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THE SIGNIFICANCE OF CERVICAL MUCUS ARBORIZATION DURING THE LAST TWO MONTHS OF PREGNANCY IN THE COW (BOS TAURUS)

by

William Harold Cusick

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Obstetrics

Approved:

Iowa State University
Of Science and Technology
Ames, Iowa

1962

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INTRODUCTION

The arborization of cervical mucus is a relatively new method of evaluating the levels of estrogens and progesterone in the body of man and animals. It has been used as an early test for pregnancy in man and animals; in determining placental insufficiency in man; for detecting ovulation in women, ewes, and cows; for studying the function of the corpus luteum; and for determining the effect of the administration of exogenous estrogens and other hormones on the reproductive system.

Arborization of cervical mucus is produced by estrogenic stimulation of the cervical glands. The presence of electrolytes, predominately sodium and/or potassium chloride, in the mucus causes the typical arborization or fern-like pattern to appear when the mucus is dried on a clean glass slide.

The presence of arborization is inhibited by the injection of progesterone into the animal or by dialysis of the mucus, but can be made to reappear by the addition of electrolytes to the dialyzed mucus or by the injection of estrogens.

The reaction appears to be specific only at the cervical site where its cyclic nature has been determined. The appearance of arborization of dried cervical mucus is greatest and most distinct during the follicular phase of the cycle, gradually losing its characteristic form and disappearing during the luteal phase of the estrous cycle. Arborization has been observed in other body fluids such as the cerebrospinal and peritoneal fluids, but at these sites no cyclic nature was noted.

The appearance of arborization of the dried cervical mucus has been well illustrated at the time of estrus in both experimental and clinical cases in man and bovidae, and in clinical cases of threatened abortion in women, but its presence or absence during late pregnancy in the bovine species has been studied only by Bone (7).

It is the purpose of the following investigation to determine the incidence of arborization during the last two months of pregnancy in the cow, and the significance of the phenomenon during this phase of the gestation period.

REVIEW OF LITERATURE

Sensitivity of the Arborization Test for Estrogens

The sensitivity of the arborization of dried cervical mucus in detecting the level of estrogen in the body has been debated but no agreement has been reached as to the amount of estrogen required to induce this reaction in man and the various animals. Zondek and Cooper (64) were the first to attempt to determine the amount of estrogen required to induce this phenomenon since its discovery by Papanicolaou (40) in 1945. They determined

> . . . one cervical mucus unit as the quantity of estrogenic hormone inducing the production of cervical mucus which crystallizes in a dried state 72-120 hours after a single injection in post-menopausal women, castrated women, or those with primary amenorrhea.

In their determination, one cervical mucus unit (CMU) was 1 mg. for estrone and 0.3-0.4 mg. for estradiol. Zondek (62) determined that the P-L test (palm-leaf) was ten times more sensitive to the presence of an estrogen than the endometrium. A comparison of the amount of estradiol benzoate required to induce estrus, cornification of the vaginal cells, and arborization in progesterone-primed, ovariectomized Romney ewes was made by McDonald and Raeside (33). They determined that to induce estrus the ED^{50} (the dose which is effective for 50 percent of the test subjects) of estradiol benzoate was 23.4 micrograms, for cornification of vaginal cells 13.2 micrograms, and for arborization of the dried cervical mucus 7.0 micrograms, and stated that the arborization reaction was more distinctive and easier to classify than vaginal cytology as a response to estrogens. This author (13) has produced the P-L reaction in an ovariectomized bovine with a 20 microgram intramuscular injection of alpha estradiol benzoate in sesame oil.

In an experiment designed to determine the effectiveness of various progestational agents, Wied and Davis (56) observed no significant difference in the sensitivity of endometrial biopsy, vaginal cytology, basal body temperature, uterine bleeding, and cervical mucus aborization in women.

Cohen and Hankin (11) compared several methods of detecting ovulation in women and concluded that arborization was present for too long a period of time to be of value as a single test for ovulation; however, Howes et al. (25) stated that arborization was the most accurate method of detecting ovulation in Hereford and Brahman cattle when compared to basal body temperature and rectal palpation.

Blair and Glover (4), working with the consistency of cervical mucus in early pregnancy, found the consistency of the mucus to be twice as accurate as cervical mucus arborization in diagnosing pregnancy at 24-28 days in the cow. The consistency test was also more accurate than the P-L test at 28 days in a later experiment (5).

The Source, Metabolism, and Elimination of Estrogens, Gestagens, and Gonadotropins during Pregnancy

The principal sources of estrogens and gestational hormones during pregnancy are the placenta (3, 17), corpus luteum of the ovary (15) and possibly the adrenal gland (2, 29, 60). At the present time only progesterone and the estrogens have received much attention because of the inability to demonstrate placental (26) and chorionic gonadotropins (6) or gonadotropins in the urine and/or serum of the cow (35). In 1940, Nalbandov and Casida (36) reported a decreasing concentration of pituitary gonadotropins in the cow with an increasing length of gestation. In 1958, Nalbandov (35) correlated the decrease of pituitary gonadotropins in cattle and swine with the increasing level of estrogen being secreted by the placenta as pregnancy progresses. Catchpole (10) also reported a gradual decrease in the gonadotropic potency of the pituitary glands of pregnant cattle; however, Day et al. (14) in the same year reported a significant increase in the LH and LTH potency of the pituitary glands from pregnant gilts over those of non-pregnant gilts.

The inactivation of estrogens and progesterone appears to occur predominately in the liver (9). Zondek (61) reported the inactivation of the alpha hormone (folliculin estrin) by enzymes of the liver in 1934. In 1940, Heller (22) demonstrated the enzymatic destruction of estrone and estradiol in the liver by oxidative processes and also the partial inactivation of estradiol by the kidney. He also showed a marked increase in estrogenic potency after the addition of estrone to uterine tissue, which indicated a conversion of estrone to estradiol. Pearlman and Cerceo (41) isolated pregnanediol-3α, 20β from ether extracts of unhydrolyzed bile from cows at least five months pregnant but could not demonstrate the 20α isomer, while in the urine the 20α isomer was present but not the 20β isomer, indicating a renal alteration of progesterone metabolites.

Wiswell and Samuels (57) studied the metabolism of progesterone by using homogenized rat and rabbit liver. They concluded that there were two liver enzymes acting on the conjugated double bond system of ring A. One of the enzymes acted on 21 carbon steroids with a substituted group on C-20 and the other enzyme acted on the steroids having an oxygen on C-17. Stucki and Glenn (51) found that the endometrial proliferating ability of progesterone was greatly inhibited by reducing the side chain to 20α or 20β hydroxyprogesterone, but the reduced compound retained its ability to produce a myometrial block. The myometrial blocking effect was retained, but the ability to induce endometrial proliferation and inhibit parturition was lost by reduction of the A ring. They suggested that the metabolic reduction of progesterone in the myometrium and/or the endometrium was necessary for establishing a myometrial block, but the reduced compounds could not inhibit parturition because of being conjugated before they reached the myometrium.

The rapid loss of radioactive C^{14} labeled progesterone from the blood stream following intravenous and intramuscular administration and its increased concentration in the adipose tissue of the body was demonstrated by Plotz (42). He concluded that progesterone and/or its metabolites were then slowly released from the tissues to be conjugated and excreted by the liver and kidney. Zander (59) also observed a high concentration of radioactivity in the adipose tissue following the injection of radioactive progesterone. The maximum time post-injection that radioactivity could be detected in the blood was 90 minutes and he attributed this to the metabolism of the molecule and the diffusion of unchanged progesterone into the tissues.

Numerous reports have appeared in the literature pertaining to the concentration of estrogens in the urine and feces in early pregnancy. Asdell (1) reported the presence of estrogenic activity in the urine of the cow after the 70th day of pregnancy, however, other workers have indicated its presence before this time. Nibler and Turner (38) reported 9 R.U. per liter of urine on the 34th day of pregnancy in the cow and 8 R.U. on the 57th day of pregnancy, with an average daily gain of 3.5 R.U. per liter per day after the 100th day of pregnancy. In the following year Turner et al. (53) reported a progressive increase in urinary estrogens after the 100th day of pregnancy in the cow. Catchpole (10) found only small amounts of estrogen in the urine of 9 pregnant cows until after the 100th day. Several workers (17, 53) have stated that the level remains low until later in pregnancy.

The low estrogen level in early pregnancy is associated with the absence of arborization of the cervical mucus (18). Higaki and Awai (24) found they could diagnose pregnancy in the cow at 35 days and be 95.1 percent correct, while Bone (7) reported that with an accurate history a tentative diagnosis could be made as early as 10 days. He was 92 percent correct at less than one month and 100 percent correct after the fourth month of pregnancy, with an over-all accuracy of 97 percent in 103 cows. Conflicting reports have been published in the human literature. Neumann and Lehfeldt (37) obtained a 56 percent correct diagnosis, Nouel and Aguero (39) 85 percent, and Sphrague (50) 99 percent. Roland (46) found no arborization during the first trimester and only a small number of cases in which atypical ferning was present in those over 4 months in

300 pregnant women. Campos Da Paz and da Costa Lima (8) reported 39.69 percent of the cases they studied showed atypical arborization during the 4th month of pregnancy. Ullery and Shabanah (54) found 30 percent of the women showed some form of arborization in the first two months of pregnancy, 15 percent from the 14th-28th week and 10 percent in those over 28 weeks. Of 226 women examined during the first trimester, 88.5 percent did not show the P-L reaction. Much of the discrepancy in the human field can be accounted for by the frequency of sampling, length of time after the last menses, and by applying corrective factors for those who subsequently abort. It is not unusual that a higher incidence of arborization is found in early pregnancy in the human than is found in the cow when consideration is given to the difference in placentae.

Short (49), in 1958, reported a constant level of progesterone in the blood of pregnant cows from the 32nd-256th day of pregnancy followed by a gradual decrease. This is not in agreement with Melampy <u>et al</u>. (29). They observed a maximal progestational activity in the corpus luteum and residual ovarian tissue at 90-129 days with the maximum levels in the placenta, allantoic fluids, and adrenal gland occurring at 170-209 days. The maximum level in the amniotic fluid did not occur until the 210-249th day of pregnancy. The progesterone activity was maximal in the plasma between the 129th-169th day. In later work, Short (48) reported that the blood progesterone level in the ewe was highest from the 40th-102nd day, which is approximately the same stage of pregnancy that Melampy <u>et al</u>. (29) found the highest levels in the cow. The occurrence of pregnanediol in the urine of pregnant cows could not be confirmed by El-Attar and

Turner (16). Further evidence for an increase in progesterone in early pregnancy is given by McDonald and Raeside (32). They were able to produce arborization with 150 micrograms of estradiol benzoate at mid-cycle, but needed 200 micrograms between the 2nd and 5th weeks of pregnancy in the ewe. Whether the resistance of the cervical mucus to arborize is due to the low level of estrogens, higher level of progesterone, or to some other inhibitory mechanism on the cervical glands (23, 64) has not been proven, however, it would appear to be the combination of a low estrogen-high progesterone level.

In 1947, Robson (45) reported that in the ovariectomized rabbit it required more progesterone to maintain pregnancy during the later stages than in early pregnancy. Wu and Allen (58) indicated an increased need for progesterone in maintaining pregnancy than for implantation in the rabbit. It should be noted here that the corpus luteum is essential throughout pregnancy in the rabbit (48) and the increased progesterone requirement could be a reflection of a rising estrogen titer. Studies involved with the dosage of progesterone required to maintain pregnancy in the ewe and cow have revealed that the corpus luteum could be removed about the 200th day of pregnancy in the cow and not result in abortion. With the supplemental use of exogenous progesterone, the corpus luteum can be removed between the 2nd and 3rd months of pregnancy and the progesterone injections stopped as early as the 143rd day without resulting in resorption or abortion (31). Hammond (21) referring to Delestres' work in 1910, stated that the corpus luteum began to degenerate after the 5th month in the cow. Hammond (21) stated, however, that the histological appearance of the corpus luteum of pregnancy maintains its organization up to at least the 8th month of pregnancy in the cow.

Raeside and Turner (43) were unable to maintain pregnancy following enucleation of the corpus luteum in cattle with daily subcutaneous injections of 25 mg. of progesterone in olive oil. With a 50 mg. injection they found that two of four cows had a resorbed fetus at midterm. Uren and Raeside (55) expressed the corpus luteum via laparotomy between the 36th and 76th day and were unable to maintain pregnancy with 25 mg., 50 mg., and 75 mg. injections of progesterone in olive oil, while in one cow, with the corpus luteum expressed on the 205th day, a live calf was born on the 273rd day. McDonald et al. (30) expressed the corpus luteum from the 58th day to the 88th day and then administered 100 mg. of crystalline progesterone in sesame oil daily for varying periods of time. The cessation of exogenous progesterone on the 102nd and 137th day resulted in resorption or abortion, but when continued until the 162nd day or longer the cows delivered live calves, although the duration of pregnancy was approximately two weeks shorter than normal and a high incidence of retained placenta occurred. Abortion occurred in all five cows in which the corpus luteum was expressed from the 92nd to the 163rd day of pregnancy. In the following year they found pregnancy could be maintained with an intramuscular injection of 500 mg. of aqueous micro-crystalline progesterone every 10 days, if given until the 143rd day of pregnancy (31). Other reports indicate the corpus luteum cannot be removed prior to the 6th month*, 200th day (44), 7th month (35), and 235th day (1). Melampy et al. (29) indicated a gradual change-over in the source of progesterone, from the corpus luteum and residual ovarian tissue to the placenta, which occurred approximately the same time the corpus luteum could be removed without causing abortion. The results of Duncan et al. (15) in gilts showed the progesterone content of the corpus luteum increased until the 48th day and then decreased. Short (49), in 1958, stated the blood progesterone level decreased in the cow after the 256th day, and in 1960 reported the decrease was predominately in the last 10 days of pregnancy in the cow (48).

The above relationship between the blood progesterone level and the time the corpus luteum can safely be removed is correlated with an increase in estrogen elimination in the urine and feces, and with the increased production of estrogens by the placenta. Nibler and Turner (38) found the greatest amount of estrogenic activity in the urine of the cow after the 270th day of pregnancy. Levin (27) reported that the estrogen content of the feces was highest during the last few weeks and that the predominant component was estradiol. This was also reported by Cowie (12). El-Attar and Turner (16) reported an increase, from 3.53 mg. during the 8th month to 12.83 mg. 4 days prior to parturition, in the total amount of estrogen excreted per 24 hours in the urine and feces, and that the ratio of fecal estrogen to urinary estrogen was 3:1, with estradiol the predominant component from each source. Gorski and Erb

*Emmerson, M. A., Department of Veterinary Obstetrics and Radiology, Iowa State University of Science and Technology, Ames, Iowa. Private communication. 1961.

(19) identified estradiol-17-β in the placentae of cows at 5 months and also estrone, and estradiol-17-α later. In earlier work Gorski et al. (20) stated that the elimination of estrogens was 10 times greater during the last four months of pregnancy than after calving. Melampy et al. (28) and Turner (52) have reported the presence of estrogenic activity in the milk during late pregnancy in the cow.

In 1954, Zondek and Cooper (64) reported that 32.3 percent of the women they studied in the last month of pregnancy exhibited a slight P-L reaction. Schwalenberg and Efstation (47) stated that the occurrence of arborization within 4 to 8 days of the onset of labor was inconsistent and present in 13 percent of the specimens examined. Bone (7) did not observe arborization after the 4th month in the cow but his work was based on single observations from slaughtered animals. McDonald and Raeside (34) did not observe a P-L reaction during late pregnancy in the ewe, but this was also based on one observation.

The high estrogen level and marked arborization of the cervical mucus at the time of estrus is followed in 10 to 12 days by a complete absence of ferning corresponding to the increased activity of the corpus luteum. In a normal estrous cycle the estrogenic increase and reappearance of arborization begins about the 18th day, but if pregnancy intervenes arborization does not recur at the expected time. Until approximately the last one-third of pregnancy, progesterone is the predominant hormone and is associated with the absence of a P-L reaction. As parturition approaches, evidence of an increasing estrogen level is given by the enlargement of the mammary glands and relaxation of the pelvic ligaments. It is therefore hypothesized that the appearance of arborization should be detectable during the last few weeks of pregnancy and possibly give some indication of the approximate time of parturition.

MATERIALS AND METHODS

For this experiment twelve dairy cows were used. All of the cows were negative to brucellosis and tuberculosis. They were stabled except for approximately a three month period when they were maintained under dry lot conditions. The ration consisted of legume hay and a grain supplement. The breeding history of each animal is given in Table 1.

Table 1. Breeding history

Cow	Breed	Age	Gestation period	Type of service	Length of gestation	Sex of calves
2	Holstein	5	3rd	Natural	283 days	Male
3	Holstein	9	8th	Natural	278 days	Female
4	Holstein	5	3rd	Natural	286 days	Male
5	Holstein	5	3rd	Natural	280 days	Female
7	Ayrshire	5	3rd	Artificial	283 days	Female
8	Ayrshire	4	2nd	Artificial	282 days	Female
13	Holstein	3	1st	Natural	277 days	Female
14	Guernsey	4	2nd	Artificial	276 days	Female
16	Brown Swiss	3	1st	Natural	288 days	Male
17	Guernsey	4	2nd	Artificial	283 days	Female
18	Guernsey	4	2nd	Natural	281 days	Female
19	Guernsey	7	4th	Natural	286 days	Male

The frequency of collection, of the cervical mucus samples, was determined by computing the expected date of parturition, based on a 283 day gestation period. Samples were taken at one week intervals from the 95th to the 60th day prepartum; at 48 hour intervals from the 60th to the 14th day prepartum; and then at 24 hour intervals from the 14th day until parturition.

Mucus samples were obtained with a bristle pipe cleaner inserted into a stainless steel infusion pipette. A glass speculum, 15 inches long and 1-5/8 inches outside diameter, with a light adapter attached, was used to expose and illuminate the cervix. The speculum was lubricated with Tomac Lubricating Jelly and inserted into the vagina. The pipe cleaner, inserted into the collecting pipette, was then passed through the speculum, inserted into the external os of the cervix, and rotated several times until the mucus was wrapped around the pipe cleaner. The mucus sample was smeared on a clean glass slide in a circular manner until the slide was covered with mucus. The smears were allowed to dry at room temperature for approximately 10 minutes and then completely dried with the flame of a Bunsen burner. A 35X magnification, with reduced, artificial daylight, was used to examine the smears. Examinations were made within one hour after collection of the samples. Classification of the degree of arborization was as follows:

N - No arborization. (Figure 1.)

P-1 - Less than 50 percent of the mucus showing arborization with indistinct palm-leaf patterns. Figure 2.

P-2 - Less than 50 percent of the mucus showing arborization with distinct palm-leaf patterns. Figure 3.

P-3 - More than 50 percent of the mucus showing arborization with distinct palm-leaf patterns. Figure 4.

P-4 - More than 75 percent of the mucus showing arborization with distinct palm-leaf patterns but not typical of estrous patterns. Figure 5.

P-5 - 100 percent of the mucus showing arborization with distinct palm-leaf patterns typical of estrous patterns. Figure 6.

The speculum and collecting instrument were cleaned with hot water and disinfected with Phenogen. The collecting instrument was then rinsed in distilled water and sterilized in a Bunsen burner flame prior to insertion of the pipe cleaner. The pipe cleaners were autoclaved for 30 minutes at 250 degrees Fahrenheit and stored in a sterile glass tube. The slides were cleaned by rinsing in 70 percent isopropyl alcohol, followed by distilled water, and allowed to dry prior to use. A stainless steel forceps, which had previously been rinsed in distilled water and sterilized in a flame, was used to bend and insert the pipe cleaner into the stainless steel infusion pipette. By making a bend in the end of the steel infusion pipette, the pipe cleaner could be passed through the glass speculum to the external os of the cervix without touching the walls of the speculum. Prior to insertion of the speculum, the vulva of the cow was washed with warm water and dried with paper towels. Care was taken not to touch the vaginal wall during collection of the mucus.

Figure 1. N (upper left)

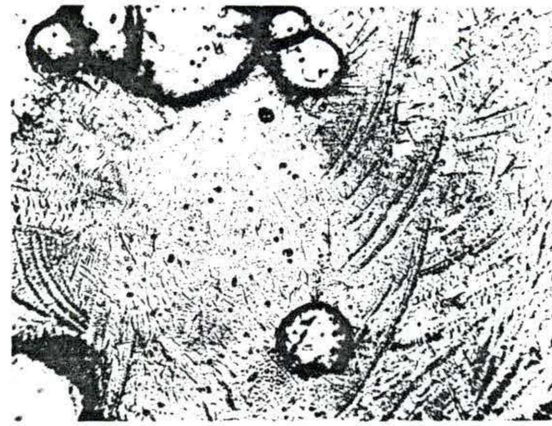
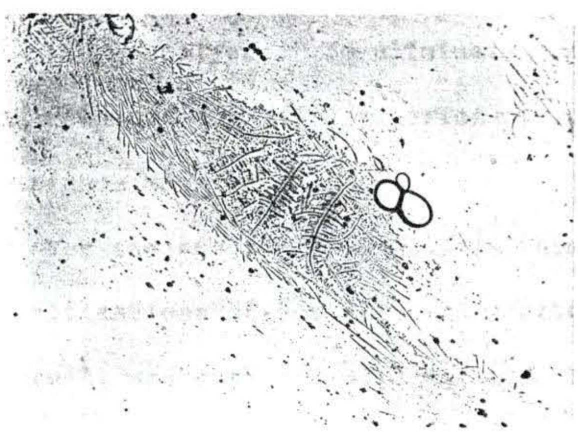
Figure 2. P-1 (upper right)

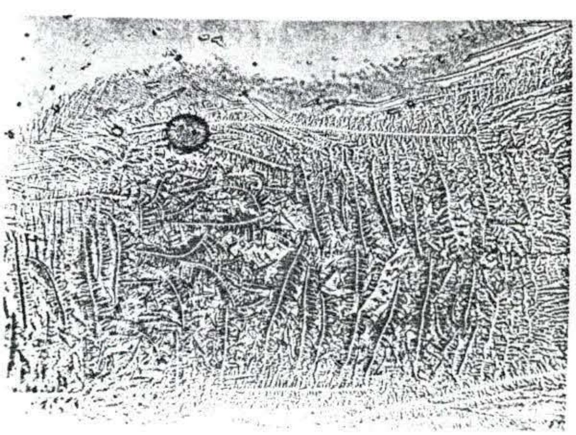
Figure 3. P-2 (middle left)

Figure 4. P-3 (middle right)

Figure 5. P-4 (lower left)

Figure 6. P-5 (lower right)




RESULTS

Classification of cervical mucus smears obtained at daily intervals indicated extreme day to day variation. For this reason, data obtained from smears collected before the 70th day prepartum were not used in the statistical analysis. The 70 day interval, prior to parturition, was divided into 10 periods of 7 days each. In a preliminary analysis, using the method of unweighted means, no interaction was found between cows and periods, therefore, in subsequent interpretations of the data, the interaction component was considered as estimating error variance in the covariance analysis. To eliminate some of the day to day variability, a mean response for a 7 day period was used as the observation for the covariance analysis.

For the statistical analysis, numerical values were assigned to the classifications of cervical mucus arborization. The N classification, Figure 1, was assigned an 0 value. The P-1 classification, Figure 2, was assigned a value of 1; the P-2 classification, Figure 3, a value of 2; the P-3 classification, Figure 4, a value of 3; the P-4 classification, Figure 5, a value of 4; and the P-5 classification, Figure 6, a value of 5.

The purpose of using analysis of covariance was to eliminate cow differences. A significant part of the variability was explained by the linear and quadratic responses. The regression of X_1 on Y, the linear response, was 0.439, and the regression of X_1^2 on Y, the quadratic response, was -0.046. The standard errors for the respective regression coefficients were 0.135 and 0.0122. The t values were, for the linear response, 3.260*, and for the quadratic response, 3.834**. The peak of the quadratic curve was on the 32nd day prepartum.

Cows 14 and 18 were not included in the statistical analysis because the data indicated a lack of response to the test compared to the other ten cows. A test for homogeneity, for cows 14 and 18, did not estimate the same variance as the other ten cows. Because of the numerous N classifications, no suitable transformation was available to adjust the variance to attain homogeneity - a major assumption in the analysis of variance technique.

Table 2. Analysis of covariance

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	76	65.20	0.858
Periods	67	39.90	0.596
Cows	9	25.30	2.811

Nine cows were used for an analysis of variance for sex differences. Four of the cows had male calves and five of the cows had female calves. The F value of 2.87, for determining sex differences, was not significant. The results indicate no significant difference between the smears obtained from cows having male calves and cows having female calves.

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* Significant at the 5 percent level.

**Significant at the 1 percent level.

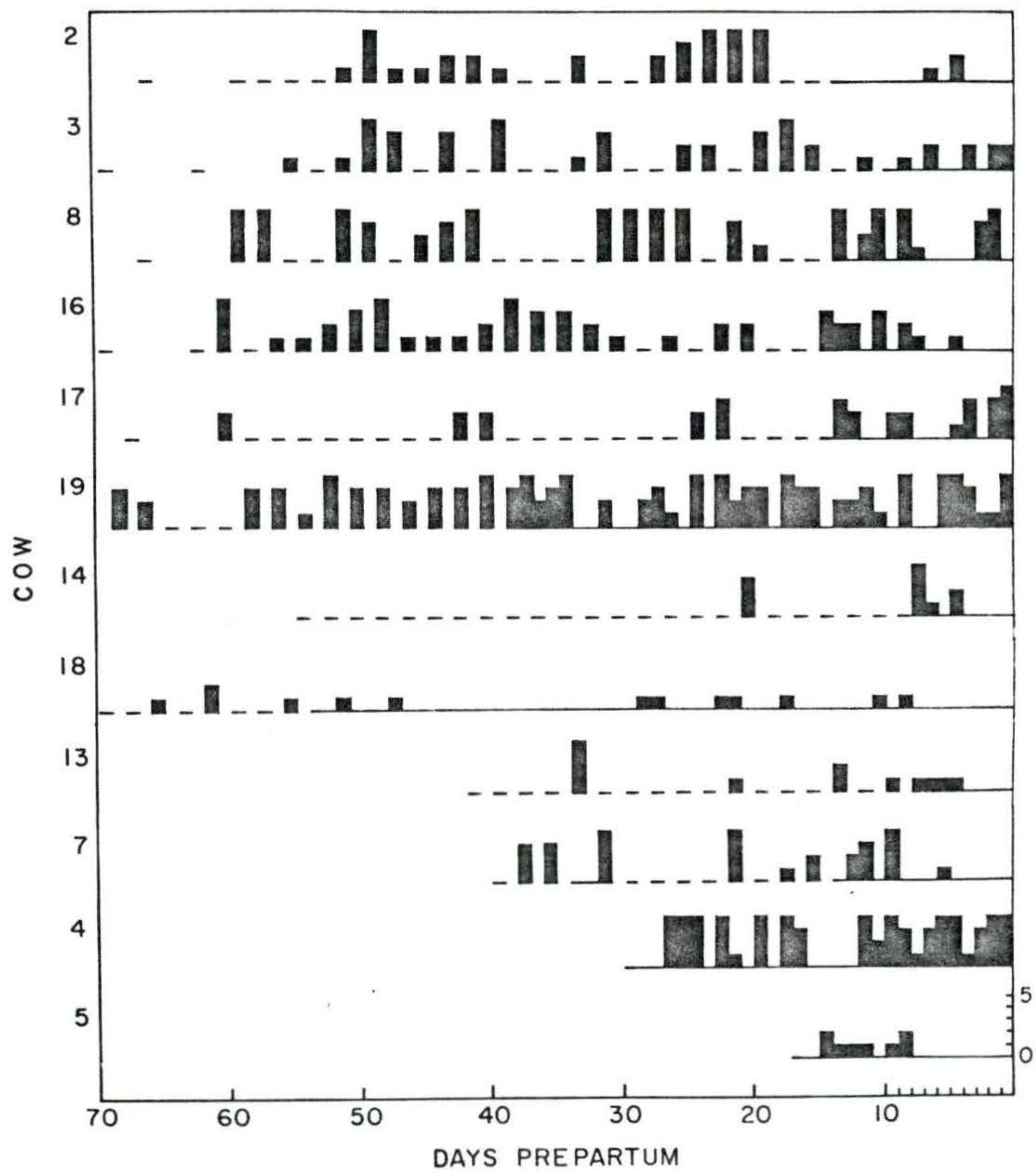
Table 3. Analysis of variance. Sex determination

Source of variance	Degrees of freedom	Mean square	F
Sex	1	3.500	2.87
Cow w/sex	7	1.217	
Periods	3	0.546	
Sex X period	3	0.350	
Whole plot (error b)	21	0.573	

Smears were obtained from four cows, number 3, 8, 18, and 19, prior to the 70th day prepartum. The first smear from each cow was obtained, respectively, on the 91st day prepartum, 95th day prepartum, 135th day prepartum, and the 120th day prepartum. The first positive smear (P-2) was obtained on the 84th day before parturition in cow number 3; on the 95th day (P-4) in cow number 8; on the 91st day P-3) in cow number 18; and on the 106th day (P-1) in cow number 19.

The longest gestation period was 288 days and the shortest 276 days with an average length of 281.9 days. The average length of the gestation period for cows having male calves was 285.7 days compared to 280 days for cows having female calves. All cows had gestation periods within the normal range.

Figure 7. Cervical mucus arborization during the last 70 days of pregnancy in the cow. Each square represents one day. Smears were taken only on the days indicated by a dash or solid block


COW
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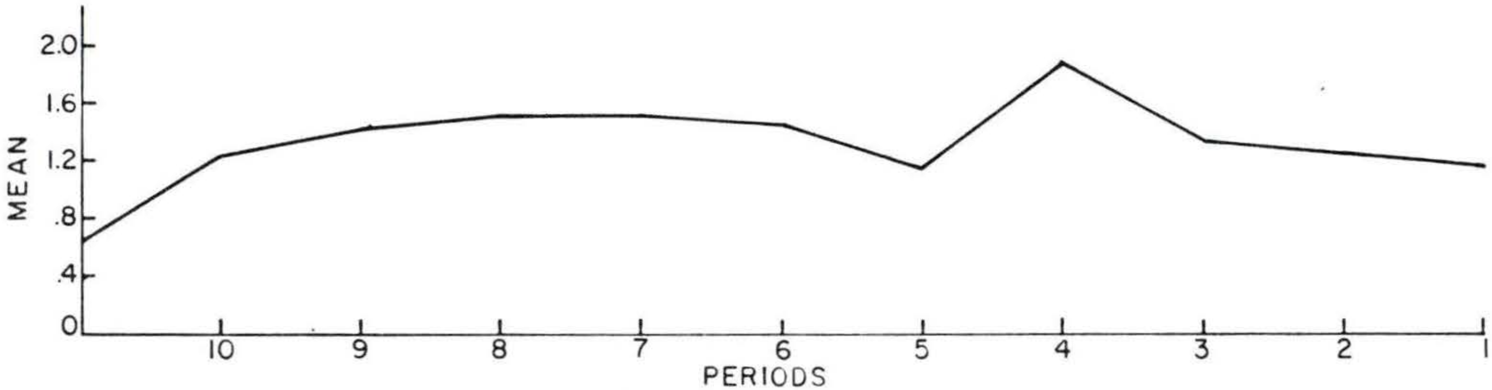
40
30
20
10
DAYS PREPARTUM

Figure 8. Arborization trends during the last 70 days of pregnancy in the cow. Each point is the observation mean for the respective period

Figure 9. Arborization trends in cows carrying male calves and in cows carrying female calves. Each point is the observation mean for the respective period. The solid line represents cows having male calves. The broken line represents cows having female calves


MEAN
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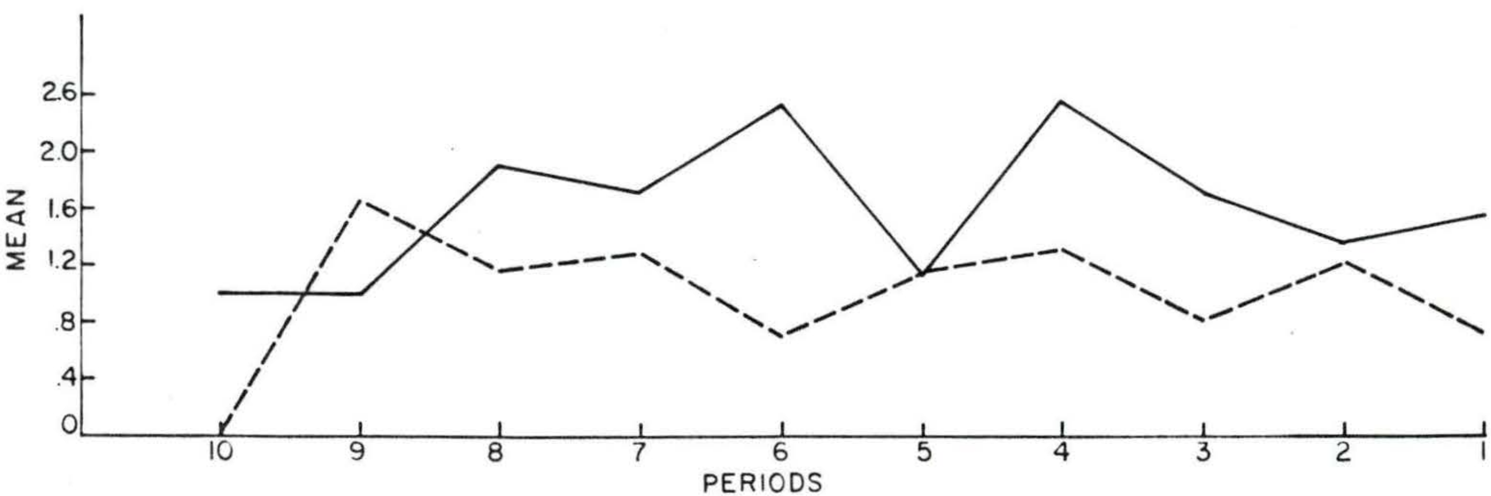
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DISCUSSION

The arborization of dried cervical mucus was studied during the last 70 days of pregnancy in the cow. All twelve cows had positive P-L reactions during this period, which does not agree with Bone's (7) work. Bone (7) observed no arborization after the 4th month of pregnancy in the cow. In the present study, 42.95 percent of the smears obtained were positive for arborization. Ten of the twelve cows had one or more positive P-L reactions during the last week of pregnancy and all twelve cows had two or more positive P-L reactions the last two weeks of pregancy. In period 1, 39.28 percent of the smears were positive for arborization; in period 2, 52.70 percent were positive; in period 3, 40.38 percent; in period 4, 54.16 percent; in period 5, 30.61 percent; in period 6, 34.09 percent; in period 7, 53.12 percent; in period 8, 46.67 percent; in period 9, 40.91 percent; and in period 10, 25 percent. A total of 447 smears were taken during the 70 day period of the study. No P-5 classifications were obtained during this time. Classifications of P-4, P-3, P-2, P-1, and N were obtained 50, 42, 45, 55, and 255 times respectively, during the 70 day period.

Insufficient animals were available to determine the number of positive smears during a 7 day period prior to the 70th day prepartum. Evidence of an increase in the estrogen level of the body, before the 70th day prepartum, was observed in cow number 19. This cow had a smear classified as P-1 on the 180th day of pregnancy. A positive smear was observed on the 187th day of pregnancy in cow number 8; on the 190th day of pregnancy in cow number 18; and on the 194th day of pregnancy in cow number 3. These dates approximate the time that Nalbandov (35) and Roberts (44) state the corpus luteum can be removed without causing abortion.

The significant linear response obtained in the analysis of covariance indicates a positive trend, or increase, in the estrogen level of the cow during the last 70 days of pregnancy. This linear response did not possess a gradient of sufficient magnitude to be used as a guide in estimating the approximate date of parturition. It, however, does show the trend of an increasing estrogen level in the body as parturition approaches.

The most significant response was a negative quadratic curve. This curve illustrates a low estrogen level on the 70th day prepartum, increasing to a maximum on the 32nd day prepartum, and then decreasing the remainder of pregnancy. No significant difference occurred between periods in the 70 day interval. This non-significant variation between periods also demonstrates the gradual increase in the estrogen level during the last 70 days of pregnancy.

Smears obtained from cows carrying male calves were not significantly different from cervical mucus smears from cows carrying female calves. Figure 9, however, shows cows carrying male calves have a tendency to show a greater response to the test than cows carrying female calves.

Visual observation of the cervix 24 hours before parturition was not noticeably different from the cervix 5 to 6 days before parturition. No dilatation was noted in cows 2 and 13, 23 hours before parturition; in cow 16, 19-1/2 hours before parturition, in cow number 19, 16-1/2 hours prepartum; in cow 7, 15-1/2 hours prepartum; in cow 5, 15 hours prepartum; or in cow 3, 13 hours before parturition. The cervical mucus plug was being extruded 5-1/2 hours before parturition in cow 18. The allantoic sac was visible, in the partially dilated cervix, at 10-1/2, 5-1/2, and 2-1/4 hours before parturition in cows 17, 8, and 14, respectively. Dilatation of the cervix is, therefore, a rapid process occurring most often between the 5th and 10th hours before normal parturition.

The low incidence of mucus arborization observed in cows 14 and 18, throughout the period of observation, is difficult to explain. Both cows exhibited normal relaxation of the pelvic ligaments, but did not exhibit fullness of the udder or erectness of the teats as early, or to the same extent, as the other cows. Both cows were four year old Guernseys and in their second gestation periods. In all other cows studied, the age, breed, lactation period, or stage of gestation, did not appear to have any effect upon the appearance of mucus arborization. In an earlier experiment, in an ovariectomized cow, the minimum amount of alpha estradiol benzoate in sesame oil, required to induce arborization of the cervical mucus following an intramuscular injection, was determined to be 20 micrograms (13). A 60 microgram injection of aqueous estradiol benzoate U.S.P., was required to induce arborization when the estrogen was given intravenously (13). While it is possible that a variance in collection of mucus, or cellular damage of the endocervix resulting from some previous injury to the external os, such as a dystocia or post parturient cervicitis, could have been responsible for the low incidence of arborization in these two cows, it is more plausible to suspect the estrogen level in cow 14 and in cow 18 was now great enough to be detected by the arborization phenomenon.

Data were available for comparing the presence and degree of arborization, during the 70 day period of study, with the first post-parturient estrus in four cows. No apparent correlation was noted between the degree and incidence of arborization during the 70 day prepartum period and the length of time between parturition and the first post-parturient estrus. Cow 19 was in heat 19 days following parturition; cow 14, 22 days after parturition; cow 8, 44 days after parturition; and cow 17, 62 days post-partum. Cow 19, which showed arborization in 74.07 percent of the smears obtained and had a male calf, was in heat only 3 days before cow 14. Cow 14 had a female calf and only showed arborization in 12.5 percent of the smears obtained. Of the three cows having female calves, cow 8 had the most positive smears, 52.63 percent, followed by cow 17 with 33.33 percent. Cow 8 came in heat 22 days later than cow 14, and cow 17, 40 days later than cow 14. The average length of time, from the date of parturition to the first post-parturient heat for all four cows, was 36.7 days.

CONCLUSIONS

Arborization of dried cervical mucus occurred in all cows during the 70 day prepartum period. Of the mucus samples obtained during this period, 42.95 percent were positive for arborization. The occurrence and degree of arborization varied in different cows and on different days. Although no significant difference was noted between cows carrying male calves and cows carrying female calves, the trend was for a higher incidence of arborization in cows carrying male calves.

The significant linear and quadratic responses demonstrate the gradual, general increase in the estrogen level prior to parturition, and the maximum occurrence of arborization on the 32nd day before parturition. This trend is not sufficient for use in predicting the approximate day of parturition.

Arborization appears for the first time on approximately the 187th day of pregnancy which coincides with the time the corpus luteum can be removed without causing abortion.

The extreme day to day variation indicated samples of mucus would have to be obtained at least at 48 hour intervals to be of value in determining the estrogen level during the last two months of pregnancy in the cow. A modification of the present technique, which would compensate for the difficulty encountered in obtaining thin smears on the slide, may eliminate some of the variation.

The test may not be sensitive enough to detect the presence of estrogens in all cows. No conclusion can be made from the present study because of insufficient animals in the experiment.

The presence and degree of arborization of the cervical mucus during the last 70 days of pregnancy are not correlated with the length of time from parturition to the first post-parturient estrus.

Dilatation of the cervix is visually noticeable 5 to 10 hours before parturition. Prior to this time, these changes do not occur, or are so gradual they cannot be detected by daily observations.

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ACKNOWLEDGEMENTS

For his special interest in this research project the author is extremely grateful to:

Dr. M. A. Emmerson for suggesting the project and for guidance during its course.

Dr. Donald Hotchkiss for consultation in the statistical analysis of the data.

Mr. Louis Facto for his careful reproduction of the figures and charts.