of vulvar tissue.

Differences in impedance spectra before and during the prepartum decline in VEI were greatest at the lower current frequencies, which indicates that an increase in extracellular fluid was the main factor responsible for the increase in total fluid volume. Similar interpretations of data were reported for sheep and cattle during the oestrous cycle, i.e., extracellular fluids accumulated during oestrus and increased total fluid volume (Adam *et al.*, 1981; Lehrer *et al.*, 1985; Lewis *et al.*, 1989).

Prepartum changes in E_2 and P_4 and postpartum changes in P_4 were similar to those reported previously (Robertson, 1974; Eley *et al.*, 1981; Humphrey *et al.*, 1983). Prepartum and postpartum changes in PGFM were similar to those in other studies (Edquist *et al.*, 1978; Lewis *et al.*, 1984).

Postpartum, the positive relationships between VEI and P_4 , and the negative relationship between VEI and E_2 , are consistent with our data on the oestrous cycle of cattle, i.e., around oestrus VEI decreased as P_4 decreased and E_2 increased (Lehrer *et al.*, 1985; Lewis *et al.*, 1989). The physiological control of the prepartum decrease in VEI is probably related to the decline in P_4 and the subsequent ability of E_2 to increase hypermia (Williams, 1948), blood flow (Resnik *et al.*, 1974) and capillary

permeability (Spaziani and Szego, 1959). Progesterone appears to suppress the oestrogen-receptor system in target tissues and attenuate oestrogen-induced water imbibition (West *et al.*, 1976). In addition, the $E_2:P_4$ ratio influenced changes in oestrogen receptor concentrations in target tissues (West and Brenner, 1985). In the present study VEI decreased, a result of water imbibition when the $E_2:P_4$ (pg/ml:ng/ml) ratio was 10:1. In oestrous cows, when VEI was low and total fluid volume was high, the $E_2:P_4$ was approximately 10:1 (Lewis *et al.*, 1989) but the absolute concentrations of both hormones were much lower. Other studies indicate that $E_2:P_4$ ratio, rather than absolute concentrations, affected uterine blood flow (Caton *et al.*, 1980). In the present study, VEI decreased when E_2 was 10 to 25 times higher than it was in oestrous cows (Lewis *et al.*, 1989). Apparently, in prepartum cows, higher concentrations of E_2 were necessary to overcome the suppressive effects of high concentrations of P_4 than were required in oestrous cows to reduce VEI.

Postpartum, the rapid increase in VEI to that measured during dioestrus, probably reflects vulvar tissue dehydration, which may be the consequence of low E_2 and the normal postpartum constriction of vulvar vasculature (Gier and Marion, 1968). The negative relationship postpartum between PGFM and VEI is consistent with other work that associated the postpartum decline in jugular concentrations of PGFM with reduced uterine blood flow (Guibault *et al.*, 1984). Because several studies indicate that during the first 3 to 4 weeks after calving changes in concentrations of PGFM are a good indicator of reductions in uterine horn diameter (uterine involution), and because we found a tendency for a negative relationship between PGFM and VEI postpartum, changes in VEI during the first 2 weeks after calving may prove to be a useful method for assessing the initial rate of uterine involution (Lindell *et al.*, 1982; Lewis *et al.*, 1984).

Results of this study indicate that measuring VEI may 1) become a valuable physiological method for studying the dynamics of hormone action on target tissues, 2) be a reliable indicator of impending calving, and 3) be a method for monitoring the rate of repair of genital tissues after calving.

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LIST OF ILLUSTRATIONS

- Fig. 1 Actual (*) and predicted (—) prepartum and postpartum changes in vulvar electrical impedance (Panel A), progesterone (Panel B), oestradiol-17 β (Panel C), and 13,14-dihydro-15-keto-PGF_{2 α} (PGFM) (Panel D). Equations used to predict the data are shown for each graph.
- Fig. 2 Least-squares regression of changes in the vulvar electrical impedance before and during prepartal depression at frequency domain of 0.1-50 KH.
- Fig. 3 Least-squares polynomial regression of vulvar electrical impedance (VEI) on progesterone (Panel A) and on oestradiol-17 β (Panel B) levels in prepartum cows.

Fig. 1

$$Y = 130.9 - 5.2X + 0.6X^2; \quad R^2 = 0.27 \quad n = 145$$

(*) VEI means (every 1 ng/ml of P_4)

$$Y = 151.5 - 0.4X + 0.0015X^2 - 0.000002X^3 + 0.0000000012X^4; \quad R^2 = 0.39 \quad n = 162$$

(*) VEI means (every 10 pg/ml of estradiol-17β)

Fig. 3

VULVAR ELECTRICAL IMPEDANCE AND ITS RELATION TO PLASMA
ESTRADIOL-17 β , PROGESTERONE AND PGFM AROUND PGF INDUCED
CALVING IN COWS.*

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ABSTRACT

Schindler D., Lewis G.S., Tadmor, A., Aizinbud, E. and Lehrer, A.R. Vulvar electrical impedance and its relation to plasma estradiol-17 β , progesterone and PGFM around PGE_{2 α} -induced calving in cows. Anim. Reprod. Sci. (in preparation).

The objectives of this study were to: (1) quantify, in vivo, by means of vulvar electrical impedance (VEI) measurements, the changes in vulvar tissue hydration around PGE_{2 α} -induced calving; (2) determine the relationship between periparturient changes in VEI and jugular blood plasma concentrations of estradiol-17 β (E₂), and progesterone (P₄) and prostaglandin F_{2 α} metabolite (PGFM); (3) compare the results with those obtained from spontaneous by calving cows.

Cylindrical bipolar electrodes were implanted inside the vulvar labia of four Israeli-Friesian cows at day 220 of gestation. VEI was measured once daily by a Hewlett Packard multifrequency impedance analyzer starting on day 245 of gestation until 15 day postpartum (pp). Jugular blood samples for hormone determinations were collected immediately after VEI was measured. Calving occurred 75 $\pm$ 18 h (mean $\pm$ s.d.) after a single I.M. injection of synthetic PGF, at day 268 $\pm$ 0.7 of gestation. There was a slight and insignificant decrease in VEI prepartum (from 148 to 138 ohm). P decreased abruptly after PGF injection from 7-8 ng/ml to 0.5 ng/ml at term. E increased from 80 pg/ml 3

days before calving to a peak of 200 pg/ml at term. PGFM increased sharply 3 days before calving and reached a peak of 4000 pg/ml 2 days pp. The pp period was characterized by a continuous decrease in VEI followed by an increase in VEI 7 days pp. E_4 remained low (<0.5 ng/ml) for the first 15 days pp. E_2 ranged from 100 pg/ml 2 days pp to 200 pg/ml at day 10 pp. All the cows retained their placenta for at least 72 h pp.

It is concluded that induced calvings, by $PGE_{2\alpha}$ injection on day 268 of gestation, are associated with lower E_2 and higher VEI levels, relative to our findings in spontaneous calving (see pages 49-67). The higher VEI levels reflected a lower degree of genital tissue hydration than that required for proper disengagement of the cow cotyledon from the caruncle, and is probably the cause of retained placentae.

INTRODUCTION

Varying degrees of genital tissue hydration around the time of calving can be identified in vivo by electrical impedometry, since a negative correlation exists between tissue hydration and tissue electrical impedance (Hoffer et al., 1969; Thomasset, 1970; Van der Veen et al., 1973; Settle et al., 1980; Segal et al., 1985). In our studies (Schindler et al., 1985; Schindler et al., 1989) the changes in vulvar electrical impedance (VEI) and their relationship to hormonal changes around the time of spontaneous calving revealed that VEI dropped almost suddenly from 152 to 118 Ω

at about 15 d prepartum and remained low until calving. The prepartum VEI decrease occurred when plasma oestradiol-17 β (E_2) and progesterone (P_4) ratio was approximately 10:1 (pg/ml:ng/ml). After calving VEI increased gradually due to genital tissue postpartal dehydration.

Corticosteroids- and prostaglandins-induced calvings are often accompanied by relatively low levels of plasma E_2 (Evans & Wagner, 1976; Henricks et al., 1977), by a lack of vulvar oedema, and by placental retention (Bosc et al, 1975; Johnson, 1981). We hypothesized that the smaller increase of plasma E levels during induced calvings relative to spontaneous calvings should be reflected by higher VEI levels in induced calvers. The objectives of this study were, thus, to (1) quantify VEI in induced calvings, (2) relate it to plasma E_2 , P_4 and PGFM levels, and (3) compare the results with those obtained by us previously, during spontaneous calving.

MATERIALS AND METHODS

The details of the experimental protocol, animal management, VEI measurements, hormone assays and statistical analyses were described by us elsewhere (Schindler et al, 1989).

Calving was induced in four cows by an I.M. injection of 25 mg of PGF (Lutelyse; The Upjohn Co., West Sussex, U.K.) on day 267-269 of gestation.

RESULTS AND DISCUSSION

The timing of the $\text{PGE}_{2\alpha}$ injection in relation to the day of gestation and the day of parturition is presented in the Table 1.

Table 1. THE TIMING OF PGF INJECTION TO PREGNANT COWS FOR INDUCTION OF CALVING.

Cow	Time of $\text{PGE}_{2\alpha}$ injection	
	After fertile insemination (days)	Prior to calving (hours)
601	267	59
677	269	76
681	268	104
751	268	61
Mean $\pm$ SD	269 $\pm$ 0.7	75 $\pm$ 18

Calving occurred an average of 75 h after injection, with a wide range of 59 to 104 h ($\text{SD} = \pm 18$).

The results of the present study are given in Figure 1. Each figure presents a polynomial regression of one of the four studied variables on time peripartum. Regressions presented in our previous study (Schindler et al, 1989) on seven cows

with spontaneous calvings are presented as well, for the sake of comparison.

Prepartal decrease in VEI at induced calving (Fig. 1, panel A) was relatively smaller (from 148 to 138 ohm) than that at spontaneous calvings (from 152 to 118 ohm). After the induced calving VEI continued to decrease slowly for a week and then started to increase. On day 15 postpartum VEI did not reach the level monitored prior to the prepartal VEI trough. In contrast, the VEI level in spontaneous calvings started to rise immediately after parturition, and on day 13 postpartum reached the levels monitored prior to the prepartal decrease.

There was no significant difference between cows with induced and spontaneous calvings in plasma P_4 profiles (Fig. 1, panel B). P_4 level on day 15 before calving was 7-8 ng/ml and dropped immediately after $PGE_{2\alpha}$ injection and down to 0.5 ng/ml at term. It remained at that level during the first 15 d postpartum. In induced calvings, the rise in E_2 level (Fig. 1, panel C) occurred later and its peak was 2.5 times lower (200 pg/ml vs. 500 pg/ml) than in spontaneous calving. E_2 decreased slower and remained higher after spontaneous calving (100 pg/ml at day 1 pp to 6 pg/ml at 4 d pp vs. 8 pg/ml to <1 pg/ml, respectively).

Figure 1- panel D shows that the PGFM level increased sharply 3 days before calving and reached a peak of 4000 pg/ml on day 2 postpartum, thereafter decreased to 200 pg/ml on day 10 pp. In spontaneous calvings PGFM reached a peak of 3000 pg/ml 2 days earlier, i.e., at term, and

decreased immediately afterward.

In comparison with spontaneous calvers, those with induced calvings were characterized by (a) relatively low plasma E_2 level; b) high VEI, and (c) retained placental membranes after delivery. (All of the four induced calvings were followed by placental retention).

Since VEI is negatively correlated with tissue hydration, the findings support the hypothesis that placental retention could be attributed to lack of prepartal genital tissue, hydration (Grunert, 1984).

The oestrogens are characterized by their ability to cause fluid and electrolyte accumulation in target tissue (for pertinent references, see Spaziani, 1975; Segal & Koide, 1979). The relatively low oestrogen level before the induced parturition apparently reduced fluid and electrolyte accumulation in the genital tissue, as was reflected in a relatively high VEI.

Grunert et al. (1976), Chew et al. (1978) and Murray et al. (1982) pointed out that lack of oestrogen causes placental retention. Grunert (1984) assumes that increased secretion of E and oestrone for a period of at least 6 days prior to calving is required for completion of placentomes maturation; the latter is characterized, inter alia, by water imbibition.

According to Grunert (1984) edematization of the placentomes is a prerequisite for separation of cotyledons from caruncles at term. In spontaneous calvers with no retained placentas, VEI troughs lasted at least 6 days and were accompanied by

high levels of E_2 (Schindler et al, 1989). It is hypothesized that placenta retention in induced calving cows could be prevented if the E level were higher and the VEI level lower for at least 6 days before prostaglandin injection. This should be examined in future trials.

LIST OF ILLUSTRATIONS

FIG. 1. Actual (*) and predicted (-) changes in vulvar electrical impedance (Panel A), oestradiol 17 (Panel B), progesterone (Panel C) and $PGF_{2\alpha}$ metabolite (PGFM, Panel D) before (-) and after (+) induced calving (0). The polynomes of our previous results on spontaneous calvings (see Schindler et al, 1989) are also presented (---), for the sake of comparison.

Panel D

Days before (-) and after (+) calving (day 0)

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VULVAR ELECTRICAL IMPEDANCE COMPONENTS RELATIVE TO CHANGES IN PLASMA PROGESTERONE AND OESTRADIOL-17 β IN THE POSTPARTUM COW.

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ABSTRACT

Raz, J., Rosenberg, J., Lewis, G.S., Dajan, H., Tadmor, A., Nikbakht, M., Aizinbud, E. and Lehrer, A.R. 1990. Vulvar electrical impedance components relative to changes in plasma progesterone and oestradiol-17 β in the postpartum cow. Anim. Reprod. Sci. (in preparation).

In eight Israeli-Friesian cows, cylindrical bipolar electrodes (28 mm long, 8 mm in diameter) were implanted in a vulvar lip at 345 ± 3 d of gestation. Vulvar tissue impedance components (VIC), namely, resistance (R) and reactance (X), were measured one to four times a day from calving through 90 ± 140 d postpartum (pp). Jugular blood samples, for quantification of progesterone (P₄) and oestradiol-17 β (E₂), were taken each time VIC were measured. R, X and the calculated angle θ increased sharply and linearly with time pp, followed by stabilisation or by a very modest increase. R increased from $95 \pm 1.9 \Omega$ (mean $\pm$ SE) at calving d to $136 \pm 1.3 \Omega$ at day 44 ± 1.4 pp. X increased from $9 \pm 2.7 \Omega$ at calving to 23 ± 0.67 at day 54 ± 2.7 pp. θ increased from $6 \pm 0.3^\circ$ to $9 \pm 0.2^\circ$ at day 56 ± 3.7 pp. During the first pp ovarian cycle, which took place during the increase, but prior to stabilisation, of VIC, no significant decrease of the VIC values at the non-luteal phase was found. In the second and third cycles this decrease was significant in comparison with the diestrous period. The rate of decrease in X was higher than that in R and θ (-28% vs. -13% and -18%, respectively).

During the pp anoestrous period, the correlation coefficient (cc) between VIC and E₂ was low and not significant; during the first pp ovarian cycle the cc ranged between -0.48 and -0.55 ($P < 0.05$) and in the second and

third cycles between -0.78 and -0.86 ($P < 0.001$). The changes in VIC reflect the course of tissue dehydration and cellular changes in the external genitalia and the resumption of the ovarian activity in the postpartum cow.

INTRODUCTION

One of the characteristic phenomena of the postpartum (pp) recovery process of the reproductive tissue in the cow is the involution of the genitalia, caused, *inter alia*, by loss of fluids (Gier and Marion, 1968; Kiracofe, 1980). The disappearance of vulvar edema at the pp, as a symptom of this process, has been described visually (Ruesse and Grunert, 1978) but never been quantified because of a lack of appropriate methods. In the last decade, biophysical methods have enabled the monitoring of hydration changes in the bovine and ovine vulvar tissue *in vivo* by measuring the tissue's electrical impedance (Adam et al., 1981; Aizinbud et al., 1984; Lewis et al., 1989). This method is based on the inverse relationship between tissue water content and its electrical impedance (Hoffer et al., 1969; Thomasset, 1965; Van der Veen et al., 1973; Gambini et al., 1980; Settle et al., 1980; Lukaski, 1987; Segal et al., 1988).

In previous studies, the changes in the bovine vulvar electrical impedance (VEI) during perioestrous and periparturient periods were clarified related to plasma progesterone (P_4) and oestradiol 17β levels (Aizinbud et al., 1984; Lehrer et al., 1985; Schindler et al., 1985; Lewis et al., 1989; Smith et al., 1989). The VEI changes, which take place in the period between calving and pp resumption of ovarian cyclicity, have not been studied previously. In the works cited above, the whole tissue

impedance (Z), or its resistant component (R), was investigated. The changes in the second component of Z , the reactance (X), and the phase angle (θ), have not been clarified. Based on the theory of electrical properties of suspension of spherical cells (Fricke, 1933; Schwan, 1957; Settle et al., 1980; Adam et al., 1981), it is accepted that R , at frequencies higher than 10 kHz reflects mainly the total tissue fluid content, X the amount of cell membrane, and θ the volume fraction of the intracellular fluids of the total tissue fluids. We hypothesised that the latter three vulvar electrical impedance components (VIC) change differently from each other during the pp recovery of the female genitalia and resumption of the ovarian cyclicity.

The aim of the present study was to serve as a bridge between previous studies, in which Z was investigated during either the peripartum period or the late pp oestrous cycle. The specific objectives were to (i) determine the VIC during pp anoestrus and the first three ovarian cycles; (ii) relate the VIC to changes in plasma progesterone (P_4) and oestradiol-17 β (E_2); and (iii) compare the pp changes among the VIC themselves.

MATERIALS AND METHODS

Cows, electrodes and surgery. In eight Israeli-Friesian cows at second or third lactation, cylindrical bipolar electrodes (28 mm long, 8 mm in diameter), with hemispherical poles (4 mm long, 8 mm in diameter) on either end, were implanted in a vulvar lip of each cow at 245 ± 3 d of gestation. The electrode cable was passed subcutaneously through the cow's back and exteriorised for connection to the measuring equipment. Details of the

electrodes' structure and of the surgical technique were described previously (Feldman et al., 1978; Lewis et al., 1989). The cows were checked for oestrus two to four times daily.

VIC measurements. The vulvar tissue impedance components, R and X, were measured by a Bio-impedance Analyzer, model BIA-101 (R.J.L. Systems, Detroit, MI, USA) at 50 kHz and 0.8 mAmp. The electrical impedance (Z) and the phase angle (ϕ) were calculated from the equations $Z = \sqrt{R^2 + X^2}$ and $\phi = \tan^{-1} X/R$.

VIC were measured routinely once a day, starting on the day of electrode implantation and up to 140 d pp. During expected perioestrous periods, VIC were measured two to four times a day.

Hormone assays. Blood samples, for quantification of P_4 and oestradiol-17 β (E_2), were taken each time VIC were measured. Radioimmunoassays, validated in the laboratory of the Institute of Animal Sciences, Bet Dagan, were used to quantify P_4 and E_2 (Rosenberg, et al. 1987).

Statistics. For each cow the daily average of the VIC was taken as a single measurement of a day in a cow, to calculate the average of the experimental cows.

The VIC data were used to characterise changes over time pp by a segmented bi-modal linear regression model, with the cows included within the group and the days assumed as independent continued variables. The slopes of the changing values and the timing of the transition from one segment to another were determined by analysis by PROC NLIN fitting to a segmented model (SAS, 1985).

The mathematical models used to evaluate changes over time around the time of oestrus included the cow as a classification variable and, depending upon the dependent variable being evaluated, the day of the ovarian cycle, with concentration of P_4 and E_2 as covariants. First- to fifth-order effects of the covariants were used to construct mathematical models that best described the data. The final models for the dependent variables contained only independent variables that accounted for significant ($P < 0.05$) amounts of variation in the data and were used to predict daily changes in VIC, P_4 and E_2 . Actual means and predicted data were plotted.

To determine if a given measure of VIC deviated from a rolling average, which for this study was considered a baseline, a Z-statistic (Steel and Torrie, 1960; Lewis et al., 1989) was calculated for each measure of VIC. The Statistical Analysis System (SAS, 1985) was used for all statistical analyses.

RESULTS

The onset of the ovarian cycle, if oestrus was absent, (day 0), was defined as the third day prior to an increase in P_4 above 1 ng/ml plasma, which lasted at least seven consecutive days.

The distribution of the cows by day of pp resumption of luteal activity was 21-40 days - 4 cows; 65 days - 1 cow; 92 days - 1 cow; 98 days - 1 cow; >140 days - 1 cow. The last mentioned cow suffered abomasal dislocation and chronic mastitis, and the results obtained with her were not included in the average calculations of the group.

Oestrus was detected at the onset of none of the first seven ovarian cycles, in contrast to detected oestruses in eight out of ten 2nd + 3rd pp ovarian cycles.

The difference between the measured R and the calculated Z was less than 2%. Z is therefore not reported.

Vulvar tissue impedance components (i.e., R, X and θ) increased with time after calving in a pattern which fitted a segmented bi-modal linear regression model (Fig. 1). A sharp linear increase in VIC was found during the first stage, followed by a stabilisation or a modest increase during the second stage (Table 1).

The rate of increase in X was greater ($P > 0.001$) than that in R and θ . The sharp increase in R ended prior ($P < 0.01$) to that in X and θ . During the second stage of the course of VIC changes, there was a progressive increase of 10% and 8% for X and θ , respectively, relative to their values at the beginning of the second stage; R was already stabilised by the end of its sharp increase.

There was a low and insignificant correlation coefficient (cc) between the time of R stabilisation and that of the resumption of luteal activity in all the cows, excluding a sick one ($n=7$). However, in the five cows in which luteal activity resumed within 65 days pp (mean $\pm$ S.E. 32 ± 5.6 d), a time interval which is considered normal, the cc was 0.86 ($P < 0.05$). In the latter five cows a cc of 0.92 ($P < 0.01$) was found between the times of R stabilisation and first pp oestrus. Luteal activity was not resumed in the sick cow. The stabilisation of R in this cow was delayed until 105 days pp.

The patterns of R, X and θ were compared among themselves during the first three pp ovarian cycles. The daily value of R, X and θ on days -2 through +2 of the cycle (the non-luteal value) was compared with the

average of the ten VIC respective daily values on days -4 to -8 and +4 to +8 (reference value). The non-luteal values of the three VIC during the first ovarian cycle did not differ significantly from the respective reference values: $R=114\pm5.8$ Q vs. 119 ± 5.5 Q; $X=16.6\pm4.2$ Q vs. 17.6 ± 3.6 Q; $\theta=8.2\pm2.1^\circ$ vs. $8.4\pm1.7^\circ$. In contrast, the non-luteal VIC values during the second and third cycles were significantly lower ($P<0.001$) than the respective reference values: $R=117\pm14.9$ Q vs. 134 ± 8.2 Q; $X=16.8\pm3.6$ Q vs. 22.9 ± 2.3 Q; $\theta=8.0\pm1.5^\circ$ vs. $9.8\pm1.1^\circ$. These findings were also confirmed by a Z-statistic test for the VIC (Fig. 2). It showed that during the first pp ovarian cycle there were no significant decreases in the Z statistic values of R, X and θ . In contrast, during the second and third cycles, significant decreases ($P<0.05$) in R, X and θ were observed as early as day 0 of the cycle, with a maximal decrease on day +2 of both cycles.

The actual means of VIC and plasma concentrations of P_4 and E_2 and data predicted with polynomial equations around the time of the first and second+third postpartum ovarian cycles, are presented in Figure 3.

Around the first ovarian cycle all VIC values were increasing, while in the following two cycles a clear perioestrous nadir ($P<0.001$) was found, in comparison with the dioestrous period. The rate of decrease in X was greater than that in R and θ (28% vs. 18%, respectively).

The cc's between R, X and θ and the levels of plasma P_4 and E_2 at the time of VIC measurements were examined. In order to test the possibility of a delayed effect of the hormone level on the VIC, the correlation coefficient between R, X and θ and the hormone level on each of the 3 days prior to the day of VIC measurement was also examined.

During the absence of ovarian luteal activity - the pp anoestrous period - there were irregular increases of up to 4 pg/ml of E_2 . However, these

increases were not accompanied by VIC changes (see representative data in Fig. 4), and the cc between VIC and E_2 were low and insignificant. The cc's between VIC and E_2 increased with time after calving. During the first pp ovarian cycle, the highest cc's were between R, X and θ , and E_2 2 days prior to, and P_4 on the same day of, the VIC measurement: -0.48, -0.52, -0.55, respectively ($P < 0.05$), with E_2 ; and 0.81, 0.83, 0.79, respectively, ($P < 0.001$), with P_4 . During the second and third cycles the highest cc's were with E_2 3 days prior to the day of VIC measurements: -0.85, -0.86, -0.78, respectively ($P < 0.001$); and with P_4 on that day: 0.64, 0.52, 0.62, respectively ($P < 0.01$).

DISCUSSION

Based on the biological meanings of the VIC (see Introduction section), we concluded that the vulva underwent a gradual loss of tissue fluids (reflected by R) during an average of approximately 6 weeks after calving. Most of the fluids were lost from the extracellular compartment, as evidenced by an increased cell density (reflected by X) and by an increased proportion of the intracellular fluid content, relative to the total tissue fluids (reflected by θ). The earlier stabilisation of R, relative to X and θ , indicated that the pp recovery of the tissue's fluid balance preceded that of the membranous components of cells.

During the pp anoestrus and the first pp ovarian cycle, no correlations were found between E_2 levels and the VIC. This apparently contradicts earlier findings in the precalving stage (Schindler et al., 1985) and during later pp ovarian cycles. We assume that the ability of oestrogens to

cause fluid and electrolyte accumulation in target tissue (Spaziani, 1975; Segal and Koide, 1979), and consequently a decrease in the VIC, cannot be fully realised during the early pp period when the genital tissues are still oedematous and in the process of involution. This assumption is supported by the fact that the VIC values during the early pp are similar to, or less than, the VIC nadirs during the non-luteal phases of the second and third pp ovarian cycles. In two cows, whose first ovarian cycle began later than 65 days pp, a VIC trough, following an increase in the E_z level, was recorded. This last observation also indicated that E_z can cause oedema (i.e., decrease in VIC), even if the target tissue was not previously exposed to the influence of P_4 ; the increase in E_z blood levels at the beginning of the first pp ovarian cycle occurs without a preceding increase in P_4 level.

To conclude, this study has added to our knowledge about the processes of pp recovery and fluid loss of the vulvar tissue and about the relationship between these processes and the resumption of the pp ovarian cyclicity. This work has also shown the potential advantage of VIC measurements, over R and Z measurements alone, for determining the hydrational and other cellular processes in the reproductive tissue in vivo.

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TABLE 1

The changes in vulvar electrical impedance components (VIC) in postpartum cows (n=7)

Component		Value at calving	Value at end of increase	Duration of sharp increase (days pp)	Rate of sharp increase (%)
R(Ω)	Mean	95	135	44	44
	$\pm$ S.E.	1.9	1.27	1.4	5.3
	Range	81-113	129-141	31-60	17-62
X(Ω)	Mean	9	23	54	146
	$\pm$ S.E.	2.7	0.67	2.7	24.3
	Range	5-13	18-28	47-69	60-263
$\theta(^{\circ})$	Mean	6	9	56	58
	$\pm$ S.E.	0.34	0.23	3.7	10.3
	Range	5-7	8-11	49-74	21-59

LIST OF ILLUSTRATIONS

Fig. 1. Actual means of vulvar electrical components and data predicted with a segmented bi-modal linear regression model in seven postpartum cows.

Fig. 2. Z-statistic calculated from mean values of vulvar electrical impedance components around the first (A, n=5) and second + third (B, n=7) postpartum ovarian cycles.

Fig. 3. Actual means of vulvar electrical impedance components, data predicted with polynomial equations, and plasma concentrations of progesterone and oestradiol-17 β around the first (A) and second + third (B) postpartum cycles. Standard errors (SE) are from the models used to predict data.

Fig. 4. Changes of reactance of the vulvar tissue, and progesterone and oestradiol-17 β plasma levels in a cow (A); and Z-statistics calculated from vulvar tissue reactance (B).

Fig. 1. Actual means of vulvar electrical components and data predicted with a segmented bi-modal linear regression model in 7 postpartum cows.

Fig. 2. Z-Statistics calculated from mean values of vulvar electrical impedance components around the first (A) ($n = 5$ cows) and second + third (B) ($n = 7$ cows), postpartum ovarian cycles.

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FOR (A):

$$R: Y = 115.51 - 2.34X + 0.50X^2 - 0.019X^3, R^2=0.91, P<0.001, SE=2.6$$

$$X: Y = 13.45 + 0.004X^2 - 0.0002X^3, R^2=0.92, P<0.001, SE=0.24$$

$$\theta: Y = 6.72 + 0.07X^2, R^2=0.85, P<0.001, SE=0.08$$

$$P_4: Y = 0.10 - 0.005X^2 + 0.0008X^3 + 0.0001X^4, R^2=0.94, P<0.001, SE=0.1$$

$$E_{17\beta}: Y = 1.52 + 1.25X - 0.05X^2 + 0.00552X^3 - 0.0001X^4, R^2=0.57, P<0.05, SE=1.44$$

FOR

(B):

$$R: Y = 134.95 + 1.14X^2 - 0.315X^3 + 0.268X^4 - 0.007X^5, R^2=0.67, P<0.05, SE=2.27$$

$$X: Y = 23.67 - 0.022X^2 + 0.0028X^3 - 0.0001X^4, R^2=0.54, P<0.01, SE=0.54$$

$$\theta: Y = 9.71 - 0.007X^2 + 0.001X^3 + 0.0001X^4, R^2=0.55, P<0.05, SE=0.14$$

$$P_4: Y = 4.01 + 1.38X - 0.81X^2 + 0.118X^3 - 0.0069X^4 + 0.0001X^5, R^2=0.97, P<0.001, SE=0.94$$

$$E_{17\beta}: Y = 1.35 - 1.79X + 1.07X^2 - 0.182X^3 + 0.0122X^4 + 0.0003X^5, R^2=0.70, P<0.01, SE=2.05$$

Progesterone ($\square$ —), ng/ml
 Estradiol-17 β (Δ - - -), pg/ml

Fig. 4. Changes of reactance of the vulvar tissue, progesterone and estradiol-17 plasma levels (A), and Z statistic calculated from vulvar tissue reactance (B) in a cow.

f. COOPERATION.

Since 1981 until present the cooperating investigators have worked together on BARD projects I-280-80 and I-872-84C. Using the BITNET computer network, the postal service, BARD-sponsored visit and sabbatical leaves, the counterparts have kept each other abreast of major aspects of the work in Israel and the United States. Through close cooperation the investigators, students and technicians of their research groups, have worked together directly to develop and standardize radioimmunoassay procedures, develop surgical protocols, electrodes, and impedance measuring procedures. They jointly wrote annual and final BARD reports, abstracts and manuscripts, and interact on numerous other vital aspects of the BARD work. The close and frequent interactions enhanced the effectiveness of the work and enabled us to constantly move toward the main goal of developing a computerized, automated system for determining when to inseminate dairy cattle.

g. EVALUATION OF RESEARCH ACHIEVEMENTS.

The present project resulted in new physiological, applied and technological knowledge.

(a) Physiological aspects. Data for better understanding of the mechanisms that control changes in bovine vulvar electrical impedance (VEI) during the reproductive cycle has been obtained. Temporal associations among progesterone, estradiol 17- β and VEI changes during spontaneous and PGE₂ induced luteolyses, which preceeded estrus and parturition, have been revealed. Cyclical changes of extra- and intra cellular fluids and electrolytes, which influence the electrical impedance of tissues, have been elucidated in the bovine vulvar tissue.

(b) Applied aspects. Additional evidences have been gained that measurements of electrical impedance in the bovine vulvar tissue enable us to identify - telemetrically and automatically - the luteal and non luteal stages of the ovarian cycle, pending parturition, and the progress of the postpartum involution of the genital tract.

(c) Technological aspects. A new hard-wired system - Bio-impedance Analyzer - (BIA-101 model, R.J.L. Systems, Detroit, MI, USA) has been successfully applied for research purposes. Its major benefits are simple measuring procedures and relatively low cost. Two telemetry systems for transmitting data of electrical impedance from inside the vulvar tissue of cows has been developed independently both by the US and Israeli counterparts.

Benefit to agriculture.

The project moved us closer to a commercializable system for determining automatically when to inseminate dairy cattle and better management of periparturient cows. Indeed, using the U.S. computer driven telemetry system in research environment, 92 % of the potential estrous periods were detected; only 67 % of the potential estrus periods were detected by visual observations (Lewis et al., 1988, plus more recent data). Improved efficiency of estrus detection and monitoring other reproductive events, without observing the cows, could increase profitability of dairying. In addition to improving the reproductive efficiency in cattle, the results of the present project provided a novel method for in-vivo studying of the 1) dynamics of hydration of the reproductive tissue and 2) action of reproductive hormones at the reproductive tissue level.

h. AN INVENTORY OF UNANSWERED QUESTIONS ARISING FROM THE PRESENT FINDINGS.

Further investigations are required in order to verify and explain a number of findings in the course of this study.

1. The delayed postpartum recovery of the vulvar tissue, as reflected by a slow increase of the vulvar electrical impedance (VEI), i.e. slow tissue dehydration, was accompanied by ovarian acyclicity (Fig.1).

The normal postpartum increase of VEI was interrupted during development of follicular cysts, detected by rectal palpation (Fig. 2). The depression of VEI at this time can be explained by the increase of estrogens which are known to stimulate the hydration of the genital tissue. Automatic and telemetric monitoring of the VEI during the postpartum opens new ways for research and diagnostics of the pathological conditions which frequently occur at this stage of the reproductive cycle.

2. Some indications have been found that the rate of VEI changes during the follicular phase could be quantitatively related to the pattern of estrogen secretion at this time (Fig. 3): high and prolonged plasma E_2 concentration were accompanied by higher peak and longer duration of the electrical conductivity of vulvar tissue (left) than during weaker secretion (right). These data are in accordance with our VEI findings at the peripartal period. Induced parturition was accompanied by minor changes of VEI and

minor estrogen secretion than around the natural parturition. The extent to which VEI changes can reflect estrogen insufficiency should be examined.

3. During periestrus the % decrease of the capacitive component (X) of the VEI ($X = 1/\omega C$, where $\omega = 2\pi f$; f-current frequency) was almost twice as higher as that of the resistive component (R) (26.2 % vs 14.6 %; $n=7$; $p < 0.001$, respectively) (Fig. 4 and 5). This phenomenon was also noted during the prepartal depression. R at 50 KHz reflects the changes of total tissue water content, whereas X reflects the changes in membranes and mitochondrias (Gambini et al, 1980; Settle et al, 1980).

It should be tested if the different degrees of response of VEI components to tissue changes throughout the reproductive cycle could be used for increasing the accuracy of detecting reproductive events.

4. The exact temporal relationships between VEI changes and the start and end of estrus is not clear. At induced luteolyses, Z-statistics were lowest 4d after $\text{PGF}_{2\alpha}$ treatments. Estrus ended approximately 4d after $\text{PGF}_{2\alpha}$ treatments (Coleman et al., 1987) about the expected time of ovulation (Louis et al., 1974). Studying Holstein cows in spontaneous estrus, Lehrer et al. (1985) found that VEI was lowest one to two days after the end of 5 of 8 estrous periods. Using telemetered data, we found that the lowest measures of impedance coincided with the first detection of estrus (unpublished data; see also pages 24-25). Also in several cases, changes in ovarian hormones and vulvar

impedance indicates that cows should have been in estrus, but twice or thrice daily heat-checks were not adequate to detect those cows in estrus.

Our opinion is that determining the relationship between VEI changes and time of ovulation is of more practical value for determining the optimal time of AI than is the relationship between changes in impedance and start of estrus. Indeed, studies indicate that: 1) some early postpartum cows ovulate without detectable signs of estrus (Hurnik et al., 1975); 2) the interval between onset of estrus and ovulations ranges from 16 to 78 hr, suggesting disassociation between onset of estrus and preovulatory release of luteinizing hormone (LH; Swanson and Hafs, 1971; Christenson et al., 1975; Bosted et al., 1976; Bernard et al., 1983); 3) changes in impedance of vulvar tissue or inside the vaginal lumen were more closely related to the preovulatory release of LH than to the onset of estrus (Schams et al., 1977; Aboul-Ela et al., 1983; Smith et al., 1989). Hence the next step to improve reproductive efficiency should be to use our telemetry systems to determine the relationship between ovulation-associated declines in impedance of vaginal tissue and ultrasound-diagnosed time of ovulation. Knowing this relationship will allow us to determine how to use telemetered impedance data to predict when to inseminate dairy cattle.

5. We found that the vulvar electrical impedance in luteal phase was lower in the morning than in the evening (Table 1).

Table 1.

Daily fluctuations in the vulvar electrical impedance

Cows No.	No. of twice daily measurements	The vulvar electrical impedance (in Ω)		P <
		At 8 AM	At 8 PM	
1	18	104.5 $\pm$ 2.4**	113.7 $\pm$ 2.6	0.02
2	24	113.8 $\pm$ 2.4	126.4 $\pm$ 1.5	0.001

* The measurements were performed in Israel from Sept. 30 through Nov. 7.

** Mean $\pm$ SE.

Telemetered data, obtained at BARC USDA indicated that there may be a circadian rhythm to changes in impedance of vaginal tissue. Whether these daily fluctuations in tissue impedance are under environmental, ovarian or other endocrine-organ control is unknown. Corticoids are known to influence fluid dynamics of tissues, and peripheral plasma concentrations of corticoids fluctuate in a circadian rhythm (Martin, 1985). Thus, circadian changes in corticoid (cortisol in cattle) concentrations may influence fluid dynamics of reproductive tissues. Fluid dynamics of reproductive tissues control changes in electrical impedance of the tissues. Nonovarian-related impedance rhythms superimposed on ovarian-related impedance rhythms probably will reduce the precision of measures of vaginal tissue impedance for determining when to inseminate cows. Therefore, we must determine if there is a circadian rhythm

to changes in impedance of vaginal tissue, and if so, determine 1) if the rhythm is ovarian dependent, 2) some factors that may affect the rhythm. Potentially, these data will improve the predictive value of algorithms derived to automatically identify cows for insemination.

6. In a preliminary work on dairy cows with one electrode in an uterine wall and one in a vulvar lip, we found that impedance changes at expected estrus in uterine walls preceeded those in vulvar labia by about 24 hr (Aizinbud et al., 1985). Impedance changes in uterine tissue may be more sensitive to hormonal changes than are those in vulvar tissue. Also, compared to vulvar vaginal tissue, the uterus is an easily-accessible source of large amounts of homogenous tissue suitable for studies of cellular mechanisms by which ovarian steroids control changes in electrical properties of reproductive tissues. Thus we should compare changes in electrical properties of uterine tissue to those in vaginal tissue during: a) ovarian cycles; b) early pregnancy. With such initial studies, a data base could be developed for future impedometric studies on cellular mechanisms of hormone actions and fluid dynamics of uterine tissue during various reproductive events.

Fig.1 Actual means of vulvar electrical resistance and data predicted with a segmented bi-modal linear regression model in 7 normal (●) and in one sick and acyclic (○) cow during the postpartum.

Fig.2 Changes in vulvar electrical impedance, P_4 , E_2 in blood plasma during the postpartum of individual cows

Fig.3 Changes in vulvar electrical conductivity, P_4 , E_2 during the follicular phase.

Fig.4 Changes of the resistive (R) and capacitive (X) components of the bovine vulvar electrical impedance around the time of estrus.

VULVAR ELECTRICAL IMPEDANCE % OF THE BASE LINE

Fig.5 Changes of the resistive (R) and capacitive (X) components of the bovine vulvar electrical impedance at peripartal period.

1. LIST OF PUBLICATIONS.

- ✓Aizinbud, E., Lewis, G. S. and Lehrer, A. R. (1985) Changes in electrical resistance of vulvar and uterine tissues during estrous cycles of Holstein cows. J. Dairy Sci. 68 (Suppl. 1):190.
- Ezov, N., Maltz, E., Yarom, E. R., Aizinbud, E., Schindler, H. and Lehrer, A. R. (1985) Cell density and volume of extracellular fluid in bovine vulvar tissue during estrus and diestrus. J. Anim. Sci. 61 (Suppl. 1):392.
- ✶Lehrer, A. R., Ezov, N., Schindler, D., Fischler, H., Tadmor, A., Schindler, H., Aizinbud, E. (1985) Electrical impedance of bovine vulvar during the ovarian cycle. J. Anim. Sci. 61 (Suppl. 1):442.
- ✶Schindler, D., Lehrer, A. R., Tadmor, A., Fischler, H., Schindler, H. and Aizinbud, E. (1985) Electrical vulvar impedance, estradiol 17 β and progesterone profiles in the periparturient cow. J. Anim. Sci. 61 (Suppl. 1):442.
- ✶Lewis, G. S., Aizinbud, E. and Lehrer, A. R. (1986) Electrical resistance (Ω) of vulvar tissue during ovarian cycles of Holstein cows. J. Anim. Sci. 63 (Suppl. 1):325.
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Additional 3 articles are in final stages of preparations for publication. They are included in this report (pp 49-99).

PATENTS

1. State of Israel, Volcani Center and Yeda R&D Co.Ltd. Device for telemetric living tissue impedance by radio means. Application for patent Addition No 86587 Juni; 1, 1988.
2. U.S. Patent. Automated estrus detector for farm animals - has two impedance measuring electrodes to determinate period between radio frequency bursts. Patent Assignee: Data Sciences Inc., US 74930 (870717).