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The Relationship Between Semen Quality  
and Conception Rate in Artificial  
Insemination of Dairy Cattle

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## CONTENTS

	Page
Introduction .....	3
Materials and Methods .....	3
Results .....	5
Pabulum vs. Egg Yolk-Citrate Diluent .....	5
Study of Seasonal Variation in Longevity and Percentage Non-Returns .....	5
Correlation Between Duration of Grade 1 Motility and Percentage Non-Returns .....	7
Discussion .....	8
Summary .....	10
References .....	11

# The Relationship Between Semen Quality and Conception Rate in Artificial Insemination of Dairy Cattle

HOWARD J. WEETH AND H. A. HERMAN

## INTRODUCTION

The extensive use of artificial insemination in dairy cattle in Missouri has made available for study numerous data concerning the relationship between semen quality and conception rate under actual field conditions. Because such data lack the definiteness of laboratory collected data, they should be interpreted with reserve; but results in the field with large populations are likely to be very indicative and are the ultimate of experimentation. The great variability in conception rates where artificial insemination as well as natural service is used, is readily observed. It is often difficult, however, to isolate the exact cause of low breeding efficiency. The quality or fertility of the semen used is only one factor. The reproductive functions of the cow are often impaired or temporarily halted and constitute the greatest factor in breeding failures. Semen of high fertility is necessary for conception. Accurate records of the quality of semen used and the results thereof are a prime requisite in attempting to evaluate the influence of any factor upon breeding results.

Herman and Swanson,<sup>10</sup> 1941, observed that undiluted semen from a group of bulls of good fertility maintained a satisfactory viability for 71 hours, which was more than twice the 26.9 hours of a group of bulls of poor fertility, and one and one-third times the 56.8 hours of a group of questionable fertility. Margolin, et al.,<sup>12</sup> 1943, found a correlation of  $+ 0.6964 \pm 0.0212$  between longevity of 207 samples of egg yolk-phosphate diluted semen and conception rate of these samples. Likewise, Swanson and Herman<sup>16</sup> demonstrated a highly significant linear correlation ( $r = + 0.84$ ) between viability of undiluted semen in storage and its ability to produce pregnancy. The present study was undertaken to determine if this criterion of fertility was applicable to storage viability of diluted semen used by three artificial breeding associations in Missouri. The data have also been studied for the effect of season on sperm viability and conception rate, and the influence of the synthetic pabulum diluent suggested by Phillips and Spitzer<sup>14</sup> on sperm viability and conception rate.

## MATERIALS AND METHODS

Three artificial breeding associations operating in Missouri routinely send portions of all diluted semen which is to be used for artificial breeding to the Department of Dairy Husbandry, University of Missouri, for measurements of quality. These samples are stored at 40° F. and examined daily until motility

rating drops to grade 1, that is less than 25% progressively motile sperm, according to the method described by Herman and Swanson.<sup>10</sup> The maintenance of motility of grade 1 or above will be referred to as longevity of the sample under storage conditions. The monthly percentage non-returns, by sires, are made available by the breeding associations. Since only monthly percentage non-returns by sires were available for the present study, in making all longevity-conception rate comparisons, average monthly sperm longevity by sires has been used. Longevity data were grouped using 12 hour mid-points, and percentage non-return data were grouped using 5% class intervals. A minority of the conception rate data was reported as 60-90 day non-returns, the majority 30-60 day; therefore, all 60-90 day non-return values have been converted to 30-60 day non-returns by using the factor 9%.

During the spring and summer of 1948 the three breeding associations began diluting semen with Phillips' pabulum diluent.<sup>14</sup> All the associations used pabulum exclusively during August, 1948. Previously equal parts egg yolk and 3% sodium citrate had been used. The pabulum was prepared as recommended by Phillips, with the exceptions that 0.03% sulfanilamide was used and the ingredients were mixed in a blender. When breeding results on the pabulum diluted semen became available, it appeared that conception rate had dropped. Therefore, in September, 1948, all associations returned to egg yolk-citrate diluent. The longevity and percentage non-return data of the pabulum diluted semen has been compared with results on egg yolk-citrate diluted semen by two methods. In the first method, paired values were obtained by including only those sires whose semen had been diluted for at least a full month in 1948 with pabulum and in the corresponding month of 1947 with egg yolk-citrate. Such a comparison should minimize the influence of season, however it does not compensate for any improvement in techniques which may have been made in the intervening year. Paired sperm longevity and breeding records were available on 33 bulls; 15 Jerseys, 11 Guernseys, and 7 Holsteins. As a second method of comparison, longevity of sperm in paired samples which were collected from the same sire within 14 days or less was studied. Since only average monthly percentage non-returns were available, no conception rate comparisons were possible. This method of comparing longevity should minimize any difference due to changes in techniques and season. Available for study were 127 paired pabulum and egg yolk-citrate diluted semen samples collected from 54 bulls; 22 Jerseys, 19 Guernseys, and 13 Holsteins.

As a second coincidental phase of the study of correlation between longevity and conception rate, the influence of season has been analyzed. Only data on bulls in use constantly for not less than one year and with not fewer than 25 breedings per month have been used. A total of 4692 semen samples contributed by 26 Jersey bulls, 16 Guernseys, and 10 Holsteins and involving 153,880 inseminations was studied. The period covered was from April, 1946 to April, 1949 for one association and from January, 1947 to April, 1949 for

the other two. The longevity and percentage non-return data have been subjected to an analysis of variance between months-within breeds and between breeds-within months. Within cells values have not been weighted, yet the smallest number of longevity observations within a cell was 61 and the smallest number of services within a cell was 696.

For the sperm longevity percentage non-returns correlation analysis the same selected data which were used in the study of seasonal variation have been used. The independent variable, longevity, and the dependent variable, percentage non-returns, were not weighted; yet the elimination of observations involving less than 25 breedings per month should minimize abnormal results. The linear correlation coefficient, conception rate on longevity, was computed.

## RESULTS

### Pabulum vs. Egg Yolk-Citrate Diluent

In the comparison of the efficiency of pabulum and egg yolk-citrate by the first method (one year interval between the semen dilutions) the mean longevity of pabulum diluted semen was significantly longer than the mean of egg yolk-citrate diluted semen ( $P < .01$ ). Furthermore, the duration of motility in pabulum diluted semen showed significantly less variation.

TABLE 1. COMPARISON OF PABULUM AND EGG YOLK-CITRATE DILUENTS\*

Diluents	No. Samples	Longevity Hours		No. Services	Percentage Non>Returns	
		Mean	S. D.		Mean	S. D.
E. Y. - Cit.	256	103.5	46.31**	5031	55.30**	8.08**
Pabulum	228	118.9**	43.13	8272	45.70	6.80

\*Pabulum used March to September, 1948; egg yolk-citrate corresponding months 1947-70-month-pairs.

\*\*Highly significant differences.

However, egg yolk-citrate diluted semen gave a higher percentage non-returns than did pabulum diluted semen. The standard deviation of the percentage non-returns of the egg yolk-citrate diluted semen was significantly greater than the standard deviation of pabulum diluted semen.

In the second method of comparison (semen samples collected from same bull within two weeks or less), the average duration of grade 1 motility of the pabulum diluted semen was 123 hours with a standard deviation of 47.7, as compared to 109 hours with a standard deviation of 45.6 hours for egg yolk-citrate. These differences are significant ( $P < .01$ ). Pabulum diluted semen stored longer than egg yolk-citrate diluted semen in 64 of the paired observations; egg yolk-citrate was better in 41 pairs; and the storage time was equal in 22 of the paired observations.

### Study of Seasonal Variation in Longevity and Percentage Non>Returns

The analysis of variance of motility duration showed the inter-month and inter-breed differences to be highly significant ( $P < .01$ ), with the inter-breed

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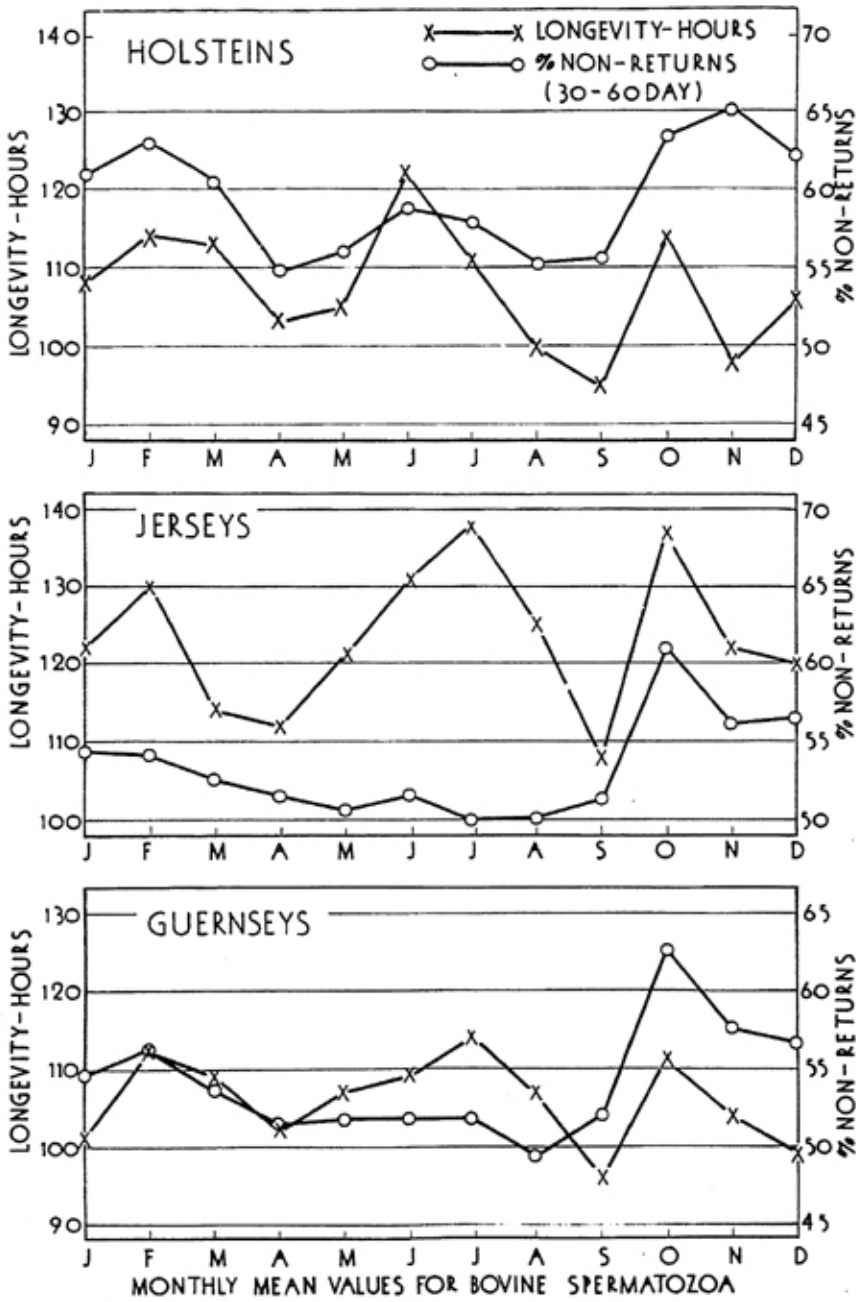


Fig. 1.—Monthly variation in sperm livability and conception rate based on 30-60 day percentage non-returns. All bulls in use for a full year and averaging more than 25 inseminations per month.

mean square being about  $8\frac{1}{2}$  times as large as the inter-month mean square. Between the breeds, the duration of grade 1 motility was significantly longer in the Jerseys than in Guernseys ( $P < .01$ ) in all months except February, and the Jersey semen stored significantly longer than Holstein semen in all months except March ( $P < .01$ ). Between the Holstein and Guernsey breeds the results were inconsistent. Holstein motility duration was longer in January, June, and December. Guernsey longevity was longer in August. Within breeds

no monthly average longevity differed significantly from the mean for the breed. Within the Jersey bulls, semen produced in September stored significantly less hours than February, June, July and October semen ( $P < .02$ ). Guernsey longevity showed less variation throughout the year than either Jersey or Holstein semen. Within the breed, only one month, July, was significantly better than the lowest month, September. (See Fig. 1.) Within the Holsteins, June rather than July was the peak month, with September again being significantly lower ( $P < .02$ ). The months of February, March, and October were also better than September at the 5% level of significance.

Comparing percentage non-returns by month, the within-breed inter-month analysis showed that no months deviated significantly below the mean of the breed; and only in the Jersey breed, during October, did the conception rate rise significantly above the mean ( $P < .01$ ). With  $P = .05$  as a level of significance, within the Jersey breed October conception rate was better than all other months. Both November and December were superior to July and August. Within the Guernsey breed, October was better in percentage non-returns than the months of March through September, and November and December were better than August. Within the Holstein breed the months of February, October, November, and December were significantly better than April, May, August, and September; January was significantly better than April, August, and September. The Holstein maintained a significantly higher conception rate ( $P = .02$ ) than either the Jerseys or Guernseys in all months except October. There was no significant difference between the Jerseys and Guernseys in any month. These results apply only to the data under observation; therefore they should not be extended to other groups of the breeds. Furthermore, the influence of the female is involved in fertility. Only a minority of the cows bred artificially were purebreds.

#### Correlation Between Duration of Grade 1 Motility and Percentage Non>Returns

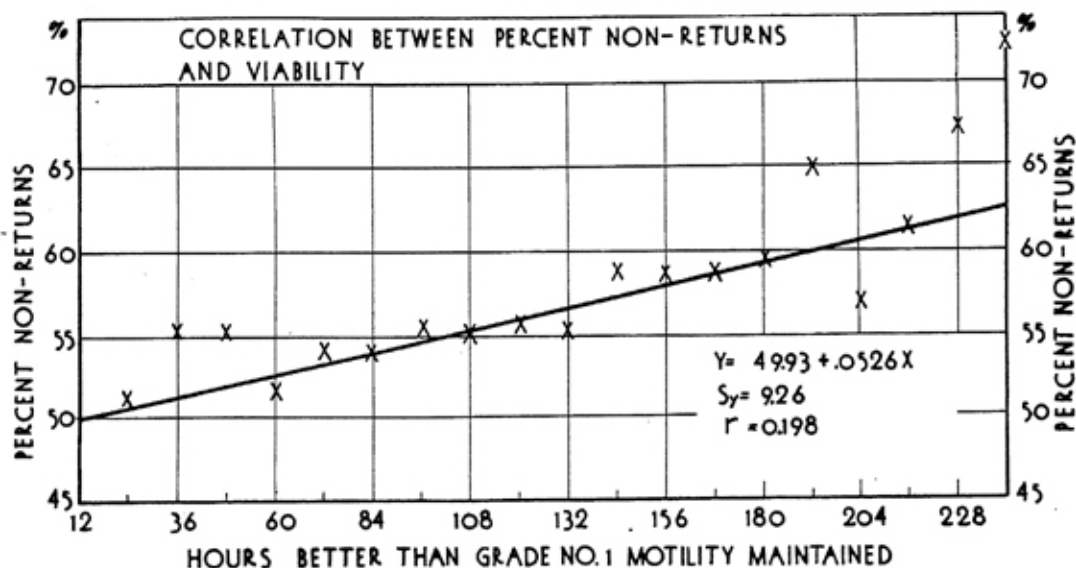


Fig. 2.—Regression-sperm livability correlated with 30-60 day percentage non-returns.

A total of 1176 bull-month average longevity-conception rate observations was available for the analysis. The results are presented graphically in Figure 2. The coefficient of linear correlation,  $r$ , was  $+ 0.198 \pm 0.028$ , which is highly significant. The regression coefficient,  $+ 0.0526 \pm 0.008$ , is also highly significant.

### DISCUSSION

The study of pabulum diluent showed that pabulum maintained semen above a grade 1 motility for a significantly longer time under laboratory storage conditions than did egg yolk-citrate. This is in opposition to the observation of Bratton, et al.<sup>2</sup> In a comparison of six different diluents they did not use Phillips' pabulum because unsatisfactory sperm livability was encountered in preliminary storage studies. The cause of the inconsistent result with pabulum diluent, improved storage time, but lowered conception rate, is not definitely known. It is apparent that some changes occurred in the pabulum diluted semen after it reached the inseminator which tended to lower fertility of the sperm. The pabulum diluent as prepared contained only 30 mgm. of sulfanilamide per 100 ml. Knodt and Salisbury<sup>11</sup> found 200 mgm. sulfanilamide per 100 ml. of sodium citrate diluent to be the minimum level of the sulfonamide necessary to prevent bacterial growth. More recently, 300 mgm. sulfanilamide per 100 ml. of egg yolk-sodium citrate has been found<sup>2, 8</sup> to be the optimum level for bacterial control and fertility. It is conceivable that 30 mgm. of sulfanilamide failed to control bacterial growth under field conditions. However prior investigations on the bacterial contamination of semen in these associations showed it to be a minor factor.<sup>18</sup>

The most consistent seasonal observation in the motility duration data was the sharp decline from a maximum during mid-summer to a minimum in September. This decline began in July with Holstein semen, but not until August with Jersey and Guernsey semen. October longevity again increased above the mean. The adverse effect of summer on longevity was delayed, for July is the mid-point of the summer season. The secretion of the thyroid gland is known to affect reproduction and a seasonal rhythm in thyroid activity has been demonstrated<sup>3</sup>, but decreasing thyroid activity in summer lags behind increasing temperature. This lag may be reflected in the late summer decrease in motility duration. The monthly changes in longevity during the winter and spring months were less consistent. It is apparent that the lack of general agreement concerning seasonal variation in semen quality and breeding efficiency<sup>1, 9, 13</sup> is due to fundamental differences in factors associated with season. However, these observations are in close agreement with those of Erb, et al.<sup>6</sup>, (Indiana) and Phillips, et al.<sup>15</sup> (Maryland), concerning seasonal changes in semen quality. The highly significant winter drop in motility duration observed by Swanson and Herman<sup>16</sup> in a small group of predominantly aged bulls was not seen in this study. Furthermore, they did not observe a marked change between September and October longevity, but rather storage duration began to increase



in September, to reach a peak in November. High mean maximum temperature plus a small diurnal variation are characteristics of Missouri's summer temperatures. Since this combination appears to be detrimental to longevity, some artificial method of cooling bull quarters is indicated.

With the conception rate data, the outstanding and consistent seasonal observation was the significant increase in percentage non-returns in October. From May to September the percentage non-returns were only slightly below the mean of the breed. The June and July peak in duration of grade 1 motility did not occasion maximum conception rate. Furthermore, neither did minimum longevity in September occasion minimum conception rate, for the two varied inversely from August to September. It appears that factors associated with climate either are different or have different effects on motility duration and the chance for successful pregnancy. Conception rate has been shown to be correlated with longevity.<sup>12, 17</sup> Therefore the wide discrepancy between the two in summer appears to have been due to some factor which limited fertility in the cow. The bulls being used artificially are given an adequate ration, mainly dry lot with some green forage in season, therefore, seasonal changes in the ration of the sires was probably not a factor. However, most of the herds being serviced are fed solely or principally on pasture during the growing season (April to October). A summer decline in pasture productivity and quality,<sup>8, 16</sup> tendency toward overgrazing,<sup>7</sup> and the declining body weight of the cows at this time may account for the longevity-conception rate discrepancy.

From the longevity-percentage non-returns correlation analysis it can be predicted that for the three associations studied, if there is no essential change in conditions, each increase of 20 hours in longevity will occasion about 1% increase in percentage non-returns. The data of Swanson and Herman<sup>17</sup> show about 2.6% increase in conception rate per 20 hours increase in longevity of undiluted semen. Margolin et al.<sup>1</sup>, did not give the functional equation of the average longevity-conception rate relationship, therefore, it is not possible to predict from their data the unit change in conception rate per unit change in longevity. The correlation and regression coefficients obtained from the present data, while highly significant, are not as high as those found by Swanson and Herman.<sup>17</sup> However, the cows involved in their study were in the Missouri Station herd and were inseminated at mid-estrus with high quality semen. Under field conditions many factors such as timing of insemination, health and nutrition of the cow, and insemination technique no doubt reduced the correlation between the two variables. The lack of parallelism is especially apparent with the Jersey and Guernsey data (Fig. 1), and these two breeds contributed 81% of the observations involved in the correlation analysis.

## SUMMARY

1. A synthetic pabulum diluent (Phillips) maintained a grade 1 motility in dairy bull semen significantly longer than did egg yolk-citrate diluent, however, conception rate in the field with pabulum was significantly less. Further studies of the diluent, using more than 0.03% sulfanilamide are indicated.
2. Inter-breed and inter-month variations in longevity and percentage non-returns were highly significant. The most consistent trend in longevity was a maximum in June for Holstein semen and in July for Jersey and Guernsey semen, followed by a sharp decline to a minimum in September; and then a significant increase in October. A delayed, detrimental effect of high environmental temperature is suggested.
3. Hours duration of grade 1 motility in diluted semen was significantly correlated with percentage non-returns ( $r = + 0.198 \pm 0.028$ ), therefore, routine determination, by artificial breeding associations, of this criterion of sire fertility is indicated.
4. A 20-hour increase in longevity occasioned a 1% increase in percentage non-returns.
5. Some factor associated with the summer season appears to have limited fertility of the cows inseminated at this time. The effect of this phenomenon on the correlation and regression coefficients has been discussed.

## REFERENCES

1. Anderson, J. 1945. "Seasonal Variation in the Reproductive Capacity of the Bull." *Jour. Agr. Sci.*, 35:184-196.
2. Bratton, R. W., R. H. Foote, S. D. Musgrave, and N. L. Vandemark. 1949. "Livability and Fertility of Bovine Spermatozoa in Different Diluents." *Jour. Dairy Sci.*, 32:604-608.
3. Brody, S. 1945. "Bioenergetics and Growth." Reinhold Publishing Corp., New York.
4. Brown, E. M. 1943. "Seasonal Variations in the Growth and Chemical Composition of Kentucky Bluegrass." *Mo. Agr. Expt. Sta. Res. Bul.* 360.
5. Crampton, E. W. and R. P. Forshaw. 1939. "Pasture Studies. XV. The Intra-Seasonal Changes in the Nutritive Value of Pasture Herbage." *Sci. Agr.*, 19:701-711.
6. Erb, R. E., F. N. Andrews, and J. H. Hilton. 1942. "Seasonal Variation in Semen Quality of the Dairy Bull." *Jour. Dairy Sci.*, 25:815-826.
7. Ethridge, W. C., C. A. Helm, and E. M. Brown. 1946. "An All-Year Pasture System For Missouri." *Mo. Agr. Expt. Sta. Circ.* 193.
8. Foote, R. H. and G. W. Salisbury. 1948. "The Effect of Sulfonamides upon the Livability of Spermatozoa and Upon the Control of Bacteria in Diluted Bull Semen." *Jour. Dairy Sci.*, 31:769-778.
9. Hammond, J. 1927. "The Physiology of Reproduction in the Cow." Cambridge University Press, London.
10. Herman, H. A. and E. W. Swanson. 1941. "Variations in Dairy Bull Semen with Respect to Its Use in Artificial Insemination." *Mo. Agr. Expt. Sta. Res. Bul.* 326.
11. Knodt, C. B. and G. W. Salisbury. 1946. "The Effect of Sulfanilamide upon the Livability and Metabolism of Bovine Spermatozoa." *Jour. Dairy Sci.*, 29:285-291.
12. Margolin, S., J. W. Bartlett, and O. L. Leopard. 1943. "The Relation of Longevity to Fertility of Bull Semen." *Jour. Dairy Sci.*, 26:983-985.
13. Mercier, E. and G. W. Salisbury. 1947. "Seasonal Variations in Hours of Daylight Associated with Fertility Level of Cattle under Natural Breeding Conditions." *Jour. Dairy Sci.*, 30:747-756.
14. Phillips, P. H. and R. R. Spitzer. 1946. "A Synthetic Pabulum for the Preservation of Bull Semen." *Jour. Dairy Sci.*, 29:407-414.
15. Phillips, R. W., B. Knapp, Jr., L. C. Heemstra, and O. N. Eaton. 1943. "Seasonal Variation in the Semen of Bulls." *Amer. Jour. Vet. Res.*, 4:115-119.
16. Swanson, E. W. and H. A. Herman. 1944. "Seasonal Variation in Semen Quality of Some Missouri Dairy Bulls." *Jour. Dairy Sci.*, 27:303-310.
17. Swanson, E. W. and H. A. Herman. 1947. "The Correlation Between Some Characteristics of Dairy Bull Semen and Conception Rate." *Mo. Agr. Expt. Sta. Circ.* 313.
18. Edmondson, J. E., Tallman, K. L., and Herman, H. A. 1949. "A Study of Bacteria Found in Bovine Semen and Their Effect Upon Livability of Spermatozoa." *Mo. Agr. Expt. Sta. Res. Bul.* (in press).