STATION TECHNICAL BULLETIN 24 JULY 1952

Microorganisms In Dairy Bull Semen As Related to Fertility

SZU-HSIAO WU
PAUL R. ELLIKER
FRED F. MCKENZIE

Agricultural Experiment Station
Oregon State College
Corvallis

ACKNOWLEDGMENTS: The authors thank Professor Ralph Bogart of the Department of Animal Husbandry, Professor W. B. Bollen of the Department of Bacteriology and Professor Hugo Krueger of the Departments of Zoology and Animal Husbandry for critically reading the manuscript. For the providing of semen samples and breeding records we are grateful to the Oregon Dairy Breeders' Association and particularly to their Chief Technician Ira F. Roberts, whose cooperation and suggestions were invaluable to this study.

Microorganisms in Dairy Bull Semen As Related to Fertility

By

SZU-HSIAO WU, PAUL R. ELLIKER AND FRED F. MCKENZIE
Departments of Animal Husbandry and Bacteriology

REDUCED FERTILITY is a complex phenomenon. It is not a disease in itself but a symptom, which may develop from a variety of conditions. It may be the result of structural defects of the reproductive systems, of nutritive conditions, of specific diseases, or, perhaps, of general constitutional factors, leading to an endocrine imbalance or lack of reproductive vigor. One of the important problems is the possible association of semen microflora with infertility. This study was undertaken with the hope of obtaining more information concerning the number and types of microorganisms in dairy bull semen and the possible relation of these microorganisms to infertility.

Review of Literature

Early in 1920 Williams and Kingsbury found *Brucella abortus,* micrococci, hemolytic and nonhemolytic streptococci and coliform organisms in the semen recovered from the vagina of cows immediately after natural service. The semen from normal bulls was relatively free of bacteria whereas that of impotent bulls usually harbored large numbers of bacteria. From the study of slaughter material, Gilman (1921) reported the presence of *Pseudomonas aeruginosa,* micrococci, streptococci, coliform organisms and unidentified rods in the genital tract of the bull. He was in agreement with Williams and Kingsbury (1920) that the semen from normal bulls was relatively free of bacteria whereas that of impotent bulls usually harbored large numbers of bacteria.

Webster (1932) noted only micrococci and diphtheroids in semen from normal bulls, while bulls from herds in which enzootic sterility was present, produced semen which contained, in addition, many alpha-hemolytic streptococci. In the semen collected aseptically by means of an artificial vagina, Hatziolis (1937) observed that almost every ejaculate yielded *Pseudomonas,* cocci, coliform organisms and spore forming rods.

Gunsalus, Salisbury, and Willett (1941), found *Pseudomonas, Escherichia coli,* streptococci, diphtheroids and bacilli. They noted that the nonhemolytic diphtheroids occurred regularly and in large

numbers, and in only one case were hemolytic diphtheroids found in bull semen. Data were also provided on the number of microorganisms in dairy bull semen. The logarithmic average of plate counts for 15 ejaculates from 12 bulls used for artificial breeding was 22,000 bacteria per milliliter (ml.), 28 ejaculates from 7 bulls for natural service was 225,000 per ml.; and the plate counts for 43 ejaculates of 19 bulls collected by means of an artificial vagina ranged from 1,000 to 22,000,000 bacteria per ml.

According to the report of the U. S. Department of Agriculture (1942) diphtheroids seem to be the predominating type of organisms in the bull semen. Staphylococci were next in frequency whereas *Pseudomonas aeruginosa* and coliform organisms were found only occasionally.

Edmondson, Tallman and Herman (1949) listed the microorganisms, isolated from bull semen, according to the order of predominance, as bacilli, micrococci, coliform organisms, hemolytic and nonhemolytic streptococci, *Pseudomonas, Actinomyces, Proteus,* and yeasts. The plate counts of 64 ejaculates from 20 bulls varied from 900 to about 2,300,000 per ml.

Almquist, Prince and Reid (1949) in a study of 202 ejaculates of semen from 36 bulls found a mean plate count of 200,000 organisms per ml., with a range from 100 to more than 3,000,000 per ml. of semen. A maximum difference in the plate count of nearly 3,000-fold has been observed between bulls and 2,000-fold between ejaculates of the same bull.

In a study of the incidence of bacteria in bull semen, Prince, Almquist and Reid (1949) classified the cultures of 19 ejaculates from 8 bulls of various fertility levels as 20 per cent micrococci, 16 per cent gram positive rods, 13 per cent gram negative rods, 5 per cent Actinomy cetaceae and a few false yeasts. *Corynebacterium* was found to be the major portion of the gram positive rods and common to nearly all samples of semen. The negative rods were identified as *Pseudomonas, Flavobacterium* and *Alcaligenes.*

Microorganisms in relation to fertility

Previous workers (Gunsalus, Salisbury, and Willett, 1941; Almquist, Prince and Reid, 1949; Edmondson, Tallman and Herman, 1949) found no apparent relationship between the average plate count and the general fertility of the bull, although Williams and Kingsbury (1920) were in agreement with Gilman (1921) that semen from normal bulls was relatively free of bacteria whereas that of impotent bulls usually harbored large numbers of bacteria. Perhaps more significant in the impaired fertility picture is the num-

ber or the effect on the female genital tract by certain organisms following insemination. There is ample evidence (Fitch, 1915; Beaver, Boyd and Fitch, 1922; Borg, 1943) that streptococci and members of the coliform group were frequently associated with ovaritis, metritis, abortion or sterility. Moreover, there is the suggestion that certain types of bacteria can produce toxins and metabolic products which may be harmful to spermatozoa. (Williams and Kingsbury, 1920; Roemmele, 1927; Krzyszkowsky and Pawlow, 1927).

Uchigaki (1927) found that *Diplococcus pneumoniae, Staphylococcus aureus, Escherichia coli,* typhoid bacillus, gonococcus, and dysentery bacillus toxins shortened the period of vitality and decreased the vitality of spermatozoa of albino rats. Ognianov (1942) reported that *Escherichia coli* caused agglutination of the spermatozoa of bull semen.

Pounden, et al. (1947) investigated a series of abortions by females previously inseminated with semen from the same bull. They found that organisms, resembling *Staphylococcus albus,* were present in the semen of the bull and the organisms appeared to be identical with those obtained in large numbers from the pus and exudates surrounding the aborted fetuses.

The possible association of *Pseudomonas aeruginosa* with low fertility has been discussed by many investigators. Gunsalus, Salisbury and Willett (1941) reported that *Pseudomonas aeruginosa* had been isolated from the semen of 5 of 19 bulls studied and 3 of those 5 bulls had shown progressively reduced fertility. Edmondson, Tallman and Herman (1949) found that 5 bulls producing semen containing *Pseudomonas aeruginosa* were low in fertility and eventually all 5 bulls became sterile. However, by the use of selective media, Prince, Almquist and Reid (1949) found that *Pseudomonas aeruginosa* was present in all ejaculates examined from 3 fertile and 2 infertile bulls. Even when present to the extent of 1,000 or more per ml., the presence of this organism was not indicative of a low level of fertility.

Materials and Methods

The semen used in this study was obtained from bulls belonging to the Oregon Dairy Breeders' Association near Corvallis, Oregon.

Collection of semen

Semen was collected with an artificial vagina according to the methods described by Lambers and McKenzie (1940). All glassware used in handling the semen was washed with "Calgonite,"

rinsed with hot tap water and sterilized by dry heat in an electric oven at 121° C. for not less than 3 hours.

After each collection of semen the inner rubber tubes and cones of the artificial vagina were rinsed with tap water, immersed in Roccal solution (1 ounce of 10 per cent Roccal solution in 1 gallon of water, i.e., approximately 8 parts per million of banzalkonium chloride) for about 1 hour, washed with "Dreft" and rinsed with hot tap water. They were then dried in a closet by an electric fan blowing over warm light bulbs. The area around the prepuce of the bull was washed with the Roccal solution before the collection of semen.

Handling of semen for shipment and insemination

Four to five ml. of freshly collected semen were diluted 1:1 with fresh egg-yolk sodium-citrate medium and left at room temperature for about 10 minutes; then it was further diluted to 1:40 (on the average) in a beaker with the same egg-yolk sodium citrate medium. It was colored differently for identification of breed according to the method described by Almquist (1946). The semen was placed in pyrex test tubes, corked, labeled and put in a water bath in the refrigerator (5° C.) for about 1 hour until packing. In packing, 8 to 10 tubes of semen were fastened around an ice can, wrapped with a carton paper bag and packed for shipping by bus to various county breeding laboratories for use in artificial insemination. The longest time required for the semen to reach the farthest county laboratory was 14 hours from time of shipment from the collecting laboratory.

Inseminations were performed by the inseminators of the Association. One ml. of the diluted semen was deposited, by using a sterilized plastic or glass pipette, into the uterus of the cow, which according to the owner's report had recently shown symptoms of estrus. The semen used was as fresh as possible, generally 1 to 2 days of age, sometimes 3 days old.

Plating, isolation, and identification of microorganisms

The bacteria were isolated from the semen by streak and plate methods. Immediately after the collection, a portion of the semen was diluted with sterilized distilled water 1:100 and plated out with brain-heart infusion agar or at the same time also plated out with blood-brain-heart infusion agar. The plates were incubated at 37° C. for 96 hours. After the incubation plate counts were made, morphologically different colonies were selected as a source of isolates for further study and identification.

Experimental Findings

The experiment was comprised of two periods of three months each. In the first period (March, April, May, 1949) 106 ejaculates of semen from 18 bulls (Holstein, Guernsey and Jersey breeds) were plated out with brain-heart infusion agar. The morphologically different bacterial colonies were isolated and identified as to species. In the second period (May, June, July, 1950) 119 ejaculates of semen from 19 bulls (11 of these had been included in the first period) were plated out with selective media for the isolation of the *Streptococcus* species and for detecting the presence of *Pseudomonas aeruginosa.*

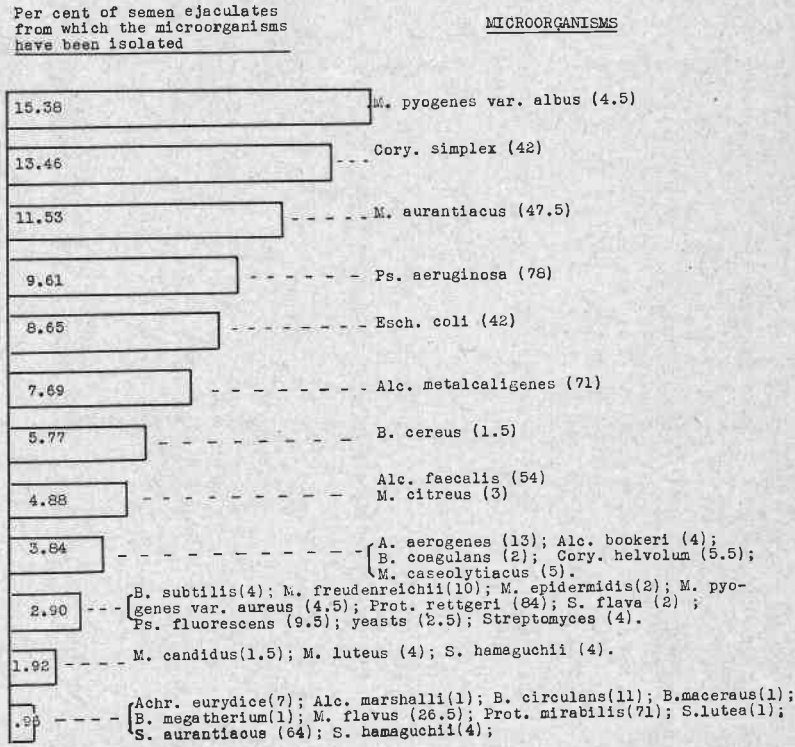


Figure 1. Incidence of microorganisms in dairy bull semen. Remarks: a. The microorganisms which have the same percentage are grouped together. b. The numbers in the parentheses indicate the average plate counts per 0.01 ml. of fresh undiluted semen; zero counts were not included in computing the averages.

Table 1. Plate Counts of Microorganisms Isolated from Dairy Bull Semen

Microorganisms	High fertility bulls				Average fertility bulls								Low fertility bulls			
	G64	J16	J20	J18	G69	H7	G59	G65	J23	J24	H8	G68	G66	J25	H3	H9
1. Achr. eurydice	....	....	....	....	7	....	....	....	....	....	....	....	....	....	....	....
2. A. aerogenes	....	....	....	6	1	....	....	....	....	....	....	....	....	22	....	....
3. Alc. bookeri	....	....	....	5	....	5	....	1	....	....	6	....	....	....	....	4
4. Alc. faecalis	....	....	....	....	....	54	....	32	....	....	9	....	....	120	....	....
5. Alc. marshalli	....	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....
6. Alc. metalcaligenes	....	....	1	13	....	....	1	....	257	....	1	....	....	....	155	....
7. B. cereus	....	....	1	....	2	....	....	1	....	2	1	....	....	....	....	....
8. B. circulans	....	....	....	....	11	....	....	....	....	....	....	....	....	....	....	....
9. B. coagulans	....	....	....	....	1	....	....	....	....	....	2	....	....	....	2	....
10. B. macerans	....	....	....	....	....	....	....	....	....	1	....	....	....	....	....	....
11. B. megatherium	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1
12. B. subtilis	....	1	....	....	....	....	....	9	....	....	1	....	....	....	....	....
13. Cory. simplex	....	4	1	5	56	....	....	1	....	....	26	9	....	2	1	....
14. Cory. helvolum	....	9	....	5	....	6	....	....	....	....	1	1	....	....	....	....
15. Esch. coli	....	7	....	49	49	....	....	....	....	....	....	....	....	61	....	....
16. M. aurantiacus	....	....	1	....	1	....	....	3	10	....	8	4	....	....	6	5
17. M. candidus	....	....	....	....	....	....	....	....	....	2	1	....	....	....	....	....
18. M. caseolytiacus	....	....	....	10	7	1	....	....	....	....	....	....	....	....	....	1
19. M. citreus	....	....	....	....	1	....	....	6	....	11	1	2	....	....	1	1

22. M. freudenreichii	....	....	....	....	....	....	26	1	....	....	....	....	....	....	....	....	2
23. M. luteus	....	....	....	....	....	....	....	2	....	....	7	....	....	....	....	....	....
24. M. pyogenes var. albus	2	....	....	....	7	1	....	2	3	....	3	18	....	....	1	....	....
25. M. pyogenes var. aureus	....	....	....	5	8	....	....	....	....	....	....	....	....	....	1	....	....
26. Prot. mirabilis	....	....	....	....	....	....	....	....	....	....	71	....	....	....	....	....	....
27. Prot. rettgeri	....	....	....	....	....	....	....	1	....	....	13	120	....	....	....	....	....
28. Ps. fluorescens	....	....	20	1	....	....	....	....	....	5	16	....	....	....	....	....	....
29. Ps. aeruginosa	....	....	....	149	....	....	5	....	....	22	....	....	....	....	63	....	....
30. Sar. aurantiacus	....	64	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
31. Sar. flava	....	....	....	....	....	1	....	....	5	....	....	1	....	....	....	1	....
32. Sar. hamaguchii	....	....	....	....	1	7	....	....	....	....	....	....	....	....	....	....	....
33. Sar. lutea	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	....	....
34. Bacterium spp.	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
35. Streptomyces	....	....	....	....	....	....	....	4	....	....	4	....	....	....	....	....	....
36. Yeasts	....	....	....	4	1	....	....	....	....	4	....	....	1	....	....	3	....
37. Streptococcus	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

Remarks: a. From 1 to 36 are the microorganisms isolated from 106 ejaculates of bull semen studied in the first period of this study (March, April, and May 1949), using brain-heart infusion agar. By using a selective medium there were only 2 samples of semen, out of 10 from one (H2) of the 19 bulls studied in the second period (May, June, and July 1950), where *Streptococcus* was found.

b. The numbers given in the body of the table are the plate counts of the microorganisms isolated from 0.01 ml. of fresh undiluted semen.

Number and type of microorganisms in dairy bull semen

The types of microorganisms isolated from dairy bull semen together with their plate counts per 0.01 ml. of fresh undiluted semen are given in Table 1, and the incidence of their occurrence in semen are shown in Figure 1. The plate count showed marked differences between samples from different bulls and between different ejaculates of the same bull. The counts varied from none to 29,700 per ml. of undiluted fresh semen with an arithmetical average of 684. It was found that *Micrococcus* species and *Pseudomonas aeruginosa* were most frequently present in the semen ejaculates while only one of the 19 bulls studied in the second period had *Streptococcus* in its semen, and *Streptococcus* did not occur at all in the semen studied in the first period.

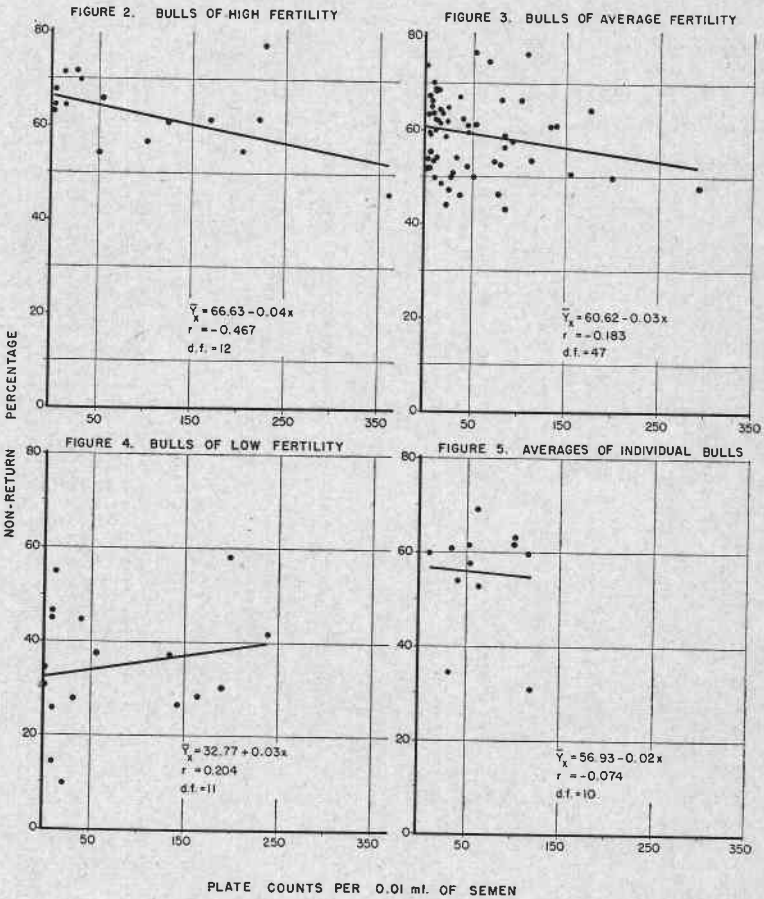
Table 2. DAIRY BULL FERTILITY

Individual bulls by fertility group	Non-return rate			Number of cows insem-inated
	50 to 80 days	8 months	Difference	
	Per cent	*Per cent*	*Per cent*	
High fertility group				
G 64	72.88	66.10	6.78	59
J 16	67.86	64.88	2.98	168
J 20	67.96	63.11	4.85	206
G 67	68.52	62.98	5.56	54
J 18	67.51	61.98	5.53	676
Group average	67.97	62.32	5.65	1,173
Average fertility group				
G 69	65.66	61.61	4.05	831
H 7	69.13	61.52	7.61	369
G 59	63.34	60.95	2.39	338
G 65	66.15	60.00	6.15	780
J 23	64.93	59.71	5.22	345
J 24	64.63	57.95	6.68	440
H 8	61.66	57.10	4.56	373
G 68	56.95	52.98	3.97	151
Group average	64.93	59.75	5.18	3,627
Low fertility group				
G 66	51.58	47.37	4.21	190
J 22	51.06	45.74	5.32	94
J 25	49.23	42.05	7.18	195
H 3	41.12	35.51	5.61	214
H 9	45.60	34.40	11.12	250
Group average	47.08	39.98	7.10	943
ALL BULLS	62.60	57.03	5.57	5,743

Number of microorganisms in relation to fertility

The fertility of the bull was estimated on the basis of per cent of non-return of estrus (non-return rate) of the cows 8 months after breeding. The non-return rate, associated with each bull, from 5,743 cows bred from 133 ejaculates in the first period of this study, was subjected to the Chi-squared test (Fisher, 1950) and the fertility of the bull was thereby classified into three levels, i.e., high, average and low (Table 2).

REGRESSION OF PLATE COUNT ON NON-RETURN RATE



The regression function of the plate count on the non-return rate of the individual semen ejaculates from individual bulls have been studied (Table 3). Regression functions were also computed for plate count on non-return rate of high, average and low fertility bulls. The associated correlation coefficients were also obtained. For 10 of the 13 bulls, the correlation coefficients between the plate counts and the non-return rate were negative and for three the correlation coefficients were positive; but none of the 13 coefficients

Table 3. THE REGRESSION OF PLATE COUNTS ON NON-RETURN RATE

Individual bulls by fertility group	r	N	d.f.	Line of regression
High fertility bulls				
J 20	—.9559	4	2	69.45 — .06 x
J 18	—.0530	12	10	63.61 — .004 x
Group average	—.467	16	12	66.63 — .04 x
Average fertility bulls				
G 69	—.2559	12	10	62.68 — .02 x
H 7	—.2639	7	5	61.66 — .04 x
G 59	—.5280	5	3	61.95 — .16 x
G 65	—.2594	12	10	61.92 — .11 x
J 23	—.4667	5	3	93.75 — .31 x
J 24	—.0384	9	7	56.88 — .04 x
H 8	—.1104	10	8	60.66 — .03 x
G 68	+.9847	3	1	45.82 + .11 x
Group average	—.1830	63	47	60.62 — .03 x
Low fertility bulls				
G 66	+.6406	3	1	44.06 + .07 x
H 3	+.3030	7	5	29.26 + .04 x
H 9	—.2223	7	5	32.28 — .06 x
Group average	+.2036	17	11	32.77 + .03 x
ALL BULLS	—.1847	96	70	55.65 — .03 x

was statistically significant. The regression functions of the average plate count of semen of the individual bulls on their non-return rates also were not statistically significant (Figure 5). Table 4 shows that if we group the plate counts and subject the non-return rate of these groups to the Chi-square test (Fisher, 1950), semen with plate counts of more than 150 bacteria colonies per 0.01 ml. of semen gave significantly lower breeding efficiency than semen with lower bacteria counts. This was true of the breeding results checked at 50 to 80 days or at 8 months after breeding. Thus the

data do not allow us to overlook entirely the suggestion that the semen which contained the larger numbers of microorganisms gave the poorer breeding results (Figures 2, 3, 4). The data, however, clearly indicated that other more potent factors are contributing to the variability of the rate of non-return. When these factors are identified and when they can be separately evaluated, the relation of total bacterial count to non-return rate should be reinvestigated.

Table 4. NUMBER OF MICROORGANISMS IN SEMEN IN RELATION TO BREEDING EFFICIENCY

Number of microorganisms	Non-return rate		Number of cows bred
	50 to 80 days	8 months	
	Per cent	*Per cent*	
Up to 10	63.49	58.22	1,290
11 to 50	63.16	57.37	1,330
51 to 100	62.75	56.95	655
101 to 150	61.11	56.44	404
151 to 200	55.67	51.67	300
201 to 250	56.58	50.00	228
251 and up	57.44	46.80	47
Up to 150	63.01	57.49	3,679
151 and up	56.17	41.91	575
Difference	6.84	15.58	.....
Chi-square	9.88	9.56	.....

Pseudomonas aeruginosa in relation to impaired fertility

The possible association of *Pseudomonas aeruginosa* with bovine infertility has been discussed by many investigators (Gunsalus, Salisbury, and Willett, 1941; Edmondson, Tallman, and Herman, 1949; Prince, Almquist, and Reid, 1949). The present study indicates that this organism was not the causative agent of bovine infertility. The evidence may be summarized as follows:

In the first period of this study, *Pseudomonas aeruginosa* was found in the majority of semen ejaculates from 3 bulls; J18, J24, and H3. The fertility level of H3 was extremely low; J24 was ranked as an average fertile bull, and J18 was among the bulls of high fertility (Table 2). In the second period (1950) the non-return rate of J18 was as high as that during the first period (1949). The bull J24 died of urinary calculi and H3 was eliminated from the herd because of extremely low breeding efficiency, thus J24 and H3 were not available for comparison. *Pseudomonas aeruginosa* was present in 50 per cent of the semen samples of J18 during both

1949 and 1950 (Figures 6, 7). Whether *Pseudomonas aeruginosa* was present or absent, the non-return rate of J18 for the months of March, April, and May of 1949 and for May, June, and July of 1950 remained near 63 per cent, as indicated in Figure 8a.

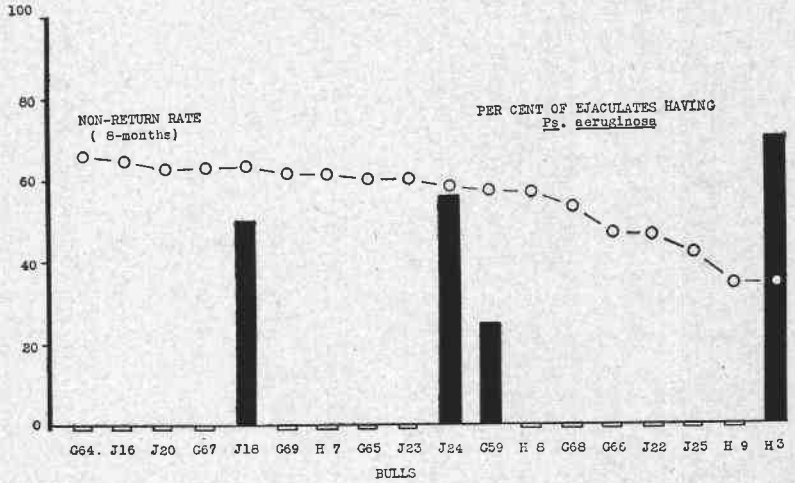


Figure 6. The occurrence of *Pseudomonas aeruginosa* in semen from dairy bulls of various fertility levels (March, April, May 1949).

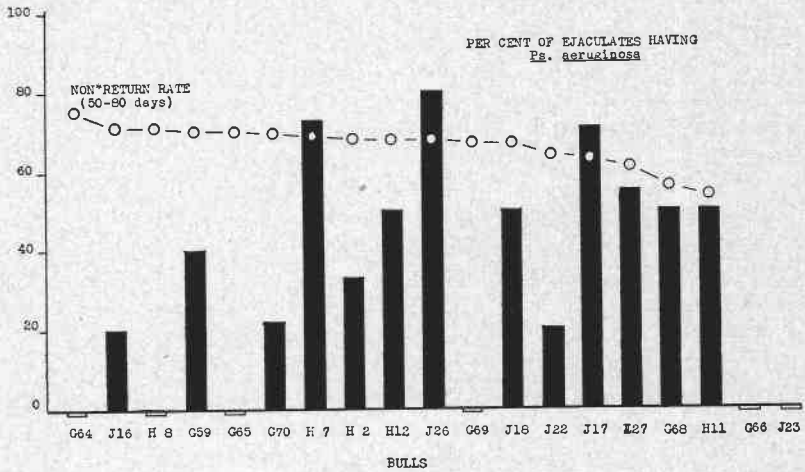


Figure 7. The occurrence of *Pseudomonas aeruginosa* in semen from dairy bulls of various fertility levels (May, June, July 1950).

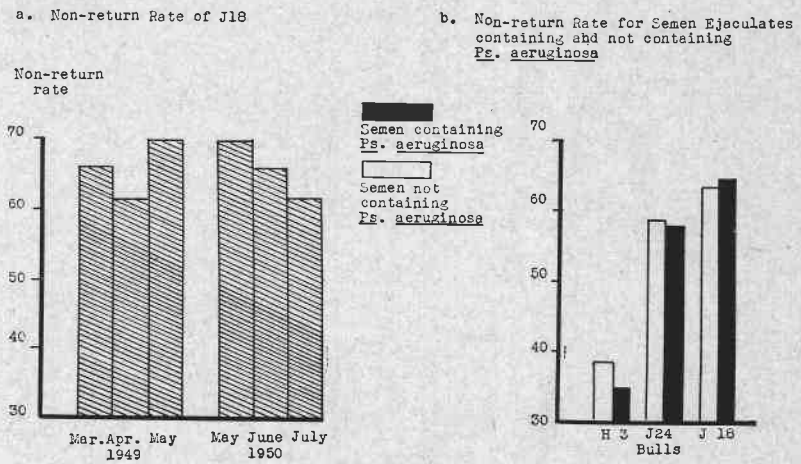


Figure 8. ***Pseudomonas aeruginosa*** **in relation to fertility (1949).**

In order to study the possible effect of *Pseudomonas aeruginosa* on semen quality, the number of semen ejaculates that qualified for artificial breeding have been compared with the total number of collections that have been made from J18 during the two periods. Qualification of semen samples depended mainly upon sperm motility and numbers. As shown in Table 5, in the first period, 88 per cent of the ejaculates were qualified for artificial insemination and

Table 5. SEMEN QUALITY OF BULL J18

Periods	Number of semen ejaculates collected	Semen ejaculates qualified for artificial breeding	
		Total number	Per cent
March, April, May, 1949	25	22	88
May, June, July, 1950	28	25	89

in the second period (14 months later) 89.3 per cent of the ejaculates were qualified. These data evidently support the interpretation that *Pseudomonas aeruginosa* had no effect on semen quality as well as no effect on the breeding efficiency of qualified semen samples.

In the second period of the study, *Pseudomonas aeruginosa* was again found in the semen of bulls of various fertility levels (Figure 7), and there was no significant difference between the plate count of *Pseudomonas aeruginosa* in the semen of high and of low fertility bulls. The breeding efficiency of the semen containing *Pseudomonas aeruginosa* has been compared with the efficiency of the semen from the same bull when not containing *Pseudomonas*. As shown in Figure 9, the differences were not significant.

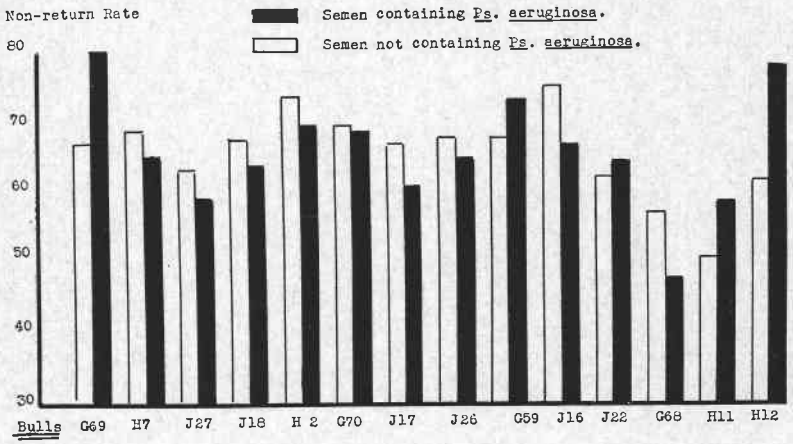


Figure 9. *Pseudomonas aeruginosa* in relation to fertility (1950).

All these lines of evidence strengthen the conclusion that *Pseudomonas aeruginosa* is not the causative agent of potential infertility in dairy bulls.

Micrococci and other microorganisms possibly associated with infertility

It is very difficult to determine the exact effect of certain organisms on fertility of semen ejaculates because fertility may be affected quite differently by different numbers of the organism, and the physiological conditions of the sperm or the nutritive state of the host may alter the response even to constant numbers of the organisms being considered.

As shown in Table 1, comparatively high percentages of semen ejaculates of bulls H9, H3, H8 and G68 contained *Micrococcus aurantiacus*. *Micrococcus pyogenes* var. *albus* was found in a great percentage of the ejaculates in G65 and H8 and the semen of G68 had large numbers of this organism. *Alcaligenes metalcaligenes* was

isolated from bulls of both high and low fertility, but the plate count for *Alcaligenes metalcaligenes* was very low in the semen of highly fertile bulls while for low fertility bulls, the counts of this organism were very high. *Corynebacterium simplex* has been isolated from the semen of bulls H8 and J16 in large numbers and in a large per cent of ejaculates. All these (H3, H8, H9, G65, G68, and J16) were low fertility bulls. The breeding history of J16 was poor prior to 1949 and the high non-return rate of J16 during the period of 1949-1950 may have been due to the addition of antibiotics to his semen.

Based on the number and the incidence of the organisms isolated from the semen of bulls of different levels of fertility, we feel that the *Micrococcus aurantiacus, Micrococcus pyogenes* var. *albus, Alcaligenes metalcaligenes* and *Corynebacterium simplex* may be associated with infertility. These organisms were not generally found

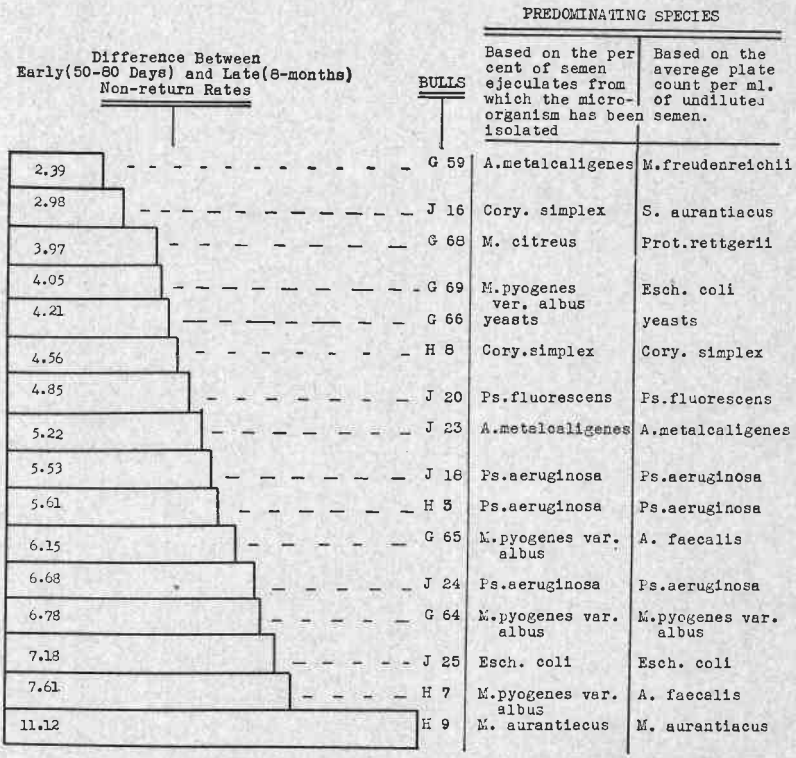


Figure 10. Possible relationship between the type of microorganisms in semen and prenatal mortality.

associated with the semen from bulls of high fertility or, if found, were present only in small numbers.

Microorganisms possibly associated with prenatal death

By comparing the non-return rate 50 to 80 days after breeding with that 8 months after breeding, differences were obtained which may be used as an approximate estimation of the percentage of prenatal mortality during that period of time. The predominating types of microorganisms in the semen of these bulls which have greater differences between the two non-return rates may be the causative agents involved in prenatal mortality.

As shown in Figure 10, *Micrococcus aurantiacus, Micrococcus pyogenes* var. *albus,* and *Escherichia coli* were the organisms present in the majority of semen ejaculates of the bulls which gave the greater differences between the 50 to 80 days and 8 months non-return rates. It is possible that these organisms may be involved in prenatal mortality.

Discussion

The findings in this study are in agreement with those of previous investigators in that the number of microorganisms found in semen varies greatly between the samples from different bulls and between the different ejaculates of the same bull. This is not surprising because even in cases of infectious diseases the specific microorganism—the causative agent of that infection—may vary greatly in numbers from time to time according to the body resistance or other physiological factors of the host. Besides, in the collection of semen, no matter how carefully accomplished, the possibilities of contamination could not be entirely eliminated.

The statistical insignificance of regression of plate count on non-return rate clearly indicated that the predictability from plate count of the breeding efficiency of semen ejaculates or the fertility of an individual bull is not practicable. However, we cannot overlook the fact that by grouping the plate count and the average non-return rate, the group with an average plate count less than 150 per 0.01 ml. of undiluted fresh semen had a significantly higher non-return rate than the group with an average count more than 150. Thus, by eliminating the semen with the higher bacteria counts one should be able to raise the over-all non-return rate in artificial breeding.

It has been found that certain bacteria, notably *Pseudomonas aeruginosa, Escherichia coli,* and *Alcaligenes metalcaligenes* usually

gave a higher plate count whenever they were found in semen; others, notably *Micrococcus* and *Bacillus,* were usually found in smaller numbers.

It is a current problem whether *Pseudomonas aeruginosa* is a causative agent of infertility. The present study clearly indicated that this organism itself did not affect the potential fertility of the bull. There are different ways that a microorganism may exert its effects on male fertility, e.g., by affecting the processes of spermatogenesis, by affecting the semen quality, or by influencing the fertilizing capacity of the spermatozoa. All these factors have not been found significantly altered by *Pseudomonas aeruginosa* as is evident from the fact that the fertility rates of the bulls which have *Pseudomonas aeruginosa* in their semen, have been maintained from year to year; moreover, in the same bull there was no difference between the non-return rates of semen ejaculates which contained *Pseudomonas aeruginosa* and those which did not contain this microorganism.

In considering the type of microorganisms in semen, the results of this study agreed with Almquist (1950) and with Prince, Almquist and Reid (1949) in that *Pseudomonas aeruginosa* was found in semen from bulls of both high and low fertility levels.

Insofar as *Pseudomonas aeruginosa* was found in bulls of low fertility as well as in bulls of high fertility, there is no essential disagreement with Gunsalus and his coworkers (1941) and with Edmondson and his coworkers (1949) who stressed the association of *Pseudomonas aeruginosa* with bulls of low fertility. Two of the five bulls reported by Gunsalus, Salisbury, and Willett (1941) having *Pseudomonas aeruginosa* were not low fertility bulls. The 5 low-fertility bulls reported by Edmondson, Tallman and Herman (1949) indicated a much closer association of *Pseudomonas aeruginosa* with low fertility than was found in this study. They found *Pseudomonas aeruginosa* in the semen of 5 out of 36 bulls and all 5 bulls with *Pseudomonas aeruginosa* in the semen were low-fertility bulls. Thus, most work reported is reasonably in agreement with the statement that *Pseudomonas aeruginosa* is found in the semen of both high- and low-fertility bulls, and of itself, *Pseudomonas aeruginosa* is not the main causative agent for low fertility of bulls. But, further confirmation from other laboratories is needed to completely clarify the problem.

Many investigators (Gunsalus, Salisbury, and Willett, 1941; and Edmondson, Tallman, and Herman, 1949) have reported the presence of *Streptococcus* in bull semen, but in the present study *Streptococcus* was found rarely in dairy bull semen.

Because of their high incidence, the presence of certain microorganisms may be associated, possibly as a causative factor, with infertility. *Alcaligenes metalcaligenes* is generally considered to be chemically inert; however, it has been reported (Parker, Smith, and Elliker, 1951) that *Alcaligenes metalcaligenes* has detrimental effects in the manufacture of cottage cheese, making the curd slimy and gelatinous. The strain of *Alcaligenes metalcaligenes,* isolated from the semen samples collected, was tested by Parker and his coworkers (1951) and found to have the same detrimental effect caused by a strain of *Alcaligenes metalcaligenes* isolated from defective cheese curd. Further, the bull J23, which usually had a high number of *Alcaligenes metalcaligenes* in its semen, gradually became of lower fertility and had eventually to be eliminated from the herd.

Based on differences between the non-return rates of 50 to 80 days and of 8 months after breeding, it was suggested that *Micrococcus aurantiacus, Micrococcus pyogenes* var. *albus,* and *Escherichia coli* may be associated with some cases of prenatal death, since these organisms appeared in a high percentage of the semen ejaculates of bulls which showed greater differences between the two non-return rates. Prenatal death, if caused by these microorganisms, may have been due to their detrimental effects on the spermatozoa that fertilize the eggs, or to the pathological changes of the uterus or of the developing fetuses induced by the bacteria.

While these preliminary studies suggest the possible association of certain microorganisms with infertility, the confirmation of these deductive suggestions awaits *in vitro* or *in vivo* studies on the effect of microorganisms on the fertility complex.

Summary

The number and type of microorganisms from 225 ejaculates (106 in one series and 119 in a second series) of dairy bull semen have been studied. The number of microorganisms was expressed in plate count on brain-heart infusion agar incubated at 37° C. for 96 hours, and the type of microorganisms in the 106 ejaculates of semen was identified to species according to Bergey's Manual and standard methods. The plate counts varied from none to 29,700 per ml. of undiluted fresh semen, with an average of 684. It varied within individual and between bulls.

Micrococcus species and *Pseudomonas aeruginosa* were the predominating microflora in bull semen studied. *Streptococcus* was found in semen only occasionally.

The correlation between plate count and per cent non-return of estrus of cows 8 months after breeding was not statistically significant. The over-all breeding efficiency may possibly be raised, however, by eliminating semen ejaculates of high bacterial plate count. The non-return rate (of estrus) for semen ejaculates having plate counts of 150 or less per 0.01 ml. was 57 per cent, and the non-return rate (of estrus) for semen ejaculates with plate counts over 150 averaged 42 per cent. The difference in non-return rate between semen ejaculates with plate counts higher or lower than 150 per 0.01 ml. was statistically significant.

On the basis of the comparatively higher incidence of certain microorganisms found in semen of low fertility bulls, it was suggested that *Micrococcus aurantiacus, Micrococcus pyogenes* var. *albus, Alcaligenes metalcaligenes* and *Corynebacterium simplex* may be associated with infertility. The higher incidence of certain microorganisms occurring in semen of bulls with great differences between the non-return rates of 50 to 80 days and 8 months after breeding suggested that *Micrococcus aurantiacus, Micrococcus pyogenes* var. *albus* and *Escherichia coli* may be associated with prenatal death.

Pseudomonas aeruginosa although often found in the semen of bulls of low fertility is apparently not the primary causative agent for the impaired fertility. *Pseudomonas aeruginosa* is often found also in the semen of high fertility bulls. Here it has no noticeable detrimental effects on spermatogenesis, semen quality or the potential fertility of the bull.

References

Almquist, J. O. The effect of certain coal-tar dyes used for semen identification on the livability and fertility of bull spermatozoa. Journal of Dairy Science 27:815. 1946.

Almquist, J. O., P. W. Prince, and J. J. Reid. Bacteriological studies of bovine semen. Journal of Dairy Science 32:543. 1949.

Beaver, D. C., W. L. Boyd, and C. P. Fitch. A contribution to the bacteriology and pathology of sterility in cows, with report of nineteen cases. Journal of American Vet. Med. Assoc. 61:469. 1922.

Borg, H. Enzootische Steriliteit by het Rund. Trydsch. diergeneesk 70: 40. 1943. (citation of Biol. Abst. 21:12214, 1947).

Edmondson, J. E., K. L. Tallman, and H. A. Herman. A study of bacteria in bovine semen and their effect upon livability of spermatozoa. Missouri Agri. Exp. Sta., Res. Bull. No. 444, 1949.

Fisher, R. A. Statistical methods for research workers. Edinburgh, Oliver and Boyd, 1950. 354 p.

Fitch, C. P. A preliminary study of the pathology and bacteriology of ovaritis in cattle. Report of the N.Y. Sta. Vet. Col. :199. 1915.

Gilman, H. L. The diseases of the oviduct of the cow and their relation to sterility. Corn. Vet. 11:14. 1921.

Gunsalus, I. C., G. W. Salisbury, and E. L. Willett. The bacteriology of bull semen. Journal of Dairy Science 24:911. 1941.

Hatziolos, B. Untersuchungen uber Konservierung von Bollensperma zum Zwecke der kunstlichen Besamung. Ztschr. F. Aucht 38 B:199. 1937.

Krzyszkowsky, K. N. and G. N. Pawlow. Beitrage zur Biologie der Spermatozon. Z. F. Tierzucht U. Zuchtungsbiol. 10:257. 1927.

Lambert, W. V. and F. F. McKenzie. Artificial insemination in livestock breeding. U.S.D.A. Circular No. 567. 1940.

Ognianov, A. La carica Batterica nello sperma Bovino Ottenuto Strumentalements nella Practica della Fecondazione Artificiale. Instituto sperimentale Italiano "L. Spallanzani" per la fecondazione artificiale. Milano :48. 1942. (citation of Ani. Br. Abst. 15:86, 1947)

Parker, R. B., V. N. Smith, and P. R. Elliker. Bacteria associated with a gelatinous or slimy curd defect of cottage cheese. Journal of Dairy Science 34:887, 1951.

Pounden, W. D., L. C. Ferguson, L. C. Knoop, and W. E. Krauss. Staphylococci abortion in cattle. Journal American Vet. Med. Assoc. 111:376. 1947.

Price, P. W., J. O. Almquist, and J. J. Reid. Bacteriological studies of bovine semen. II. The incidence of specific types of bacteria and the relation to fertility. Journal of Dairy Science 32:849. 1949.

Roemmele, O. Biologische and Phisiologische untersuchungen am Sperma and am Scheidensekret des Rindes im Himblink suf die Kunstliche Besamung. Zoo. Jahrb. Abt. F. Allg. Zool. U. Physiol. 44:85. 1927.

Uchigaki, S. Biological research of sterility. Japanese Jour. Obst. and Gyn. 10:5. 1927. (citation of Biol. Abst. 4:15364, 1930)

U. S. Department of Agriculture. Report of the chief of the bureau of animal industry. U.S.D.A., p. 5. 1942.

Williams, W. W. and B. F. Kingsbury. Technique of collecting semen for laboratory examination with a review of several diseased bulls. Corn. Vet. 10:87. 1920.