THE EFFECT OF OVARIAN HORMONES UPON THE SUSCEPTIBILITY OF THE BOVINE UTERUS TO BACTERIAL INFECTION

by

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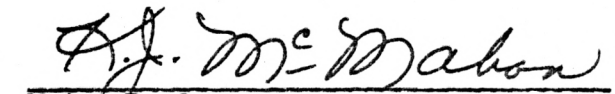

Major Professor

TABLE OF CONTENTS

INTRODUCTION..... 1

REVIEW OF LITERATURE..... 2

MATERIALS AND METHODS..... 10

Animals Employed, Hormonal Treatments and the Bacterial Cultures Used..... 16

Experiments in Ovariectomised Cows Under the Influence of Exogenous Betaestradiol Only..... 17

Processing of Samples, Media Employed and Incubation Method..... 20

Identification of Bacteria..... 23

EXPERIMENTAL RESULTS..... 23

DISCUSSION..... 33

SUMMARY..... 39

ACKNOWLEDGMENTS..... 41

BIBLIOGRAPHY..... 42

APPENDIX..... 46

INTRODUCTION

Infertility in cattle is recognized as one of the serious problems affecting the economy of the cattle industry. Various factors responsible for reproductive failures are (1) noncontagious bacterial infections of the reproductive tract caused by a variety of microorganisms; (2) contagious infectious diseases; (3) endocrine disorder; (4) anatomical defects of the reproductive parts; (5) nutritional deficiencies due to imbalanced feeds and (6) genetic defects.

Infertility has been attributed to the early embryonic mortality in the dairy cow. Since infectious agents have been suggested as a possible cause of this loss, the importance of bacterial infection of the uterus of the cow which has been reported by many workers, is of great significance. It is also emphasized that ovarian hormones influence the susceptibility of the uterine endometrium of the cow, to bacterial infection. Investigators have employed various methods for sampling the uterus of live cows for bacteriological studies and have reported the bacterial flora of the normal postpartum and the repeat breeding cow. The objectives of the present study were two-fold (1) to get representative intrauterine samples from the cow without contaminating either the uterine lumen or the sample itself from other parts of the reproductive tract; (2) to study the influence of exogenous ovarian hormones on the natural postpartum or induced bacterial infection, in intact and ovariectomised cows. The instrument designed for taking swab samples for bacteriological examination was slightly modified to inject bacterial cultures directly into the uterus and to obtain uterine biopsies.

REVIEW OF LITERATURE

Hatch et al., (1949) used a glass speculum and a swab for collection of intrauterine samples for bacteriological study of the reproductive tract of infertile cows. Easley et al., (1951) designed an instrument for carrying out bacteriological studies of the reproductive tract of cows. The instrument consisted of glass speculum 15" long, 8 mm dia, glass pipette 16" long 4 mm diameter. The pipette was attached to a syringe of two ml capacity. Using this instrument they obtained samples from 148 normal cows and 40 repeat breeders. They reported identification of the following genera. Neisseria, Micrococcus, Streptococcus, Pseudomonas, Corynebacterium, Bacillus, Flavobacterium, Sarcina, Coliaerogenes, Actinomyces, Proteus, Bacterium, Gaffkya, Bacteroides, yeast, Mycobacterium, and Salmonella. Kampelmacher (1952) designed an instrument to study the microbiology of the uterus. It consisted of metal speculum 30 cm long, 7 mm dia, metal tube 52 cm long, 4 mm dia, and a copper rod 56.5 cm long exactly fitting the conical part of the 52 cm tube. The instrument was also used to take biopsy samples for histological examination. Lindley and Hatfield (1952) used a fiber speculum 8" long and a glass pipette for collection of intrauterine samples. Frank and Bryner (1952) designed an instrument for bacteriological studies of the reproductive tract of cows which consisted of two telescoping metal tubings and a wire carrying a swab for obtaining samples. A rubber diaphragm was fixed over the end of the outer tube to avoid contaminating the instrument. The inner tube could also be attached to a syringe for sucking out mucous from the uterine lumen. Gunter et al., (1955) employed a modification of Frank and Bryner's instrument. They omitted the rubber diaphragm and replaced it with a plug of 2 per cent sterile agar (after

sterilization of the instrument).

Elliott (1961) examined 110 postpartum uteri obtained from the slaughter house. Out of the 110 uteri cultured, 67 (60.9%) showed microflora. A correlation between the length of postpartum period and the presence of microorganism in the uterus was reported. He concluded that the normal bovine uterus becomes essentially free from bacteria at 60 days postpartum. He isolated Proteus, Staphylococcus, Micrococcus, Streptococcus, Escherichia, Aerobacter, Pseudomonas, Corynebacterium, Microbacterium, Actinomyces, Alcaligenes, Arthrobacter, Clostridium, a non-spore forming anaerobe and Vibrio. He also isolated a mold Aspergillus. Abo-Ahmed (1963) examined uteri of 20 normal healthy postpartum cows for the presence of bacteria. He took swab samples from the uterine lumen of each cow on the 8th, 15th, 23rd, 35th and 50th day postpartum. He reported a distinct correlation between the length of postpartum period and the presence of microorganisms in the uterus of the cow. Bacteria were isolated from 86.4% of the cows examined on the 8th day postpartum. By the 50th day all the uteri examined were bacteria free. Bacteria isolated were members of the following genera: Staphylococcus, Micrococcus, Streptococcus, Corynebacterium, Pseudomonas, Sarcina, Gaffkya, Aerobacter, Escherichia, Alcaligenes, Achromobacter, Flavobacterium, Serratia, and Bacillus. He concluded that the normal bovine uterus is bacteria free. Apparently microorganisms enter the uterus at the time of parturition but are eliminated within 50 days.

Black et al., (1954) found that the uterus of estrous and ovariectomised rabbits exhibited a high degree of bactericidal activity. They also reported that repeat breeding cows in the luteal phase of the estrous cycle generally exhibited a greater defense towards bacterial infection than did first service

animals. Black et al., (1953) compared the development of pyometra in follicular and luteal phases employing first service heifers and repeat breeder cows. They inoculated three estrous and three 12 day luteal phase virgin heifers with bovine semen by laprotomy. No response was found in estrous females at slaughter 24-36 hours after insemination, however the uteri of 2 luteal phase heifers contained pus. The reaction in the third was not typical. Six repeat breeding cows injected by cervical insemination, 8-12 days after heat, exhibited highly variable response ranging from no gross reaction in two to as much as five ml of pus in others. None of the uteri of 4 cows in luteal phase showed pyometra when inseminated with identical semen while 3 heifers treated similarly demonstrated this condition.

Tanabe and Casida (1949) reported embryonic death rate of 65.1% in genitally normal cows 34 days after breeding. They studied reproductive performance of 104 cows (49 Guernsey and 55 Holstein) each of which was bred 4-13 times without conceiving. They concluded that (1) breed, (2) number of previous calvings, (3) herd size and (4) herd breeding index had appreciable effect on fertilization, however Bang's disease and the last three factors were considered to have influence on embryonic mortality.

Willett et al., (1948 and 1952) observed pyometra in all of the 6 heifers inseminated ten days after the last heat. Weinstein et al., (1936) reported that estrogen treated mice developed pyometra. Of the 45 control animals, two showed bacteria in the uterus while most of the estrogen treated had bacterial infection. Gardner and Allen (1937) indicated that estrogenic hormones injected for long periods of time in large amounts induced change favoring uterine infections or preventing the control or removal of infection in mice. They also indicated that pyometra cannot be attributed

to a direct action of estrogenic hormones.

VonHaam and Rosenfeld (1943) reported remarkable increase in specific agglutinins and protective antibodies following administration of estrone and pneumococcal vaccine in castrated and noncastrated rabbits of both sexes. Estron injected rabbits showed an early response to antibody production and slow disappearance, but no significant rise in antibody titer appeared after reimmunization. Foley and Aycock (1944) found a stilbestrol induced factor which prevented infection in mice with hemolytic streptococci following a single dose of 40-50 I.U. of stilbestrol.

Broome et al., (1959a) found no bactericidal factor in the blood plasma of the cattle and rabbits in various stages of the estrous cycle. Broome et al., (1959b) observed satisfactory growth of Escherichia coli contained in semi-permeable sac, when placed in the uterus of estrous and diestrous cows and rabbits. Ovarian status of the animal did not influence the growth of bacteria in washings obtained from the uteri of both cows and rabbits when tested in vitro. No difference could be detected in the rate of growth of bacteria in extracts of uteri from estrous and pseudopregnant rabbits. Broome et al., (1959c) reported that no significant local uterine antibody response developed until 8 days, following intrauterine immunization with E. coli. Antigen significantly increased antibacterial activity against E. coli in the uteri of both estrous and pseudopregnant rabbits. Winter et al., (1960) reported a noncellular factor supposed to account for the bactericidal activity in the uteri of leucopenic rabbits in the follicular phase. Rowson et al., (1953) found the bovine uterus remarkably resistant during estrous to infection introduced either as infected semen or as a culture of Corynebacterium pyogenes. It was shown to be equally susceptible

to such infection in the luteal phase. The above observations were duplicated employing ovariectomised cows and exogenous hormones. Of the 11 cows inseminated during the luteal phase, eight had pyometritis at slaughter. No such condition was observed in six cows inseminated during estrous, with semen containing C. pyogenes.

Black et al., (1951) artificially ovulated estrual and pseudopregnant rabbits by administration of gonadotrophin and inseminated the animals either by the vaginal route or deposited the semen into the uterus by laprotomy. It was demonstrated that the percentage of corpora lutea at autopsy, 15 days later, was 39% and 49% in estrual animals inseminated by the vaginal and direct uterine route respectively. It was shown to be 0% in uterine inseminated pseudopregnant rabbits. Theyalso concluded that failure of fertilized luteal phase ova to survive in the oviducts of other artifically ovulated estrual and pseudopregnant host does was due to factors associated with the development of pyometra in the pseudopregnant uterus into which semen was directly placed. McDonald et al., (1952) failed to produce significant reaction in estrous or pseudopregnant rabbits after injection of sterile semen while contaminated semen produced marked pyometra in pseudopregnant and a mild inflammatory condition in estrous rabbits.

Black et al., (1953) reported that pyometritis resulted from the inoculation of pyogenic microorganisms in luteal female rabbits but no appreciable reaction resulted from follicular phase injections. The fact that pyogenicity varies with changes in tissue medium as well as with inherent nature of the organism was indicated when Vibrio fetus failed to induce appreciable response. It was also shown that pymetra was produced in untreated pseudopregnant females and in ovariectomised animals treated

with progesterone, however, no response in untreated or estrogen treated ovariectomised rabbits was noticed. There was little response seen in rabbits treated with both the ovarian hormones.

Teunissen (1952) carried out studies on the development of endometritis following betaestradiol and progesterone inoculation. Out of the 20 dogs inoculated with estrogen, six showed little hyperplasia. Out of the 15 dogs inoculated with progesterone, 14 showed proliferation and 11 of these developed endometritis of which six had the bacterial infection.

Hawk (1958) injected ligated uterine horns of the estrous and pseudopregnant rabbits with E. coli. He observed more leucocytic response and bactericidal activity in estrous rabbits than the pseudopregnant ones during the early stages of infection. Hawk et al., (1960a) reported that ovarian hormones influenced the uterine defenses through an effect on leucocytic response. Progesterone inhibited the bactericidal activity of the uterus against E. coli while estrogen stimulated its clearance from the uterine lumen. Hawk et al., (1960b) attributed the bactericidal property of rabbit uteri to the presence of leucocytes. Cell free uterine exudates obtained four hr after experimental inoculation of leucopenic estrous rabbits had little or no bactericidal activity in vitro. In control estrous and pseudopregnant rabbits, the bactericidal activity was evident. Leucopenia was induced by N-N'-N" triethylenethiophosphoramide, N-(3-oxapentamethylene)-N', N"-diethylenethiophosphoramide, benzene, or nitrogen mustard. In another group of estrous rabbits in which leucocytic migration was delayed by alcoholic stupor, no bactericidal activity was apparent in the uterus. Hawk et al., (1960c) observed that regardless of the endocrine state of the rabbit, the bactericidal activity of the cell-free uterine exudate in vitro

was proportional to the number of leucocytes in the uterine lumen. The concentration of the noncellular bactericidal substance was affected by the ovarian hormones which influenced the rate of leucocytic response. The leucocytic response exhibited by the uteri of estrous rabbits four hr after injection with E. coli was more as compared to pseudopregnant rabbit uteri and contained more bacteriocidal cell free exudate. However at 16 hr after injection pseudopregnant rabbit uteri contained more leucocytes than estrous ones and their exudates were the more bactericidal.

Lamming and Heap (1960) reported that there was no significant difference in the bactericidal activity of the pseudopregnant and ovariectomized rat uteri, under the influence of exogenous betaestradiol. Pseudopregnant rabbits, however, were particularly susceptible to infection but estrous animals were resistant. Uteri of sheep were shown to be resistant to experimental uterine infection during the estrous and the luteal phase. Bactericidal activity was attributed to leucocytic infiltration and its activity in rabbit uteri. Close correlation was also established between the susceptibility to infection and the presence of an acid soluble fraction. The later was considered to inhibit the leucocytic infiltration and thus increase the susceptibility of the uterus to infection. Broome et al., (1960) found that the difference in the number of leucocytes circulating in the blood stream during various phases of estrous cycle were not responsible for the amount of bactericidal activity present in the uteri of rabbits. They reported that the activity was due to the release of some leucocytic factor by the uterus during the follicular phase. They observed that the leucocytes had difficulty in passing through the uterine endometrium during luteal phase. Phagocytosis was considered to play an important role in

cleaning the uterus of Staphylococcus aureus infection. There was no difference found in the phagocytic activity of the uterus in follicular and luteal phases. It was pointed out that a nonleucocytic defense mechanism may also exist in uteri during the follicular phase. Winter et al., (1960) reported that more bacteria survived in the uteri of rabbits in the luteal phase than in the follicular phase under different conditions of uterine ligation, when compared at four intervals following treatment (5, 10, 20, and 40 hours). The organisms employed were E. coli and S. aureus. It was interpreted that more bacteria were lost through cervical drainage under the influence of estrogen especially in the first few hr after infection. A direct correlation existed between the presence of bacteria and the number of leucocytes in the uterus in the luteal phase only. The possibility of a nonleucocytic bactericidal factor in the uterus during the follicular phase was indicated. Bactericidal activity against E. coli in the cell free uterine exudate in vitro was shown to be more pronounced during the follicular phase, five hr after inoculation but no overall difference in the activity was noticed in different hormonal states.

Hawk et al., (1961) observed the influence of ovarian hormones on the water and electrolyte content of the uteri of animals. The effects were found to be more pronounced in follicular phase of the estrous cycle. The cow exhibited water uptake, shift in water from intra to extracellular phase, increase in sodium and decrease in potassium contents of the endometrium. In myometrium, progesterone influenced water content distribution and ion concentration. Estrogen, however, affected only water content. Exogenous betaestradiol given to ovariectomised cows and rabbits did not affect water distribution and ion concentration, however, progesterone did influence

these conditions. The influence of ovarian hormones on water and electrolyte concentration in the uteri of different species of animals was found to be variable. Cow and rat endometrium showed less variability than monkey or rabbit, however it was reverse for myometrium.

Heap and Lamming (1960) pointed out differences in the concentration of chemical constituents present in the uteri of various species of animals at different phases of the estrous cycle. An increase in sodium, potassium, chloride, nitrogen and carbohydrates was observed during estrous in rats while rabbits and sheep exhibited increase in K, phosphorous, N and CHO in luteal phase. The increase in the concentration of these electrolytes was also observed under the exogenous influence of betaestradiol in rats and progesterone in rabbits and sheep. In the cow Na, K, N, CHO increased during the luteal phase. A material detected and extracted with trichloracetic acid "Acid Soluble Fraction" was present under the influence of progesterone in rabbits and occurred during the latter half of the estrous cycle in cows.

MATERIALS AND METHODS

An instrument was developed to obtain uncontaminated intrauterine samples from dairy cows during various stages of the estrous cycle. Photographs of the unassembled and assembled instruments are shown in Plates I and II. All the stainless steel tubings used were of 18-8 type 304.* Tube C was slightly filed uniformily throughout its surface before

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*Metal Goods Corporation, St. Louis, 14, Missouri.

EXPLANATION OF PLATE I
(Unassembled Instrument)

A = The speculum consisted of aluminum tube 39 cm long and 1.27 cm outer dia. Its front end was spun into a taper to reduce the dia to 6.5 mm.

B = The outer stainless steel tube which was 48 cm long and 6.25 mm outer dia. The inner dia and wall thickness measured 4.5 mm and .875 mm respectively.

C = The inner stainless steel tube 55 cm long and outer dia 4.7 mm. Measurement for the inner dia and the wall thickness was 3 mm and .875 mm respectively.

D = The rod (stainless steel) was 71 cm long. This carried a cotton swab at one end and had a handle at the other end.

E = The collet made of stainless steel served as a locking device for the set of telescoping tubings and was provided at the proximal end of the speculum.

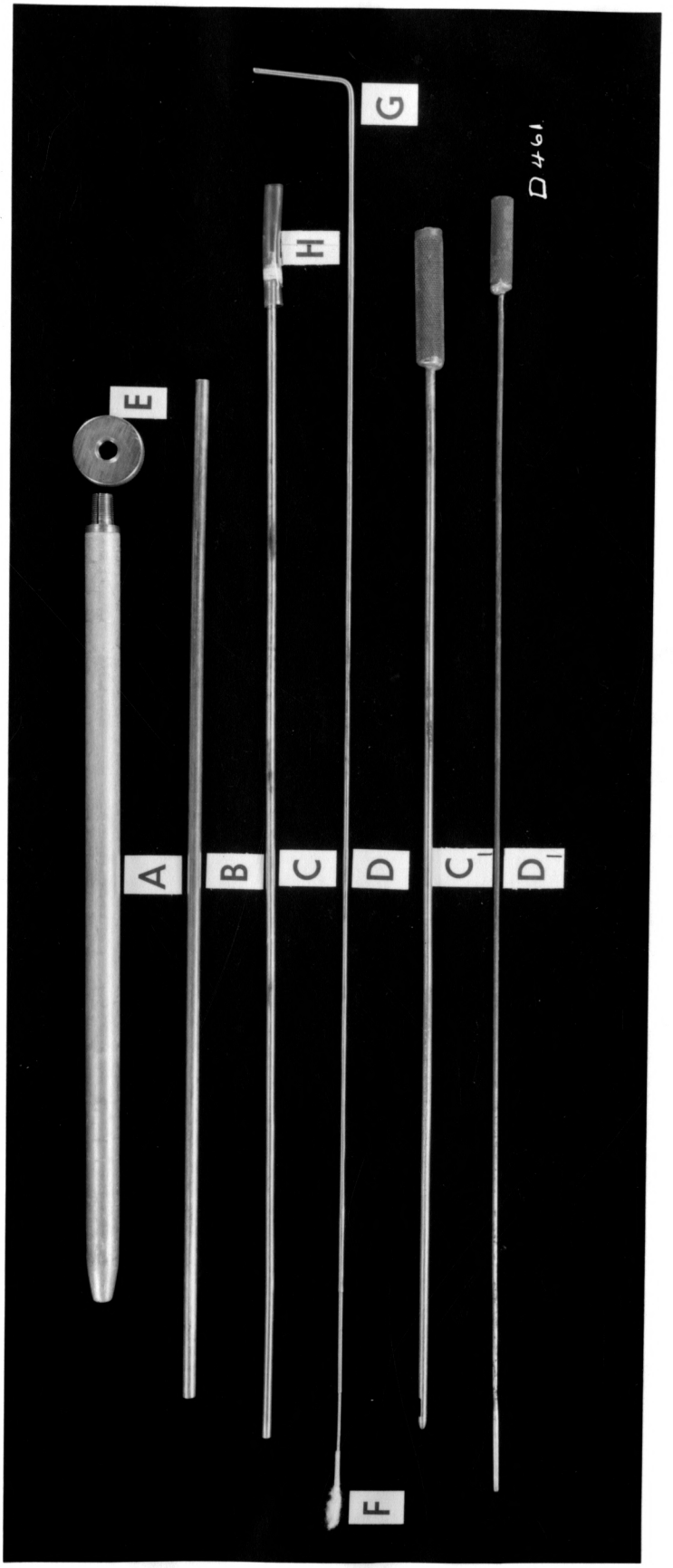
F = The cotton swab was secured on rod D with the help of adhesive tape.

G = The handle of rod D was made to facilitate the manipulation of the swab.

H = The tygon tubing ten cm long was tied on to the proximal end of tube C.

C_1 and D_1 = Tubes of the biopsy instrument corresponding to tube C and rod D.

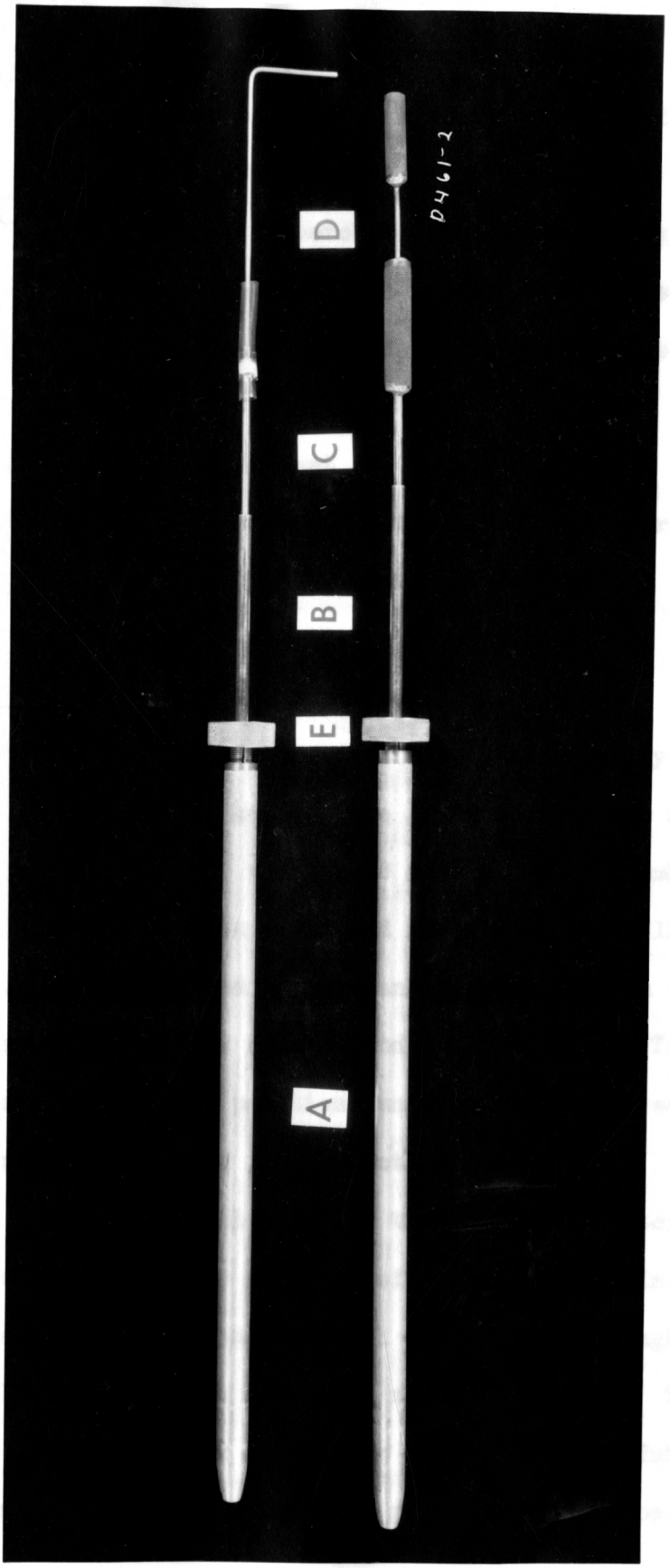
PLATE I



EXPLANATION OF PLATE II
(Assembled Instrument)

The explanation is the same as given for unassembled one except that the tubes B and C in both the instruments have been designated by the same letters.

PLATE II



it could be inserted into the outer tube B. Gelatin caps which were the longer part of gelatin capsules aseptically separated from the other part, were subjected to ultraviolet rays for two hr. They were then tested for sterility in aerobic and anaerobic media before being fitted to the stainless-steel tubes of the sterilized instrument. Sealed boxes of gelatin capsules No. 1 and 5 were employed to prepare caps to fit tubes B and C respectively.

To prepare the instrument for taking samples, a cotton swab was fixed with the help of adhesive tape to the distal end of rod D which was then inserted into the tube C so as to rest 2 cm from the distal end of the tube. A piece of tygon tubing was tied at the proximal end so as to avoid contamination of the end during manipulation.

Tube C was then inserted into tube B and finally the set of two telescoping tubes and the rod were inserted into the speculum. The collet E was tightened to grip the tube B firmly. The assembled instrument (Plate II) was wrapped in paper and autoclaved at 15 lb pressure for 15 minutes. It was allowed to cool and sterile gelatin caps were fitted to the anterior ends of tubes C and B aseptically. The tubes were then drawn back to original positions and the instrument was wrapped again in the paper until used for collecting sample.

At the time of collection of the sample the external genitalia were disinfected with 1/1000 solution of Roccal and the instrument was inserted into the vagina until the proximal end of A was brought in contact with the external opening of the cervix by rectal palpation. Tube B was advanced forward into the cervix by working on the collet. Tube C and rod D were pushed ahead gently in succession guided by rectal palpation and a

representative sample from the horns and body of the uterus was obtained. The swab was first withdrawn into tube C and then tubes C and B were withdrawn in succession before the instrument was finally taken out.

A modification of the instrument in which rod D was replaced by a stainless steel tube was used for the intrauterine inoculation of various cultures of microorganisms. The exact amount of culture could be inoculated with the help of a syringe attached to the innermost tube.

Animals Employed, Hormonal Treatments and the Bacterial Cultures Used

Five dairy cows with known reproductive histories were employed in this study. These cows were ovariectomised five days after calving to eliminate endogenous hormonal effects and rested for 15-21 days before the experiment was started. In addition ten cows that had been employed to study the effects of progesterone on postpartum uterine regression, were sampled for bacterial infection and endometritis during the course of this treatment. Three of the five cows were injected with physiological levels of betaestradiol and progesterone and two cows served as controls. Intrauterine samples were obtained from these cows and processed for the isolation and typing of the bacteria.

The levels of exogenous ovarian hormones as presented in Table 1 were selected so as to represent the amount of hormones considered to be present during various phases of the estrous cycle (Norwood, 1963).

Three cows were injected im with the above mentioned levels of hormones. Intrauterine samples obtained during different phases of the cycle (estrous, metestrous, diestrous and proestrous) were examined for any bacterial infection that may have occurred after inoculation of exogenous hormones.

Table 1. Levels of hormones administered.

Day of Inoculation	Dose of Hormones (im)		Remarks
	Betaestradiol	Progesterone	
	mg	mg	
1	.06		Estrous
2	.06		Estrous
3	.06		Estrous
4	--	5	Metestrous
5		5	Metestrous
6	.01	5	Metestrous
7	.01	10	Metestrous
8	.01	15	Diestrous
9	.01	20	Diestrous
10-11	.01	25	Diestrous
12	.01	30	Diestrous
13-16	.01	35	Diestrous
17-18	.02	25	Diestrous
19	.02	20	Diestrous
20	.02	18	Diestrous
21-23	.06	18	Proestrous

After approximately two months of the previous treatment, the uteri of three cows under the influence of ovarian hormones were injected with the organisms previously recovered from the uteri of repeat breeder cows by Mr. Joseph Poerio, a graduate student in bacteriology. These organisms, i.e., Streptoccoccus faecalis, C. bovis and S. epidermidis were supplied by him for this work.

Experiments in Ovariectomised Cows Under the Influence of Exogenous Betaestradiol Only

The supply of betaestradiol (in sesame oil) was obtained from Mr. James S. Norwood, who was then a graduate student in the Department of Dairy Science.

Inoculation of Betaestradiol. Three cows were inoculated im each with 0.06 mg of betaestradiol on 3 successive days. On the 4th day bacteria were injected into the uterus. This was followed by injections of .01 mg of betaestradiol on alternate days until the intrauterine samples became negative. One cow which was not injected with the hormone acted as a control for the experiment (Tables 2 and 3).

Table 2. Betaestradiol and bacterial inoculations I.

No. of cows	Treatment period (days)	Dose of beta-estradiol	Type of organism deposited	Dose of viable organisms
		mg		
3	1-3 Alternate days	.06 .01	C. bovis	750 millions/2ml
3	1-3 Alternate days	.06 .01	S. epidermidis	700 millions/2ml
3	1-3 Alternate days	.06 .01	S. faecalis	600 millions/2ml

Preparation and Inoculation of Cultures. S. faecalis and C. bovis were grown on blood agar medium while S. epidermidis furnished good growth on tryptose agar. Twenty-four hour slant cultures were washed with normal saline solution in separate tubes and the opacity of each suspension was adjusted between nephlometer tube 2 and 3.

Approximately two ml of each suspension was kept in the refrigerator and the remainder was packed in ice to be taken to the barn, for intrauterine inoculations. Two ml of the suspension was injected into each cow. The number of viable bacteria injected was assessed by the plate count of

Table 3. Betaestradiol and bacterial inoculations II.

Cow no.	Treatment period (days)	Dose of beta-estradiol	Types of organisms deposited	Dose of viable organisms
		mg		
136C	1-3	.06	A mixture of S. epidermidis S. faecalis and C. bovis	Between Neptometer tube 1-2 containing each of 300-400 million organisms/ml
	5,7,9,11	.01	" "	" "
225C	1-3	.06	" "	" "
	5	.01	" "	" "
208C	1-3	.06	" "	" "
	5,7	.01	" "	" "
216C	Nil	Nil		

the sample kept in the laboratory, within one hour after inoculation. Serial 10 fold dilutions of each of the bacterial suspension were made in normal saline. Previously incubated sterile blood agar plates were inoculated with 0.2 ml of the suspension each from 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilutions. Three plates were used for each dilution and the suspension was spread with the help of a sterile bent glass rod on the surface of the plates. The colonies were counted after 48-72 hr incubation at 37°C. The uterus of each of the three cows was injected four times using different cultures for three consecutive inoculations while a mixture of the three cultures was injected in the final inoculations carried out. A rest period of at least 21 days was allowed between each of these inoculations after the uterus was found clear of the infection introduced.

The hormone injection schedules and the type and dose of bacteria inoculated are presented in Tables 2, 3, and 4.

Table 4. Progesterone and bacterial inoculations.

Cow no.	No. of days	Dose of progesterone each day	Types of organisms injected	Dose of viable organisms
		mg		
136C	1-20	30	S. faecalis S. epidermidis and C. bovis	Each, of 300-400 million organisms/ml
225C	1-20	30	" "	" "
208C	1-20	30	" "	" "
216C	Nil	Nil	" "	" "

A dose of 30 mg of progesterone was injected im daily for 20 days. On the 5th day, a mixture of the 3 cultures was injected. Samples for bacteriological examination were taken up to 40 days. Table 4 shows the levels of progesterone employed in the cows and the microorganisms introduced.

Ten normal postpartum cows which were treated with 30 mg of progesterone each daily during the first 30 days of the postpartum period were examined for the persistence of postpartum bacterial infection and the development of endometritis and pyometra during the treatment period. Uterine samples were obtained at regular intervals during the postpartum period.

Processing of Samples, Media Employed and Incubation Method

All the samples were collected in the dairy barn attached to the University and situated at a distance of about 400 meters from the main campus. As soon as the sample from the reproductive tract of the cow was collected, tube 'C' containing the swab, was removed from the apparatus. The piece of tygon tubing was removed from the end of tube 'C' and the swab was drawn out of the tube. The swab was immediately placed in a tube containing 1 ml of

0.1% glucose phosphate broth. It was rinsed, removed from the broth and smeared on a blood agar plate before being discarded. A control blood agar plate was inoculated with sterile medium. The tubes representing the different samples were immediately transported to the laboratory, appropriate media inoculated and incubated under suitable condition for a proper length of time as indicated in Table 5. The time between sampling and culturing in the laboratory ranged between 1 to 1 1/2 hr. One blood agar plate from each of the samples was inoculated within 15 minutes after taking the sample.

Anaerobic conditions were maintained by incubating the inoculated media in an anaerobic jar and for incubation under increased CO_2 tension, a candle jar (sealed pressure cooker) was employed.

To check sterility all media employed were incubated 48 hours before use. The blood agar contained 5% sterile defibrinated ovine blood. The media employed are listed in Difco Manual 9th Edition, (1953) and Determinative Bacteriology Laboratory Manual (Lord, 1959).

The plates and other inoculated culture media were examined every two days for growth of the organisms. Growth appearing in liquid media was streaked on blood agar and Tryptose agar plates and incubated aerobically and anaerobically. Different colonies appearing on the plates were isolated. The cultures which were found to be aerobic spore formers on examination of stained smears, were discarded.

Table 5. Cultural methods.

Serial no.	Media inoculated	Time of incubation (days)	Temperature of incubation °C	Purpose of inoculation	Method of incubation
1	Blood agar plates				
	(a) 2	4	37	For organisms like Streptococcus, Corynbacterium, and Staphylococcus	Incubated aerobically
	(b) 1	5	37	Streptococcus, Brucella, Vibrio	Incubated under 10% CO_2 tension
	(c) 1	5	37	Anaerobic species	Incubated in anaerobic jar
	(d) 2	5	37	For isolation from liquid media	Aerobic and anaerobic 1 each
2	Nutrient Broth (2 tubes)	4	37	Some aerobic species	Aerobically
3	Thioglycolate Broth (2 tubes)	12	37	Anaerobic organisms	Aerobically
4	Tryptose Agar 1/700,000 crystal violet (1 plate)	21	37	Brucella	Under 10% CO_2 tension
5	Thiol Broth Medium	5	37	For Vibrio	2 tubes aerobically and 2 tubes under 10% CO_2 tension
6	Tryptose Agar (2 plate)	5	37	For isolation from liquid media	Aerobic and anaerobic one each

Identification of Bacteria

The cultures isolated from four cows after intrauterine inoculation were identified. Optimum growth temperature, morphology and gram reaction served as bases for further classification.

Separation into Micrococcaceae and streptococci was established by morphology and catalase characteristics. Anaerobic fermentation of glucose was used as the test to distinguish staphylococci from micrococci.

After the completion of initial tests, each culture isolated was subjected to biochemical tests. These tests along with the chart showing the reactions of the organisms isolated are given in the Appendix (page 51-53).

EXPERIMENTAL RESULTS

A total of 127 samples from the reproductive tract of seven nonpregnant postpartum cows was examined bacteriologically. The results presented in Table 6 indicate the efficacy of the instrument for collecting uncontaminated samples from various parts of the genital tract of cows.

Data indicating influence of ovarian hormones on the susceptibility of uterine lumina of ovariectomised cows to bacterial infection under natural conditions are presented in Table 7. The uterine lumina under the influence of physiological levels of exogenous hormones were found to be free of the bacteria. These animals under the influence of either betaestradiol or progesterone exhibited individual variations of uterine endometrial response to the induced bacterial infections. S. faecalis was observed to predominate and survive for a comparatively longer period in the uterus than S. epidermidis

Table 6. Bacteriological examination of samples to observe the efficacy of the instrument.

Serial no.	Sample days postpartum	Nature of sample: Intra-uterine	Intra-cervical	Intra-vaginal	Remarks
1	10 and 15	+++			Intact
	20	+++			
	35 and 50	-		+++	
2	5 and 10	+++		+++	Intact
	15	+++		+++	
	23 and 40	-			
3	5 and 10	+++		+++	Intact
	15	-		+++	
	23 and 35	-			
4	13	++		+++	Ovariectomised
	15, 17, 27	-	-	+++	
	40, 43, 50,	-		+++	
	57, 60	-		+++	
5	60 and 64	-	-	+++	Ovariectomised
	74, 77, 81, 85	-		+++	
	88, 94, 96	-		+++	
	100, 104	-		+++	
6	70,74,78,80	-	-	+++	Ovariectomised
	84, 93	-	-	+++	
	102,105,108	-		+++	
	111	-		+++	
7	56, 60, 66	-	-	+++	Ovariectomised
	68,69,70,77,	-		+++	
	79,85,86,88	-		+++	
	96	-		+++	

- = No growth on plate (no infection)

++ = 10-100 colonies on plate (moderate infection)

+++ = More than 100 colonies on plate (heavy infection)

Table 7. Influence of betaestradiol and progesterone on uterine endometrium.

Representative phase	No. of sample	RESULT OF CULTURAL OBSERVATIONS: Intrauterine samples: Cow no. 136C	Cow no. 225C	Cow no. 208C	Cow no. 252B (Control)	Intravaginal samples *: Cow no. 136C	Cow no. 225C	Cow no. 208C	Cow no. 252B (Control)
Before inoculation	1	-	-	-	-	+++	++	+++	+++
	2	-	-	-	-	+++	+++	++	++
	3	-	-	-	-	+++	++	+++	+++
Heat period	1	-	-	-		+++	++	+++	
	2	-	-	-	-	++	++	+++	
Metestrous	1	-	-	-	-	++	-	++	++
Diestrous	1	-	-	-		-	++	+	
	2	-	-	-	-	+++	+++	+++	++
	3	-	-	-		+++	-	-	
	4	-	-	-	-	+++		+++	+++
Proestrous	1	-	-	-		+++	+++	+++	
	2		-		-		+++		++
Post-hormonal	1	-	-	-	-	+++	++	-	+++
	2	-	-	-	-	+++	-	-	
	3	-	-	-		+++	-	+++	
	4		-				+++		
	5		-				-		

- = Samples negative for bacteria (no infection)
+ = Samples showing 5-10 colonies on agar plate (slight infection)
++ = Samples showing 10-100 colonies on agar plate (moderate infection)
+++ = Samples showing more than 100 colonies on agar plate (heavy infection)

*While intrauterine samples were negative, most of the intravaginal samples taken at the same time were found to be positive.

or C. bovis. It was interesting to observe that C. bovis disappeared from the uterine lumina within 24 hr after injection of the bacteria. The data presented in Tables 8-12 indicate that the bacteria of one type or combination of three types could persist in the uterine lumina for a period of nine days maximum. Reinfection of the uterus in animals was noticed when inoculated the first time with C. bovis and S. epidermidis whereas no such recurrence was observed when a combination of three types of bacteria was introduced. This may be attributed to the possible resistance of the uterine lumina developed during its early association with the three types of bacteria injected separately at three different times. It was also seen that S. epidermidis could not persist in the uterine lumina in the presence of C. bovis, the latter being present as a result of reinfection at the time of S. epidermidis inoculation. No appreciable difference in the endometrial response to bacterial infection under the influence of betaestradiol and progesterone was evident. The control also did not respond differently than the experimental cows to the bacterial infection. However it was observed that the uterine susceptibility to induced bacterial infection following first injection of microorganisms in these animals, was comparatively higher than following subsequent inoculations.

The data presented in Table 13 list the number and types of bacteria recovered following inoculation with different bacteria. It was observed that the organisms recovered had the same morphological, cultural and biochemical characteristics as observed prior to their intrauterine inoculations.

A total of 70 intrauterine samples from ten postpartum intact normal cows under the influence of exogenous progesterone were collected. These samples were examined for the presence of bacteria. The cows were examined

Table 8. Influence of betaestradiol on the susceptibility of uterine endometrium to C. bovis inoculation.

Sample no.: Days post-inoculation	Cow no. 136C	Cow no. 225C	Cow no. 208C
0 th day	-	-	-
6 hr	+		
1 day	-	-	-
2 "	-	-	-
3 "	-	-	-
4 "	-	-	-
5 "	-	-	-
8 "	-	-	-
12 "		-	-
16 "		-	-
20 "	-	-	-
24 "		-	-
28 "		-	-
32 "		-	-
36 "		-	-
40 "		-	-

C. bovis was eliminated within 24-36 hr from the uteri of all the cows.

- = Samples negative for bacteria (no infection)

+ = Samples showing 5-10 colonies on agar plate (slight infection)

Table 9. Influence of betaestradiol on the susceptibility of uterine endometrium to S. epidermidis inoculation.

Sample no.: Days post-inoculation	Cow no. 136C	Cow no. 225C	Cow no. 208C
0 th day	+++	-	-
1 day	+++	+++	+++
2 "	++	++	-
3 "	-	-	-
4 "	-	-	-
5 "	-	-	++
6 "	-	-	-
12 "	-	-	-
16 "	-	-	-
20 "	-	-	-
24 "	-	++	-
28 "	-	-	-
32 "	-	-	-
36 "	-		-
40 "	-		-

C. bovis was observed on the 0th day and S. epidermidis was not isolated at 24 hr and later after inoculation in Cow no. 136C. Reinfection of S. epidermidis was noticed in the other two cows.

- = Samples negative for bacteria (no infection)
+ = Samples showing 5-10 colonies on agar plate (slight infection)
++ = Samples showing 10-100 colonies on agar plate (moderate infection)
+++ = Samples showing more than 100 colonies on agar plate (heavy infection)

Table 10. Influence of betaestradiol on the susceptibility of uterine endometrium to S. faecalis inoculation.

Sample no.: Days post-inoculation	Cow no. 136C	Cow no. 225C	Cow no. 208C
0 th day	-	-	-
1 day	+++	+++	+++
2 "	+++	+	+++
3 "	+++	-	+++
4 "	+++	-	++
5 "	++	-	+
8 "	+	-	-
12 "	-	-	-
16 "	-	-	-
20 "	-	-	
24 "	-	-	
28 "	-	-	
32 "	-	-	
36 "	-	-	
40 "		-	

- = Samples negative for bacteria (no infection)
+ = Samples showing 5-10 colonies on agar plate (slight infection)
++ = Samples showing 10-100 colonies on agar plate (moderate infection)
+++ = Samples showing more than 100 colonies on agar plate (heavy infection)

Table 11. Influence of betaestradiol on the susceptibility of uterine endometrium to inoculation with a mixture of S. faecalis, S. epidermidis, and C. bovis.

Sample no.: Days post-inoculation	Cow no. 136C	Cow no. 225C	Cow no. 208C	Cow no. 216C (Control)
0 th day	-	-	-	-
1 day	+++	+	+	+++
2 "	+++	-	+++	
3 "	+++	-	++	++
4 "	+++	-	-	+
6 "	+++	-	-	+
9 "	++	-	-	-
14 "	-	-	-	-
18 "	-	-	-	-
28 "	-	-	-	-
36 "	-	-	-	-

Streptococcus was predominant. Cow no. 136C showed a few colonies of S. epidermidis along with a large number of Streptococci colonies. Staphylococci, however, disappeared within 48 hr.

- = Samples negative for bacteria (no infection)
+ = Samples showing 5-10 colonies on agar plate (slight infection)
++ = Samples showing 10-100 colonies on agar plate (moderate infection)
+++ = Samples showing more than 100 colonies on agar plate (heavy infectio

Table 12. Influence of progesterone on the susceptibility of uterine endometrium to a mixture of S. faecalis, S. epidermidis, and C. bovis inoculation.

Sample no.: Days post-inoculation	Cow no. 136C	Cow no. 225C	Cow no. 208C	Cow no. 216C
0 th day	-	-	-	-
1 day	+++	+++	+++	+++
2 "	+++	++	++	+++
3 "	++	-	-	+
4 "	-		-	-
5 "	-	-	-	-
8 "	-	-	-	-
12 "	-	-	-	-
16 "	-			
20, 24, 28, 32, 36 and 40 day	-	-	-	-

- = Samples negative for bacteria (no infection)
+ = Samples showing 5-10 colonies on agar plate (slight infection)
++ = Samples showing 10-100 colonies on agar plate (moderate infection)
+++ = Samples showing more than 100 colonies on agar plate (heavy infection)

Table 13. Typing of the organisms.

Cow no.	Table no.	Organisms injected	Day of sample collection	Culture number allotted	Organism typed
136C	8	C. bovis	6 hr	1	C. bovis
"	9	" "	0 th day	2	" "
"	9	" "	1	3	" "
"	9	" "	2	4	" "
208C	9	S. epidermidis	5	5	S. epidermidis
225C	9	S. epidermidis	24	6	S. epidermidis
"	10	S. faecalis	1	7	S. faecalis
"	"	" "	2	8	" "
136C	10	S. faecalis	1	9	S. faecalis
"	"	" "	2	10	" "
"	"	" "	3	11	" "
"	"	" "	4	12	" "
"	"	" "	5	13	" "
208C	10	S. faecalis	1	14	S. faecalis
"	"	" "	2	15	" "
"	"	" "	3	16	" "
"	"	" "	4	17	" "
"	"	" "	5	18	" "
"	11	S. faecalis, C. bovis, S. epidermidis	1	19	" "
"	"	" "	2	20	" "
"	"	" "	3	21	" "
136C	11	" "	1	22	S. faecalis
"	"	" "	1	23	S. epidermidis
"	"	" "	2	24	S. faecalis
"	"	" "	3	25	" "
"	"	" "	4	26	" "
"	"	" "	6	27	" "
225C	11	" "	1	28	S. faecalis
216C (control)	"	" "	1	29	S. faecalis
"	"	" "	3	30	" "
"	"	" "	4	31	" "
136C	12	" "	1	32-35	S. faecalis
225C	"	" "	1	36-39	S. epidermidis
208C	"	" "	1		
216C	"	" "	1		
136	"	" "	3	40	S. faecalis
216	"	" "	3	41	" "

Cultures No. 2, 3 and 4 indicate reinfection of Cow no. 136C inoculated with C. bovis (Table 8).

at the time of sampling for any abnormality of the uterus developing or pus formation in the uterine lumina, during the course of progesterone treatment. Data in Table 14 indicate the persistence of postpartum infection under the influence of ovarian hormone in three of ten animals and subsequent development of endometritis and pyometra. It was not possible to obtain samples from these three cows for a longer period as the uteri became hard and swollen. There was no appreciable difference observed in the period of persistance of postpartum bacterial infection in normal intact cows under the influence of exogenous progesterone and in normal untreated intact animals. (Studies of normal postpartum untreated cows were made by Abo-Ahmed, 1962). The cows that did not pick up infection at the time of parturition showed negative uterine samples later on. Thus a correlation existed between the presence of infection in the uterus of intact postpartum cow and the development of endometritis, under the influence of exogenous progesterone.

It was observed that the bacteria isolated from the uterine lumen of ovariectomised postpartum cows were the same types as were used for inoculation.

DISCUSSION

An instrument was designed to obtain bacteriological samples from the bovine uterus. This instrument enabled the investigator to obtain samples from the desired portion of the reproductive tract with a minimum chance of contamination from other parts of the tract. The instrument consists of a speculum fitted with a collet at the proximal end. A set of two telescoping tubings and a rod carrying the swab in the innermost tube, can

Table 14. Influence of exogenous progesterone on the susceptibility of the uterine endometrium of normal intact postpartum cows to natural bacterial infection.

Serial no.	Cow no.	Sample days postpartum	Bacteriological examination	Remarks
1	179C	5 days	++	Endometritis and
		14 "	+++	Pyometra
		16 "	+++	
		18 "	+++	
		21 "	+++	
2	483B	17,24,30,35,36	+++	
		27,38,42	++	
		51 and 56	-	
3	73B	5,10,15,19	+++	Endometritis and
		24,30,31	+++	Pyometra
4	489B	9,14,24,26	+++	
		28,31,34	+	
		45	-	
5	213C	7, 9	+	
		14,18,20	++	
		22,23,26,40	-	
6	169B	16,20,25	+++	
		27,29,36,42,52,60	-	
7	376C	6,9,11,15,22,27	+++	Endometritis and Pyometra
8	364C	11,21,24,29,35	-	
9	377C	6	+	
		9,15,22,25	-	
10	190C	5,8,12,17,22,26	-	

pass through the collet and be secured in position by this locking device. The tygon tubing is tied to the proximal end of innermost tube. Both the telescoping tubes carry sterilized gelatin caps. Hatch et al., (1949), Easley et al., (1951), Lindley and Hatfield (1952) obtained samples from the reproductive tract of cows employing a speculum and a swab or a pipette and found bacteria in most of the nongravid uteri of the living animals. The assumption that swab may have been contaminated by coming in contact with other parts of the reproductive tract cannot be excluded.

Kampelmacher (1952) employed an instrument which was used for taking a biopsy as well as mucous samples from the uterus for microbiological and histological studies, and reported success in collecting intrauterine samples aseptically. This instrument is primarily a biopsy instrument that also collects mucous for bacteriological examination. The speculum, the tightening mechanism and the gelatin cap are lacking. Frank and Bryner (1952) designed an instrument consisting of 2 telescoping tubings with a thin wire plunger carrying the swab or an arrangement for sucking the mucous. The outermost tube has a piece of thin rubber over the mouth to avoid contamination during passage of the instrument through the vagina. The instrument does not have a speculum which will protect it from becoming contaminated while it is being passed through the vaginal lumen. The use of a piece of rubber at the mouth of the outer tube poses problems of being too stiff to be pierced through by the innter tube creating chance of injuring the uterine wall if more force is applied. The rubber end might become detached and be retained in the uterus. The use of 2% sterile agar plug used by Gunter et al., (1955), omitting the rubber diaphragm may increase the chances of contaminating the sample. This instrument also has

no collet which will hold the set of telescoping tubes in position. Furthermore the use of a thin flexible wire in the instrument may present difficulty in taking a representative sample from the horns of the uterus. It was experienced that a rod could be guided more easily into the horns of the uterus by rectal palpation and thus facilitated taking a representative sample.

Variation in uterine response to bacterial infection which might have been due to ovarian activity in animals has been reported by a number of workers, Broome et al., (1960), Rowson et al., (1953), Black et al., (1953, 1954), Hawk et al., (1960) and Winter et al., (1960). These investigators agreed that the susceptibility of the uterine lumina of animals to bacterial infection during the luteal phase of the estrous cycles is comparatively greater than during the follicular phase. The present studies indicate that no natural uterine infection took place in ovariectomised cows which were receiving a combination of exogenous ovarian hormones (Table 7), however when the bacteria were deposited in the uterus of the cow under the influence of exogenous betaestradiol or progesterone the infection did occur but remained for only short periods. It is assumed that rapid destruction of the deposited bacteria in the uterus may be due to the influx of leucocytes in the lumen of the uterus. Studies done in rabbits by Hawk (1958) and Hawk et al., (1960a,b,c) showed that leucocytes were responsible for the bactericidal activity of the uterine endometrium.

Black et al., (1953 and 1954) reported that the bovine endometrium of the repeat breeding cow was comparatively inhibitory to an induced bacterial infection. Three of the cows included in this study had been bred at least four times prior to ovariectomy and the remaining cow had received two services during the preoperative period. It was interesting

to note that the cow which had been bred only twice prior to ovariectomy proved to be the most susceptible to bacterial infection. These results support the observations of Black et al., (1953, 1954) who used first service heifers and repeat breeder cows in the luteal phase of the estrous cycle. Two of the six repeat breeding cows exhibited little response to the infected semen injected into the uterus. They also found from experiments with repeat breeder cows and virgin heifers that none of the four repeat breeding cows exhibited infection following insemination with infected semen, however three virgin heifers developed endometritis and pyometra.

The present investigations involving four ovariectomised cows indicated that repeat breeding cows could tolerate a heavy dose of bacterial infection. It was observed that reinfection in three cows occurred after treatment with betaestradiol was discontinued. It is logical to assume that bacteria were eliminated from the uterine lumina after short periods following their inoculation, but they managed to survive in the submucous tissue and reappeared free in the uterus under suitable environmental conditions at a later stage. When the same animals under the influence of progesterone were injected with bacteria, reinfection was not observed. Broome et al., (1959c) indicated significant increase in antibacterial activity in the uterine lumina against E. coli following administration of these organisms in rabbits. As the present experiments were conducted in ovariectomised cows employing the same animals for each of the experiment (at least 21 days rest period was allowed between two successive experiments), a certain degree of resistance was observed in the uteri against the organisms. It was noticed that bacteria persisted for comparatively shorter period in the uterine lumina under the influence of progesterone which may also be attributed to the same

reason (Table 12).

Three of the ten intact postpartum cows that were under the influence of exogenous progesterone developed endometritis and pyometra (Table 14). This type of variability of infection in repeat breeding animals in the luteal phase was also observed by Black et al., (1953) who found pus formation in four out of the six cows injected with the contaminated semen. The results were based on the formation of pus in the uteri of these animals. Similarly in another experiment already stated, he observed that none of the four repeat breeding cows in the luteal phase showed pus formation following intrauterine injection of a similar semen sample.

Elliot (1961) and Abo-Ahmed (1962) found that the uteri of the intact normal postpartum cows were bacteria free within 60 and 50 days postpartum, respectively. The present investigation conducted in intact cows under the influence of exogenous progesterone indicates that the bacteria in postpartum normal uteri may persist up to 42 days after parturition. It can be concluded that there is no appreciable difference in the time during which the microorganisms persist in the uterus of postpartum intact cows under the influence of progesterone or untreated normal postpartum cows. It was also evident that the uteri of three postpartum cows which did not get infected at the time of parturition subsequently remained free of bacteria (Table 14). This fact may be correlated with the persistence of a bacterial free condition of the uteri of three ovariectomised cows under the influence of ovarian hormones (Table 7).

The problem of ovarian hormones in relation to uterine bacterial infections could further be investigated through well controlled experiments, employing animals of various categories with known reproductive histories.

Comparative studies in non repeat and repeat breeding ovariectomised and intact heifers and cows may suggest a possible answer to this problem.

SUMMARY

An instrument to obtain uncontaminated bacteriological samples from the reproductive tract of cows was designed. A total of 127 samples from seven cows was taken to establish its efficacy. It was found that the instrument was effective in obtaining representative aseptic samples from the various parts of the reproductive tract of cows.

Five ovariectomised repeat breeding postpartum cows were employed to determine the influence of exogenous betaestradiol or/and progesterone on the susceptibility of the uterine lumina of cows, to natural or introduce bacteria. The uterine lumina of the ovariectomised cows under the influence of physiological levels of exogenous ovarian hormones were found to be free of bacteria under natural conditions. S. faecalis was observed to survive for a longer period in the uterus than S. epidermidis or C. bovis. Under these conditions, the uterine susceptibility following first injection of microorganisms in these animals was comparatively higher than following subsequent inoculations. Three of the ten intact postpartum cows under the influence of exogenous progesterone developed endometritis and pyometra. No appreciable difference was observed in the length of time the bacteria survived in these animals and normal untreated intact postpartum cows. A correlation was found to exist between the presence of bacteria in the uterus after parturition and the development of endometritis and pyometra in normal postpartum cows under progesterone treatment. It was also observe

that the bacteria isolated from the uteri of cows following bacterial inoculation were the same which were introduced.

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APPENDIX

COMMON BIOCHEMICAL TESTS EMPLOYED

Anaerobic fermentation of glucose and mannitol.

Tubes of mannitol Bromo-cresol-purple agar and glucose agar were inoculated by stabbing. The tubes were observed for growth of the culture and aerobic or anaerobic fermentation of the sugars which was indicated by yellow color.

Sodium hypurate hydrolysis test.

To 0.8 ml of culture in 1% sodium hypurate broth, 0.2 ml of ferric chloride reagent was added. A positive test was indicated by a permanent precipitate.

Starch hydrolysis test.

Cultures grown on starch agar plates were flooded with grams iodine and observed for utilization of starch.

Oxygen relationship and optimum temperature determination.

Melted 0.1% glucose yeast extract agar tubes cooled to 45°C in a water bath were stabbed and solidified immediately in cold water. The tubes were incubated at 10°C, 28°C, 37°C and 45°C and observed for a week to determine the optimum temperature for growth, oxygen relationship and growth of the organisms.

Catalase test.

Forty-eight hr nutrient agar cultures were covered with 3% hydrogen peroxide. A positive test was indicated by the release of bubbles from the surface of the cultures.

Nitrate reduction test.

Five-tenth ml each of sulfanilic acid and dimethylalphanephthylamine reagents were added to 5 ml of culture in 0.1% potassium nitrate broth. Development of a red color indicated a positive test. If no color observed, addition of a small quantity of fine zinc dust would decide between a negative test and denitrification.

Gelatine hydrolysis test.

Growth of cultures on plates of modified gelatin agar (Lord, 1959) was flooded with Frazier's gelatin developer and observed for the hydrolysis of gelatin.

M.R.-V.P. test.

Six-tenth ml of a 5% alcoholic solution of alphanephthol was added to 1 ml of culture in glucose phosphate broth medium and mixed. A red color within 15 minutes on addition of 0.4 ml of 40% aquous potassium hydroxide indicated a positive Voges-Proskauer test.

Appearance of a red color on adding a few drops of methyl red indicator to the other portion of the culture was considered a positive methyl red test.

Urea hydrolysis test.

Tubes of urea broth were incubated 48 hours before use. A change of color of the medium from pink to deep red after inoculation of the cultures and incubation was considered a positive test.

Action on litmus milk.

Tubes containing litmus milk were inoculated with cultures and incubated 14 days. Changes in the medium were observed on 2nd, 4th, 7th, 10th, and 14th day.

Reduction of methylene blue.

One-tenth % methylene blue milk was observed for the reduction of the dye, after inoculation with the culture.

Test for motility.

Motility agar tubes were stabbed down to one-third of the column of the medium and observed for spreading growth at 4, 8, 24 and 48 hr intervals.

Sodium chloride tolerance test.

Various percentage of sodium chloride in agar slants and broth were used for this test and the inoculated media were observed for growth.

Potatoe slant test.

The slants were observed for growth and pigment production after inoculation.

Hemolysis of red blood cells.

Blood agar plates made by incorporating 5% sterile ovine blood in tryptose agar, were streaked with the cultures. Alpha, beta or gamma type of hemolysis was observed after 48 hr incubation.

Sugar fermentation test.

Five-tenth % of the desired sugar was incorporated in brom-thymol-blue fermentation broth base. The medium was tubed (each tube containing a Durham tube) and autoclaved at 120°C for 10 minutes. Observations were made for fermentation of sugar and gas production up to 14 days after inoculation of cultures.

Metachromatic granule stain.

Cultures were stained with Loefflers alkaline methylene blue and observed for the presence of metachromatic granules.

Indole test.

Several drops each of Ehrlich's reagent #1 and #2 were put to moisten the underneath surface of the swabs of the culture tubes containing tryptone broth medium. The swabs were pushed down until they were at a distance of about one inch from the surface of the medium. The tubes were then placed in the boiling water bath for 10 minutes. The appearance of a red color on the moist surface of the cotton swab indicated a positive test.

Bile solubility test.

To 0.4 ml of yeast extract broth culture, 0.1 ml of filtered sterile 10% sodium desoxycholate was added. Solubility of the culture in bile was indicated by clearing of the turbidity in the serological tube after incubation for one hour at 37°C. A control with sterile water was also set up.

All media employed and techniques followed are listed in Lord's Manual of Determinative Bacteriology (1959).

Typing of Streptococcus faecalis.

Culture number	Morphology	Oxygen relationship	Optimum temperature	Growth at various temperature: 10°C	28°C	45°C	Motility	Gelatin hydrolysis	Starch hydrolysis	Litmus milk	Catalase test	NO_3 reduction test	Bile solubility	1% Sodium hippurate broth	5% Sucrose broth	2% Sodium chloride broth	6.5% Sodium chloride broth	PH 9.6 Nutrient broth	0.1% Methylene blue milk	Glucose	Lactose	Maltose	Sucrose	Xylose	Inulin	Trehalose	Raffinose	Glycerol	Sorbitol	Hemolysis	H_2S production	Arabinose	Dulcitol	Cellubiose
Control	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
7	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
8	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
9	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
10	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
11	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
12	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
13	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
14	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
15	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
16	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
17	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
18	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
19	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
20	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
21	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
22	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
24	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
25	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
26	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
27	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
28	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
29	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
30	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
31	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
32	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
33	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
34	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
35	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
40	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+
41	Gram (+) cocci	Faculative	37°C	+	+	+	-	+	-	Acid	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	Alpha	-	+	-	+

Typing of Staphylococcus epidermidis.

Culture number	Morphology	Oxygen relationship	Glucose agar (B.C.P.) stab	Optimum temperature	Gelatin hydrolysis	Starch hydrolysis	Litmus milk	Catalase test	NO_3 reduction test	Motility	Potatoe agar slant	7% Sodium chloride	12% Sodium chloride	Urea glucose agar	Mannital agar (B.C.P.) stab	H_2S production	Xylose	Lactose	Sucrose	Maltose	Inulin	Glycerol	Arabinose	Raffinose	Sorbitol	Urea broth
Control	Gram (+) cocci	Facultative	+	37°C	+	-	No change	+	+	-	Whitish growth	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+
5	Gram (+) cocci	Facultative	+	37°C	+	-	No change	+	+	-	Whitish growth	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+
6	Gram (+) cocci	Facultative	+	37°C	+	-	No change	+	+	-	Whitish growth	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+
23	Gram (+) cocci	Facultative	+	37°C	+	-	No change	+	+	-	Whitish growth	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+
36	Gram (+) cocci	Facultative	+	37°C	+	-	No change	+	+	-	Whitish growth	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+
37	Gram (+) cocci	Facultative	+	37°C	+	-	No change	+	+	-	Whitish growth	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+
38	Gram (+) cocci	Facultative	+	37°C	+	-	No change	+	+	-	Whitish growth	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+
39	Gram (+) cocci	Facultative	+	37°C	+	-	N_o change	+	+	-	Whitish growth	+	+	-	-	-	-	+	+	+	-	+	+	-	-	+

Typing of Corynebacterium bovis.

Culture number	Morphology	Granule stain	Oxygen relationship	Optimum temperature	Gelatin hydrolysis	Starch hydrolysis	Litmus milk	Catalase test	NO_3 reduction test	H_2S production	V. P. test	M. R. test	Indol production	Glucose	Lactose	Sucrose	Maltose	Mannitol	Motility
Control	Gram (+) rods	Barred	Facultative	37°C	+	-	No change	+	-	-	-	-	-	-	-	-	-	-	-
1	Gram (+) rods	Barred	Facultative	37°C	+	-	No change	+	-	-	-	-	-	-	-	-	-	-	-
2	Gram (+) rods	Barred	Facultative	37°C	+	-	No change	+	-	-	-	-	-	-	-	-	-	-	-
3	Gram (+) rods	Barred	Facultative	37°C	+	-	No change	+	-	-	-	-	-	-	-	-	-	-	-
4	Gram (+) rods	Barred	Facultative	37°C	+	-	No change	+	-	-	-	-	-	-	-	-	-	-	-

THE EFFECT OF OVARIAN HORMONES UPON THE SUSCEPTIBILITY OF THE BOVINE UTERUS TO BACTERIAL INFECTION

by

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One of the causes of infertility in the dairy cow is attributed to early embryonic mortality. The possible role of microorganisms in this condition has been the focus of many investigations throughout the past years. Since the development of the embryo proceeds mainly under the influence of progesterone, the ovarian hormones are believed to alter the susceptibility of the bovine uterus to bacterial infection.

To carry out these studies in live animals, an instrument to collect uncontaminated samples from the reproductive tract of the cow was designed. The instrument consists of an aluminum speculum fitted with a collet at the proximal end. A set of two telescoping stainless-steel tubings and a rod carrying a swab in the innermost tube, passes through the collet into the speculum and is secured in position by tightening the collet. A piece of tygon tubing is tied to the proximal end of the innermost tube. Both the stainless steel tubings carry sterilized gelatin caps. A total of 127 samples from various parts of the reproductive tract of seven postpartum cows was collected. The instrument was found to be effective in obtaining representative aseptic samples from the various parts of the reproductive tract of cows. Slight modifications of the instrument enabled the investigator to inject bacteria directly into the uterus.

To study the influence of ovarian hormones on the susceptibility of uterine lumina to bacterial infection, five ovariectomized and 10 intact normal postpartum cows were employed. It was found that uterine lumina, under the influence of physiological levels of exogenous ovarian hormones in ovariectomized cows, were free of bacteria. In induced infections with Streptococcus faecalis, Staphylococcus epidermidis and Corynebacterium bovis in these animals, under the influence of either betaestradiol or progesterone,

individual variations were observed. Streptococcus faecalis was found to predominate and persisted for a comparatively longer period in the uterus than the other two organisms.

A total of 70 intrauterine samples from ten postpartum intact cows under the influence of exogenous progesterone was collected. Three of these ten animals developed endometritis and pyometra. The maximum period of postpartum bacterial infection in these cows was observed to be 42 days (excluding those which developed endometritis). Three cows which were found to be free of uterine bacterial infection immediately after parturition, remained negative. A correlation was found to exist between the presence of postpartum uterine bacterial infection and the development of endometritis and pyometra in cows, under progesterone treatment.