THE EFFECT OF NITROFURAZONE AND PROTEOLYTIC ENZYMES ON THE POSTPARTUM BOVINE UTERUS

by

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INTRODUCTION

Lowered fertility shortly after parturition in the bovine is a factor of great economic importance to the beef industry. If the postpartum interval from parturition to conception could be shortened to 30 days it would be possible for a cow to produce one more calf during her normal production years. A great deal of research has been conducted to explain this period of infertility; however, no treatment has been successful in shortening the postpartum interval. Gier and Marion (1968) reported that the uterus must be free of infection and have a complete lining of epithelium to maintain a pregnancy, and that a variety of environmental factors can affect uterine involution.

The present study involved an attempt to shorten the postpartum interval from calving to conception by infusing proteolytic enzymes and nitrofurazone intrauterine to eliminate uterine infection and maintain the uterus in a histologic condition necessary for pregnancy.

Uterine biopsies were obtained to determine cytological and histological changes of the uterus in treated and non-treated cows in an attempt to ascertain the mode of action of the treatment and its relation to the length of the interval from parturition to conception.

REVIEW OF LITERATURE

Casida et al. (1968) defined uterine involution as being complete when the uterus has returned to its normal, non-pregnant position and when the two horns are similar in diameter and show normal consistency and tonus. According to Gier and Marion (1968), a variety of histological and physical changes must take place in the uterus before involution may be termed complete. Rasbeck (1950) reported this process complete by 20 to 25 days. Gier and Marion (1968) stated that under normal conditions involution was not complete until 50 days postpartum. The difference may be explained by the fact that histologic regeneration of the endometrium and epithelium may occur at an earlier date than does physical reduction in size of the uterus.

Differences arise when it is not clear where histological and physical measurements have been taken. Gier and Marion (1968) reported that uterine epithelium had re-covered the entire intercaruncular luminal surface by 8 days postpartum. Rasbeck (1950) reported that by 20 days after parturition the epithelium had regenerated over the caruncle. Larson (1965) found in the early postpartum cow it is extremely difficult to determine intercaruncular areas from caruncular areas when obtaining a biopsy. Likewise, palpation measurements vary greatly among researchers, and it appears that the point of measurement creates the discrepancies. Casida and Venzke (1936) and Casida and Wisnicky (1950) measured uterine diameters with the uterus lying on the pelvic floor in a near natural position. Had the uterine horns been in a position where measurements could have been made mesometrially-antimesometrially as well as transversely, different values may have been obtained.

Gier and Marion (1968) describe uterine involution as having 3 overlapping processess: (1) reduction in size, (2) loss of tissue, and (3) repair. The greatest reduction in size was described as occuring during the first few days postpartum. The uterus regressed to its normal size by 40 to 50 days after parturition. This is contrary to Morrow (1966) who reported the regression was slow during the first few days postpartum, then accelerated 10 to 14 days postpartum. The main reduction in size was complete by 25 days postpartum.

Sloughing of necrotic uterine tissue is an important factor in involution. Postpartum necrotic tissue, if not eliminated, may cause a great deal of variance in the postpartum interval to the next pregnancy. Gier and Marion (1968) described this loss of tissue as being complete by 19 days postpartum. During this period leucocyte invasion played a significant role in proper tissue repair. It was also reported that under normal conditions the caruncular surface was re-covered with epithelium by 25 days postpartum. Nellor and Brown (1966) reported in cattle that leucocytes passed from the endometrium through the epithelium. In small concentrations the invasion of leucocytes passed between epithelial cells and did not interrupt the epithelial lining. Gier and Marion (1959) reported that following parturition, intercaruncular epithelium was erroded by bacterial lysis in some cases. If it were possible to eliminate uterine infection, it may be possible to have the epithelium regenerate over the caruncle earlier than 25 days postpartum.

Gier and Marion (1968) described the process of uterine involution as complete by 50 days postpartum. This appears to be an accepted length of time for the interval from parturition to the completion of uterine involution. Foote et al. (1960) reported 43.7 days, Foote and Hunter (1964) 47 days and Buch et al. (1955) 47 days; however, Buch et al. (1955) broke the figure down further by calculating other factors that affect uterine regression. Buch et al. (1955)

reported the difference between primiparous and pluriparous cows was 42 to 50 days, respectively, for the interval from parturition to uterine involution. Season also affects uterine involution (Buch et al., 1955). He stated that the period from calving to involution was shortest in the summer and longest in the winter. Marion et al. (1968) reported that involution intervals for pluriparous and primiparous cows during the 4 seasons were: fall, 40.1 and 36.4; winter, 44.1 and 36.5; spring, 38.2 and 30.4; and summer 37.8 and 31.1 days. Besides parity and season, retained placenta and progesterone may also significantly affect the interval to involution. Norwood (1963) described retained placenta as extending the interval from calving to involution by a period of approximately 4 days in both parity groups. Marion et al. (1968) reported that retained placenta significantly increased the time required for uterine involution; however, they gave intrauterine infusions of nitrofurazone or oxytetracycline, both antibacterials, in all cases. Norwood (1963) not using antibacterials, reported that the length of time for uterine involution in cows with retained placenta was 39.75 days for primiparous and 44.38 days for pluriparous cows respectively, a figure seemingly not significantly different than in the clinically normal cow.

VanDemark and Salisbury (1950) described postpartum fertility as increasing to 100 to 120 days postpartum. Graves et al. (1968) recorded no fertilizations or pregnancies occured within the first 10 days after parturition in the bovine. In a study of inseminations at various intervals postpartum, Shannon et al. (1952) reported that a minimum interval from calving to first insemination of 50 days was required for satisfactory fertility. In order to obtain higher levels of fertility it appeared that the cow needed to cycle normally several times after parturition.

Hormone treatment has been attempted at various times postpartum to induce earlier uterine regression and estrus. Casida and Wisnicky (1950) using diethylstilbestrol dipropionate on cows with retained placentae, pyometra and mummified fetuses reported involution as being complete by 29.4 days after parturition. They concluded there was no effect due to treatment on rate of involution, time to first ovulation, or first estrus postpartum. Ulberg and Lindley (1960) and Foote and Hunter (1964) using progesterone or estrogen or both did not significantly shorten the interval from parturition to uterine involution. Ulberg and Lindley (1960) found that estrogen significantly shortened the interval from parturition to first estrus (46 to 29 days) by decreasing the overall variance within the experimental herd. Foote and Hunter (1964) also decreased the interval to first estrus (49 to 27 days) in estrogen and estrogen plus progesterone treated cows. In all cases progesterone alone did not significantly affect uterine involution, or the interval to first estrus or first ovulation. Foote and Saiduddin (1964) also found estrogen to be the most satisfactory treatment, but failed to decrease the interval from calving to conception. Fosgate et al. (1962) found that variation for the interval from parturition to involution was significantly increased after treatment with 17-alpha-hydroxyprogesterone-N-caproate, but the variation for the interval from parturition to ovulation was significantly decreased. If no effect on estrus was noted, it would appear that an early ovulation in the presence of a non-regressed uterus would be of little practical value. Norwood (1963) administering beta-estradiol, progesterone and Provera (6alpha-methyl-17alpha-acetoxy-progesterone) did not significantly affect uterine involution in primiparous cows and progesterone and Provera did not significantly affect the uterine involution interval in pluriparous cows.

Saiduddin et al. (1968) treated postpartum Hereford cows with progesterone and estradiol-17beta. Estradiol-17beta alone or following progesterone resumed ovarian activity earlier, but the interval from parturition to uterine involution was not significantly altered. A trend towards decreasing the variance in the interval to first ovulation, first estrus and conception was noted.

It appears that although hormone treatment significantly decreases the variance in ovarian activity, the treatment has no beneficial effects on the interval to uterine involution. If the uteri were fully regressed and ovarian activity was induced earlier, beneficial effects may ensue; however, at the present time hormone treatment is not practical from an economic and management standpoint.

The time from parturition to first ovulation, first estrus and uterine involution is essential to early postpartum rebreeding. In addition to environmental factors there are breed differences which affect first estrus and first ovulation after calving. Casida et al. (1968) stated that beef cows tend to have a longer interval from parturition to the first succeeding estrus than dairy cows. Data from several studies (table 1) are summarized in table 2. These data indicate a significant difference between beef and dairy herds. A dairy herd is usually more closely observed than a beef herd and a poorly expressed estrus would most likely be detected in a dairy herd as opposed to a beef herd. Clapp (1937) and Wiltbank and Cook (1958) found a longer interval to first estrus in suckled cows than in milked cows.

Data from table 2 concerning first ovulation postpartum indicates the first ovulation is not accompanied by estrus. Marion and Gier (1968) reported from a herd of 250 cows, 214 ovulations were palpated 11 to 20 days after parturition with only 12 observed heats. Casida et al. (1968) stated that in every case where both the time to first ovulation and first estrus are known, the average is shorter to first ovulation than to first estrus. Casida et al. (1968) also reported that dairy cows appear to show the first ovulation earlier

TABLE 1. SUMMARY OF STUDIES CONCERNING POSTPARTUM REPRODUCTIVE FUNCTION IN THE BOVINE

Author		Breed	Uterine Involution	First Ovulation	First Estrus
Saiduddin *et al.*	(1968)	Hereford	46	38	46
Ulberg & Lindley	(1960)	Hereford	46	38	46
Foote & Hunter	(1964)	Hereford	47	44	49
Casida & Wisnicky	(1950)	Holstein	28	35	63
Fosgate *et al.*	(1962)	Holstein	28	45	51
Fosgate *et al.*	(1962)	Jersey	27	35	45
Menge *et al.*	(1962)	Holstein	45	20	34
Wiltbank & Cook	(1958)	Shorthorn	56	53	84
Wiltbank & Cook	(1958)	Shorthorn	44	36	54
Foote *et al.*	(1960a)	Angus	41	61	86
Foote *et al.*	(1960a)	Shorthorn	43	62	76
Foote *et al.*	(1960b)	Hereford	44	38	59
Foote & Saiduddin	(1964)	Hereford	46	38	46
Warnick	(1953)	Hereford	--	--	58
Warnick	(1953)	Angus	--	--	51
Warnick	(1955)	Angus	--	--	59
Warnick	(1955)	Hereford	--	--	63
Buch *et al.*	(1955)	Holstein*	42	--	--
Buch *et al.*	(1955)	Holstein**	50	--	--
Perkins & Kidder	(1963)	Hereford	36	--	--
Perkins & Kidder	(1963)	Angus	39	--	--
Casida & Venzke	(1936)	Holstein	26	41	--

*Primiparous
**Pluriparous

than beef cows.

TABLE 2. A COMPARISON OF BEEF AND DAIRY POSTPARTUM REPRODUCTIVE FUNCTION

(averages and ranges in days calculated from data in table 1)

	Av. Uterine Involution	Range	Av. First Estrus	Range	Av. First Ovulation	Range
Beef	44.4	36-56	59.2	46-86	45.3	36-62
Dairy	35.1	26-50	45.2	33-63	35.2	20-45

Casida *et al.* (1968) stated that the necessity of the uterus being completely involuted for conception is not clearly demonstrated. Buch *et al.* (1955) estimated the proportions of cows at given postpartum intervals which had shown a heat and also had an involuted uterus. He stated at 30 days postpartum only 6 percent of the cows observed had shown a heat. It was not until 60 days postpartum that 75 percent of the cows with involuted uteri had shown a heat. Marion and Gier (1968) also reported that in the period 36 to 56 days postpartum, only 51 percent of the cows had showed heat. They pointed out that absence of ovulations before 11 days after parturition may have been due to the inhibiting influence on each distended uterine horn. As mentioned previously, Gier and Marion (1968) stated that regression in uterine horn size is an important process in uterine involution. If involution is delayed then possibly distension would also be present. With distension present then ovarian activity begins at a later date postpartum, in turn, first estrus may also be affected.

The interval from parturition to conception is the most important interval in relation to the producer. Casida *et al.* (1968) reported that variation in this interval is due to variation in the interval to first estrus. Since estrus is a function of ovarian activity, and uterine involution may affect ovarian function, the importance of a shortened involution period becomes evident. Casida et al. (1968) reported fertility data for 6 successive months in dairy cattle and for 4 successive months in beef cattle after parturition. The averages for conception at first service were 39.3, 53.2, 61.6, 62.2, 64.7, and 64.3 percent for consecutive months 1 to 6 in dairy cattle and 33.4, 58.1, 68.6 and 74.4 percent for months 1 to 4 in beef cattle. Casida et al. (1968) stated, "It is doubtful that there is any great change after the third month." Uterine involution should be complete before the third month postpartum in the normal cow. It appears then that early postpartum infertility is due to the condition of the uterus.

Lindley (1954) indicated that much of the problem of postpartum infertility may be caused in intrauterine bacterial infection. It has been only in the past few years that techniques have been developed to detect uterine bacteria. Fitch and Bishop (1932) studying a large group of bovine uteri concluded that "The healthy bovine uterus is, in general, free of bacteria." Clark and Stevenson (1949) using flushing techniques also stated that the normal gravid and non-gravid bovine uterus is essentially bacteria free. Dawson (1950), using more desirable growing mediums, isolated Pseudomonas, Neisseria and Proteus for the first time. He reported that the most commonly observed bacteria were Staphylococcus and heamolytic golden colonies. It was his conclusion that the healthy uterus was not germ free. Wulf and Dracy (1952) however reported that when flushing the uterus with sterile techniques the number of colonies obtained were very small, but recorded that the predominant genera observed were Bacillus, Corynebacterium, Actinomyces, Staphylococcus and Cocidioides. Gunter et al. (1955) indicated that indeed the uterus was not free of bacteria, and bacteria may play a role in infertility. The most common bacteria reported were Streptococcus sp; Micrococcus pyrogenes strains and Corynebacterium.

Elliot et al. (1968) using endometrium samples as well as uterine flushings, isolated 33 different species. In some cases he failed to isolate organisms from uterine lumen flushings while cultures from the endometrium were positive. This may explain why some of the previous workers failed to recover bacteria. The most common isolations made contained Staphylococcus epidermidis, Pseudomonas sp., Corynebacterium bovis, Streptococcus faecalis, Microccus sp., Staphlococcus aureus, Corynebacterium pyogenes, Escherichia coli, Microbacterium flavum and Pseudomonas boreopolis. Elliot et al. (1968) reported that the cervix remained relaxed in the cow for several days after parturition and provided entrance for bacteria into the uterine cavity, where sloughed tissue, tissue fluids and a small amount of blood maintained a liquid medium favorable for bacterial growth. It was also reported that a rapid decrease in percentage of detectable infections of the uteri accompanied progression of time after parturition, with 93% of the uteri found infected within 15 days after parturition and 9% infected between 46 and 60 days after parturition. Singh (1962) found large concentrations of leucocytes in the endometrium under certain conditions of postpartum regression, indicating active or recent bacterial infection. Elliot et al. (1968) concluded that bacteria of certain species can be considered normal inhabitants of the uterus of the postpartum cow, probably causing little if any damage while normal sloughing of the caruncles and regeneration of the epithelium are taking place. Excessive infection may delay epithelial regeneration and return of the uterus to normal. It was also indicated that the fluid medium in the uterus may secondarily erode the uterine epithelium and bacteria in the uterus 60 days after parturition may cause reproductive failure in future breeding. Lukert (1961) reported that virological examinations of the bovine uterus indicated that it is free of viral infections.

With the reported occurrence of uterine bacteria, an additional attempt was made to solve the problem of postpartum infertility with the intrauterine infusion of antibiotics. Roberts (1955) evaluated intrauterine infusions for impaired fertility using 4 basic treatments: (1) Perservisal, which was an isotonic Ringers solution and dextrose in distilled water, (2) an iodine solution prepared by adding 3 to 15 ml of Lugol's solution to 250 to 500 ml distilled water, (3) a solution of thyrothricin, and (4) penicillin and streptomycin in solution. No marked differences in conception rate occurred after treatment when compared with figures tabulated on large numbers of cows recorded by workers in the field of artificial insemination. Lindley and Hatfield (1952) concluded that low-grade bacterial infections appeared to be a cause of infertility in dairy cows, and felt that proper antibiotic or bacteriostatic treatment restores the reproductive capacity of repeat breeders. Lindley (1954) divided an experimental herd of dairy cows into 3 groups and infused antibiotics or sulfonamides 1 or 2 days following service. The 3 treatment groups consisted of: (1) streptomycin sulfate and penicillin in sterile saline, (2) streptomycin sulfate and penicillin in distilled water, or (3) streptomycin sulfate and penicillin in a 12% sulfopyridine solution. Results indicated that in all treatment groups fertility was increased in the instance of repeat breeding cows; however, the period of time postpartum was not given. Jones et al. (1956) using an antibacterial for retained placenta, recommended a 90 day sexual rest period, therefore no data on how early postpartum the cow rebred was available.

Witter (1960) reported on the usefulness of Furacin (Eaton Lab; Norwich, N. Y.) for fertility problems in the mare, and stated the nitrofurans which are neither sulfonamides nor antibiotics, possess a broad antibacterial spectrum and induces little, if any, bacterial resistance. He also reported the drug is effective in the presence of pus, serum or organic detritus. Rude (1959) treated cows having retained placenta with nitrofurazone. He reported that

Furea: Veterinary (Eaton Lab; Norwich, N. Y.) combines the antibacterial activity of nitrofurazone and the proteolytic effect of urea to produce dissolution of necrotic tissue. The results were that of 30 cows with retained placentae after treatment a 93% conception rate was recorded. However, the interval from parturition to conception was not stated. Cramer and Dodd (1946) reported on the mode of action of nitrofuran compounds and concluded that the product adversely affects the metabolism of a bacterial organism, but the nature of the effect or the substrate affected was not known.

Jones (1965) described the nitrofuran derivatives as a group of synthetic compounds which possess antibacterial activity and are primarily used for their activity against gram negative organisms, but they also have some activity against gram positive bacteria. The exact mechanism of action of the nitrofurans is not known, although, it is probable that they inhibit an enzymatic oxidative process. Apparently bacteriostatic activity results from a reversible inhibition of enzymes involved with the dissimilation of pyruvate.

Witter (1960) described nitrofurazone superior to all other compounds, synthetic, antibiotic or sulfonamide in nature, for the treatment of retained placenta or impaired fertility. However, it is significant to note that no data is available on the effect nitrofurazone may have on the interval from parturition to conception.

METHODS AND MATERIALS

Forty-five pluriparous Hereford cows weighing an average of 320.6 ± 5.6 kg. were placed in four uterine treatment groups which were stratified among 4 different levels of nutrition. All cows had access to Bluestem winter pasture and 1.35 kg. of alfalfa hay per day. In addition, group 1 (high-energy, high-protein) received 1.35 kg. of milo and 0.68 kg. of soybean meal; group 2 (high-energy, low-protein) received 1.35 kg. of milo and group 3 (low-energy, high-protein) received 0.68 kg. of soybean meal. Group 4 (low-energy, low-protein) had only pasture and alfalfa hay.

All cows were corralled once a week and cows that had calved the previous week were placed in one of 4 uterine treatment groups: (1) controls, (2) nitrofurazone, (3) proteolytic enzymes and (4) nitrofurazone plus proteolytic enzymes. The antibacterial used was a 0.2% Furacin solution (Eaton lab; Norwich, N. Y.) and the proteolytic enzymes used were alpha-chymotrypsin and collagenase (Worthington Bio. Chem. Corp. Freehold, N. J.) used at the rate of lmg/ml of each. The first, second and third week postpartum treatment was administered in 100, 50, and 25 ml respectively (table 3), with the first uterine treatment occurring on an average of 4 ± .5 days postpartum. Crystalline proteolytic enzymes were dissolved and combined not more than 24 hours prior to intrauterine infusion via breeding catheter.

Daily observations, heat mount detectors and rectal palpation of the ovaries made at 7 day intervals was used to aid in the detection of estrus. All cows were rectally palpated weekly after parturition to determine the time of ovulation and uterine size, with the first palpation occurring not more than 7 days postpartum and palpations were discontinued at conception. Bulls were placed with the cows on May 8, 1968.

Palpation data was statistically analyzed using t and F tests for variation with averages calculated for the interval from calving to first postpartum estrus and ovulation, and date of uterine regression, (table 4).

Three or 4 uterine biopsies were obtained from each animal from the previously gravid horn with the first biopsy taken less than 7 days postpartum and the last biopsy obtained not more than 28 days postpartum. The first biopsy was obtained prior to any treatment and used as a histological control. Tissues were immediately fixed in chrom-acetic fixative consisting of 15 ml of 1.0% chromic acid and 1.0 ml glacial acetic acid. The tissues were dehydrated in a series of isopropyl alcohol and infiltrated with paraffin. They were embedded in fresh paraffin and sectioned at 10 microns and mounted with Mayer's albumin on glass slides. Representative sections were stained with Mallory's Triple for histological studies.

The slides were examined and measurements of the epithelium were made with the aid of an eyepiece micrometer. Photomicrographs were taken at 400x or 100x to show the effect of the various treatments on uterine epithelium.

TABLE 3. TREATMENT SCHEDULE

Treatment Groups	Days After Parturition 0-7	7-14	14-21
Control	no treatment	no treatment	no treatment
Nitrofurazone[a]	25 ml nitrofurazone in sterile saline to make a volume of 100 ml	25 ml nitrofurazone in sterile saline to make a volume of 50 ml	25 ml nitrofurazone
Alpha-chymotrypsin[b] and collagenase	100 mg alpha-chymotrypsin plus 100 mg collagenase in 100 ml sterile saline	50 mg alpha-chymotrypsin plus 50 mg collagenase in 50 ml sterile saline	25 mg alpha-chymotrypsin plus 25 mg collagenase in 25 ml sterile saline
Nitrofurazone plus alpha-chymotrypsin and collagenase	100 mg alpha-chymotrypsin plus 100 mg collagenase and 25 ml nitrofurazone made up to 100 ml with sterile saline	50 mg alpha-chymotrypsin plus 50 mg collagenase and 25 ml nitrofurazone made up to 50 ml with sterile saline	25 mg alpha-chymotrypsin plus 25 mg collagenase in 25 ml nitrofurazone

[a]Furacin solution (0.2% nitrofurazone in squeejet dispenser), Eaton Lab., Norwich, N. Y.

[b]Crystalline form

RESULTS AND DISCUSSION

The effect of uterine treatments on the intervals from parturition to first ovulation, first estrus and uterine involution is shown in table 4.

TABLE 4. THE AFFECT OF ALPHA-CHYMOTRYPSIN, COLLAGENASE AND NITROFURAZONE ON THE INTERVALS FROM PARTURITION TO OVULATION, ESTRUS AND UTERINE INVOLUTION[a]

Treatment Groups	First Ovulation	First Estrus	Uterine Involution
Control	18.81 ± 3.32	26.99 ± 3.84	25.84
Nitrofurazone	25.62 ± 3.09	36.11 ± 4.18	31.55
α-chymotrypsin and collagenase	24.53 ± 3.02	31.85 ± 3.96	30.46
Nitrofurazone plus α-chymotrypsin and collagenase	21.96 ± 3.16	30.70 ± 3.61	32.92

[a]Values represent days postpartum ± standard error

The interval from parturition to first ovulation was shorter than the interval to first estrus in all groups. This is in agreement with Casida *et al.* (1968) and consistent with the data in table (2). There was no significant difference between the four treatment groups in relation to ovulation. Marion and Gier (1968) stated that 93% of the dairy cattle palpated in that study ovulated 11 to 20 days after parturition. Casida *et al.* (1968) stated dairy cattle tend to ovulate earlier after parturition than do beef cattle. The average number of days from parturition to ovulation for the four groups was 22.73 ± 1.99. This interval appears only slightly longer than what was reported by Marion *et al.* (1968); the slightly longer interval obtained in this study may be due to breed difference.

There was no significant difference between the four groups in relation to the first postpartum estrus. The average time of first estrus was 31.41 $\pm$ 2.48 days after parturition. The interval to first estrus was longer than the interval to first ovulation. This is in agreement with Marion and Gier (1968) who reported that only 12 heats occurred in 214 ovulations after parturition. It is possible that endogenous estrogen levels occurring at first ovulation are not high enough to cause an observable heat. The actual first heat postpartum is probably coupled with the second ovulation postpartum.

Uterine regression in size was determined by palpation measurements with regression being termed complete when the uterus had returned to its normal, non-pregnant position and when the previously gravid horn had regressed to a diameter of 35 mm or less. There was no significant difference among the four groups. The treatments had no effect on the muscular contractions and reduction in size of the uterus. Although the previously gravid horn had decreased in diameter to a normal non-pregnant size, histological repair may not have been complete at that time. This observation is indicated in the data on the interval from parturition to conception (table 5). If the uterus had been histologically involuted as well as physically regressed, there should have been no significant difference among the four treatment groups.

Saiduddin et al. (1968), Ulberg and Lindley (1960), and Foote and Hunter (1964) all reported on the interval from parturition to conception for Hereford cattle to be from 57 to 59 days. The interval from parturition to conception in the control, alpha-chymotrypsin and collagenase and the alpha-chymotrypsin and collagenase plus nitrofurazone groups agrees with the normally accepted value of 50 to 60 days postpartum. It is of significant importance to the beef producer to have a postpartum interval to conception of 60 days or less.

TABLE 5. THE EFFECT OF ALPHA-CHYMOTRYPSIN PLUS COLLAGENASE AND NITROFURAZONE ON THE INTERVAL FROM PARTURITION TO CONCEPTION[a]

Treatment Groups	Interval	Significant Differences
(1) Control	53.86 $\pm$ 4.35	(1) vs (2) P < .01
(2) Nitrofurazone	72.76 $\pm$ 4.71	(1) vs (3) n.s.
(3) α-chymotrypsin and collagenase	60.13 $\pm$ 4.68	(1) vs (4) n.s.
(4) Nitrofurazone plus α-chymotrypsin and collagenase	53.24 $\pm$ 4.37	(2) vs (4) P < .01

[a]Forty of the 45 cows rebred for an 88.9% conception.

The cows in the group receiving nitrofurazone had an interval from parturition to conception significantly longer (P<.01) than the control group or the group receiving alpha-chymotrypsin and collagenase plus nitrofurazone. It is significant to note that nitrofurazone is a commonly recommended treatment for bovine impaired fertility, retained placenta or uterine bacterial infection. In most cases in which nitrofurazone has been infused intrauterine, a period of sexual rest is recommended. For this reason it has been impossible to obtain data on the interval from parturition to conception in nitrofurazone treated cows. Lindley (1954) indicated that postpartum infertility was due to a low-grade bacterial infection, and therefore prescribed nitrofurazone as the most logical treatment. With the fact that nitrofurazone had no significant affect on the intervals from parturition to first ovulation, first estrus and uterine reduction in size after parturition, the only explanation is that nitrofurazone directly or indirectly affected the uterus histologically.

Uterine biopsies obtained during the treatment period were observed for histological abnormalities that may have caused delayed conception. Observations of the histological preparations indicated that epithelial erosion

EXPLANATION OF PLATE I

Fig. 1. Uterine biopsy from Nitrofurazone treated cow 22 days postpartum showing typical epithelial erosion (400x).

Fig. 2. Uterine biopsy from Nitrofurazone treated cow 22 days postpartum showing a lack of epithelial regeneration from uterine gland (400x).

Fig. 3. Uterine biopsy from Nitrofurazone treated cow 11 days postpartum showing typical epithelial "blow off" (400x).

Fig. 4. Uterine biopsy from Nitrofurazone treated cow 29 days postpartum showing leucocyte invasion and consequential massive epithelial loss (400x).

Fig. 5. Uterine biopsy from Control cow 11 days postpartum demonstrating typical uniform epithelium (400x). Compare with Plate I, Fig. 3.

Fig. 6. Uterine biopsy from Control cow 27 days postpartum showing uniform epithelium and lack of epithelial "blow off" (400x). Compare with Plate I-Fig. 4 from nitrofurazone treated cow 29 days postpartum.

PLATE I

Fig. 1

Fig. 2

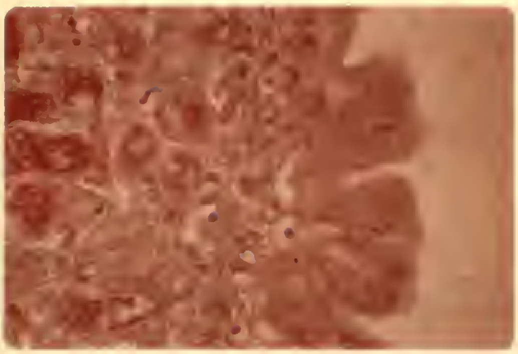

Fig. 3

Fig. 4

Fig. 5

Fig. 6

(Plate I, Fig. 1) and delayed epithelial regeneration (Plate II, Fig. 2) were more common in the nitrofurazone treated group. Most notable and particularly unique to the nitrofurazone treated group was uterine epithelial "blow off" (Plate I, Fig. 3 and 4) which occurred in this group for a prolonged period of time and to a greater degree than in the other three treatment groups. In comparing Plate I, Fig. 3 and 4 to Plate I, Fig. 5 and 6 from cows at similar times postpartum respectively, a definite difference in the uniformity of the epithelium can be observed. In comparing Plate II, Fig. 1 and 2 from cows receiving alpha-chymotrypsin and collagenase, or to Plate II, Fig. 3 and 4 from cows receiving alpha-chymotrypsin and collagenase plus nitrofurazone, a lack of epithelial uniformity and predominance of epithelial "blow off" can be noted in the nitrofurazone treated cows.

To establish a quantitive difference between the histological observations from the nitrofurazone treated group and the other three groups an arbitrary scale was devised to measure the occurrence and degree of epithelial "blow off". The following is a description of the scale established to estimate the average degree of epithelial loss.

Degree of Loss[a]

(1) Minor epithelial loss, from 5 to 10 cells or 25 microns across or less.

(2) More severe epithelial loss, 10 to 20 cells or from 25 to 50 microns across.

(3) Severe epithelial loss, from 20 to 30 cells or from 50 to 75 microns across.

(4) Most severe epithelial loss, from 30 to 40 cells or from 75 to 100 microns across.

(5) Extreme epithelial loss, greater than 40 cells. or greater than 100 microns across.

[a]All measurements and photomicrographs were made at 400x.

EXPLANATION OF PLATE II

Fig. 1. Uterine biopsy from alpha-chymotrypsin and collagenase treated cow 11 days postpartum showing uniform lining of epithelium (400x). Compare with Plate I, Fig. 3 from nitrofurazone treated cow also 11 days postpartum.

Fig. 2. Uterine biopsy from alpha-chymotrypsin and collagenase treated cow 27 days postpartum showing typical uniform epithelium (400x). Compare with Plate I, Fig. 4, and Plate I, Fig. 6.

Fig. 3. Uterine biopsy from alpha-chymotrypsin and collagenase plus nitrofurazone treated cow 11 days postpartum showing uniform epithelial lining (400x). Compare with Plate I, Fig. 3.

Fig. 4. Uterine biopsy from alpha-chymotrypsin and collagenase plus nitrofurazone treated cow 27 days postpartum demonstrating complete epithelial regeneration and showing no areas of damage due to leucocyte invasion. Compare with Plate I, Fig. 4.

Fig. 5. Uterine biopsy from degree (1) of epithelial loss (400x). Nitrofurazone treated cow 21 days postpartum with epithelial loss less than 25 microns across.

Fig. 6. Uterine biopsy from degree (2) of epithelial loss (400x). Nitrofurazone treated cow 28 days postpartum with "blow off" from 25 to 50 microns across.

PLATE II

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Plate II, Fig. 5 shows an example of degree 1 of epithelial loss. Plate II, Fig. 6 shows degree 2 of epithelial loss. Degrees 1 and 2 may be found in many cases and observed frequently in all treatment groups. However, the frequency of observable degree 3 "blow offs" (Plate III, Fig. 1); degree 4, (Plate III, Fig. 2) and degree 5, (Plate III, Fig. 3) is greater in the nitrofurazone treated group.

TABLE 6. AVERAGE DEGREE OF "BLOW OFF" FOR EACH UTERINE TREATMENT GROUP AS CALCULATED FROM SCALE FOR DEGREE OF LOSS

Treatment Group	Number of Biopsies	Number of Eruptions	Average Degree
Control	10	46	1.13
Nitrofurazone	14	112	2.49
α-chymotrypsin and collagenase	11	67	1.37
Nitrofurazone plus α-chymotrypsin and collagenase	11	67	1.86

Table 6 shows that both the average degree of uterine epithelial "blow off" and the total number of eruptions is highest in the nitrofurazone treated group. Histological observations indicated that the possible cause of the epithelial loss was due to abnormally high concentrations of leucocytes migrating from the endometrium which in turn caused displacement of the epithelium from the basement membrane. In small concentrations leucocyte invasion of the epithelium is not detrimental, (Nellor 1966), however in larger concentrations (Plate I, Fig. 4) a significant loss of epithelial cells occurs. If this situation were continuous throughout the entire uterus a factor seriously detrimental to blastocyst implanation would exist.

The loss of epithelium continued in the nitrofurazone treated group for

EXPLANATION OF PLATE III

Fig. 1. Uterine biopsy from degree (3) of epithelial loss (400x). Nitrofurazone treated cow 30 days postpartum showing severe epithelial loss especially for this period of time after parturition.

Fig. 2. Uterine biopsy from degree (4) of epithelial loss (400x). Nitrofurazone treated cow 21 days postpartum showing epithelial loss of from 75 to 100 microns across; a situation seriously detrimental to blastocyst implantation.

Fig. 3. Uterine biopsy from degree (5) of epithelial loss (400x). Nitrofurazone treated cow 22 days postpartum showing extreme epithelial loss of greater than 100 microns across.

PLATE III

Fig. 1

Fig. 2

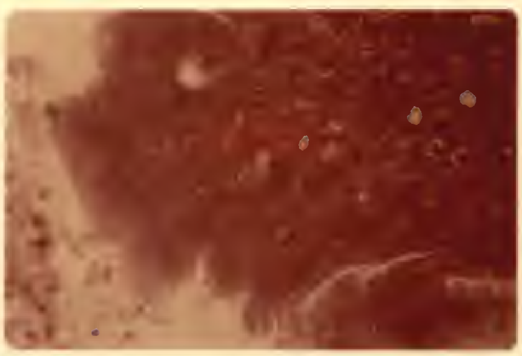

Fig. 3

a longer period of time after parturition than in the control group, and histological observations indicated the degree of "blow offs" increased after the third treatment of nitrofurazone. This also may have caused the interval from parturition to conception.to be extended in the nitrofurazone treated group. The total number of eruptions and average degree of epithelial loss were less in the alpha-chymotrypsin and collagenase plus nitrofurazone group and also in the alpha-chymotrypsin and collagenase treated group. Since the proteolytic enzymes alone or in combination with the nitrofurazone caused less detrimental effects it is probable that the enzymes tended to counteract the effect of the nitrofurazone.

It is hypothesized that some bacteria common to the postpartum bovine uterus may produce beneficial proteolytic enzymes (McMahon, 1969) aiding in the breakdown and eventual sloughing of necrotic tissue. Nitrofurazone eliminates uterine bacteria and in turn prevents the production of proteolytic enzymes from such bacteria. Leucocyte invasion occurs in response to necrotic tissue in the uterus, and causes epithelial loss. Since a normal interval from parturition to conception and a lack of leucocyte invasion was observed in the nitrofurazone plus alpha-chymotrypsin and collagenase treated group, probably the proteolytic enzymes aided in the breakdown of necrotic tissue, which eliminated the need for leucocyte invasion and therefore tissue repair was complete at a normal postpartum interval.

The interval from parturition to conception in the proteolytic enzyme treated group was not significant at the (P .01) level because of considerable variation. However, histological observations revealed that tissue repair occurred at a near normal rate and with a lack of leucocyte invasion.

The original hypothesis of shortening the postpartum interval from calving to conception by the intrauterine infusion of proteolytic enzymes was rejected. It was concluded however that nitrofurazone in the postpartum cow produces a detrimental effect in relation to tissue repair because of its effect on proteolytic enzyme producing bacteria in the uterus. This detrimental effect of lengthening the postpartum interval from parturition to conception did not occur when nitrofurazone was combined with alpha-chymotrypsin and collagenase, or when alpha-chymotrypsin and collagenase were used alone. It is therefore considered that if nitrofurazone is to be infused intrauterine in the postpartum cow, it should be combined with proteolytic enzymes in order to produce an interval from calving to conception that is not greater than that which is expected from the clinically normal cow.

SUMMARY

Forty-five Polled Hereford cows received intrauterine infusions of proteolytic enzymes, nitrofurazone or a combination of both in an attempt to shorten the postpartum interval from calving to conception. None of the treatments affected the intervals from calving to ovulation, estrus or decreased uterine size. The interval from parturition to conception was not shortened, causing rejection of the original hypothesis. The control, proteolytic enzyme and proteolytic enzymes plus nitrofurazone groups had an interval from calving to conception that coincided with that of clinically normal cows. The nitrofurazone treated group had an interval from parturition to conception significantly longer than the other three groups.

Uterine biopsies studied for histological differences between the four groups revealed that the uterine epithelium of the nitrofurazone treated cows was being eroded for a significantly longer postpartum period than in the other treatment groups. Since the interval from calving to conception was significantly shorter in the proteolytic enzyme plus nitrofurazone treated group, it was hypothesized that the proteolytic enzymes diminished the detrimental effects of the nitrofurazone. Histological observations also revealed that epithelial repair had progressed at a normal rate and without a significant loss of tissue in the proteolytic enzyme plus nitrofurazone treated group. It was therefore concluded that if nitrofurazone was to be infused intrauterine for retained placenta or impaired fertility that it should be combined with proteolytic enzymes if the interval from parturition to conception is to be kept at a minimum.

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THE EFFECT OF NITROFURAZONE AND PROTEOLYTIC ENZYMES ON THE POSTPARTUM BOVINE UTERUS

by

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This study was conducted to further elucidate postpartum ovarian and uterine function in the bovine and to determine the effects of various compounds infused intrauterine on involution after-parturition. Forty-five Polled Hereford cows were divided into four groups at parturition and given intrauterine infusions of nitrofurazone, alpha-chymotrypsin and collagenase or alpha-chymotrypsin and collagenase plus nitrofurazone. A control group was not infused. Infusions were given in 100, 50 and 25 ml on the first, second and third week postpartum respectively. The enzymes were given in a concentration of 1 mg of each per ml of sterile saline. Twenty-five ml of a 0.2% nitrofurazone solution was diluted to the above volumes. At seven day intervals after parturition, time of ovulation, diameter of pregnant and non-pregnant uterine horns were determined by rectal palpation. Uterine biopsies were taken on the second, third and fourth week after parturition from 20 cows. Other uterine biopsies were taken at selected times on some cows. The interval from calving to ovulation, estrus, uterine involution and conception was determined. Uterine tissue was prepared for histological study by standard procedures. The uterine treatments had no statistically significant effect on the intervals from parturition to ovulation, estrus or uterine involution. The average interval from parturition to ovulation and estrus for the 45 cows was 22.7 and 31.4 days respectively. If ovulation was observed within the first 20 days postpartum it occurred on the side of the previously non-gravid horn in 92% of the cases. Nitrofurazone significantly lengthened the interval from parturition to conception over the controls, enzyme treated and enzyme plus nitrofurazone treated groups (72 vs 54, 60 and 53 days, respectively). The percent conception for each treatment group was 92%, 100%, 80% and 85% for the control, nitrofurazone, alpha-chymotrypsin and collagenase and the alpha-chymotrypsin and collagenase plus nitrofurazone groups respectively.

Although palpation data indicated that uterine regression was complete by an average of 30 days after parturition, histologically the uterine epithelium was eroded or "blown off" in the treated groups. This was more prevalent in the nitrofurazone treated group, and the degree of tissue loss increased after the third treatment of nitrofurazone. The interval from parturition to conception in the nitrofurazone plus proteolytic enzyme treated group was not significantly different from that of the controls, indicating that the enzyme may cancel the detrimental effect of nitrofurazone. This was apparent in histological calculations in the degree and number of epithelial erosions.