JULY, 1947 RESEARCH BULLETIN 407

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
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# *The Relationship Between Percentage of Live Spermatozoa and Motility, Longevity, and Fertility of Semen of Dairy Bulls*

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(Publication authorized June 19, 1947)


COLUMBIA, MISSOURI

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# The Relationship Between Percentage of Live Spermatozoa and Motility, Longevity, and Fertility of Semen of Dairy Bulls

F. W. MADDEN, H. A. HERMAN, and E. R. BEROUSEK*

Widespread use of artificial insemination of dairy cattle and the need for highly fertile spermatozoa have brought out many tests to determine the quality of the semen to be used. Some of the more widely used tests are motility rating, concentration of spermatozoa, longevity in storage, morphological examination, cold shock, and determination of the percentage of live spermatozoa by use of a differential stain. Most of these tests have been correlated with actual fertility or other fertility indices and their value as a quality test determined.

Swanson and Herman (10), Trimberger and Davis (11), and Donham and co-workers (1) have found no significant correlation between average morphological abnormalities and conception rate. They also found no correlation between concentration of spermatozoa and conception rate.

Motility rating as suggested by Herman and Swanson (5) (as revised (10) was significantly correlated with conception rate (10) and showed a curvilinear relationship. Ellenberger and Lohmann (3) found little or no relationship between conception rate and motility where motility ratings were made by a scale of 0 - 100% motility which was subdivided into 10% increments. They did, however, find a direct relationship between conception rate and motility ratings of very progressive, progressive, and slightly or non-progressive motility. Donham, Simms, and Shaw (1) also found a good correlation betwen motility and conception rate when motility was classified as good or poor only.

Swanson and Herman (10) and Margolin, et al. (8) have found a highly significant linear correlation between the length of time a motility rating of 2 or better was maintained in storage and conception rate. Herman and Swanson (5) showed that longevity in storage of several different ejaculates gave a good index of the fer-

This study was aided in part by grants from M.F.A. Artificial Breeding Association, Springfield, Missouri, and Midwest Breeding Farms, Trenton, Missouri.

The authors wish to express their appreciation to Dr. Eric W. Swanson for reading and criticizing the manuscript, and to Mr. Kenneth Bowers for his assistance in collecting the samples and counting many of the differential slides.

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tility of a bull. The cold shock test as used by Lasley and Bogart (6) was significantly correlated with fertility when yolk-buffer-diluted semen was used for the test. The basis of the cold shock test is the difference between the percentage of live spermatozoa before and after the shock as shown by a differential stain.

Lasley, Easley, and McKenzie (7) developed a staining method for the differentiation of live and dead spermatozoa and showed the reliability of the method when used at different temperatures and by different workers. This differential stain has been used by Easley, et al. (2) to show the influence of diluters, rate of cooling, and storage temperatures on the survival of bull sperm. Lasley and Bogart (6) compared the percent of live sperm with the percent of conceptions and found a linear correlation up to about 50% live sperm and little difference in conception rate with higher percentages of live sperm. However, their data showed a relatively high percentage of live spermatozoa and a high mean motility with no motility rating below 2 when a motility scale of 0-6 was used.

This study was made to show the relationship between the percentage of live spermatozoa, as shown in the staining method of Lasley, et al. (7), and longevity, motility, and fertility of the semen. Since motility is a widely used quality index and both motility and longevity in storage have been correlated with actual fertility, these tests have been selected to use as comparisons with the percentage of live sperm.

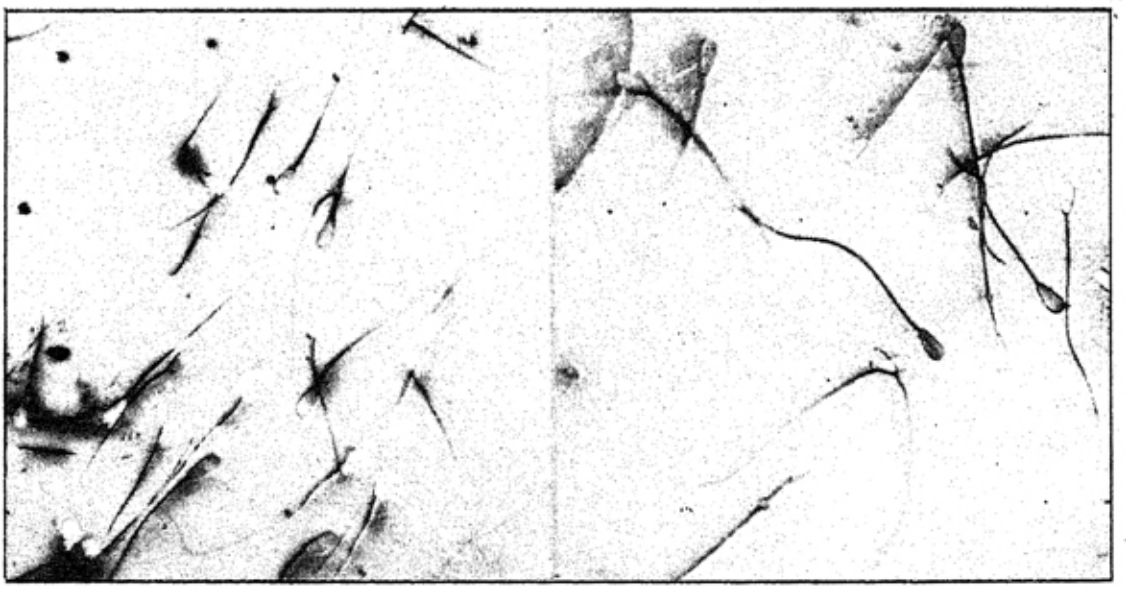

Figure 1.—Photomicrographs showing typical slides of differential staining of bull sperm. Unstained sperm were alive and those that are partially or completely stained were dead when the slide was made. Opal blue-eosin stain (x450).

## PROCEDURE

Semen from bulls in the Missouri Station dairy herd was used. Over a 12-month period studies were conducted on a total of 13 bulls, consisting of four Jerseys, eight Holsteins, and one Guernsey. The bulls ranged in age from one and one-half to sixteen years and all were in good breeding condition. Bulls were not used according to any definite schedule but collections were made as the breeding requirements and laboratory facilities permitted. Collections were made throughout the year at fairly constant intervals each week. The greater portion of the data was obtained during the period of January 1, 1946 to December 16, 1946. However, a few samples are included that were collected and studied by the junior author during the summer of 1942. These samples include some bulls outside the Station herd. Where these samples are included in the results a notation is made to this effect.

All collections were made by means of the artificial vagina as described by Herman and Ragsdale (4). Immediately after collection the ejaculates were placed in insulated vials and then placed in a thermos bottle of water at 65° F. After they were taken to the laboratory, they were gradually cooled down to 40° F. at which temperature they were stored. Storage was made in water placed in a refrigerator which maintains a temperature of 40° F. $\pm$ 4°.

Motility ratings were made immediately after collection, using a scale of 0 - 5 (10). Motility was rated each 24 hours until after the samples dropped below a 2 motility rating. At that time most samples were discarded.

The percentage of live spermatozoa was determined by means of the differential stain described by Lasley, et al. (7). Stains were dried rapidly by placing them on a hot plate (150° F.) in front of an electric fan. This dried the stain rapidly so that sperm which died during the process of making the slide could not absorb the stain after dying. A total of 333 sperm were counted on each slide in random fields over the entire slide. Differential stains were made on all undiluted samples soon after collection (within 30 minutes) and each 24 hours, at the time the motility ratings were made.

A total of 305 collections were included in the study. Some of the analyses are made on 196 samples due to incomplete data on more than this number. All analyses include samples that have complete data concerning the comparisons made. The fertility studies do not include all services made in the University herd during the year but do include all services made to cows that were known breeders and on which complete data had been obtained on the semen.

## RESULTS

### Percentage Live Sperm Compared with Motility

The initial motility ratings of fresh semen were compared with the initial percentages of live spermatozoa in the same samples. A total of 305 pairs were compared (solid line in Figure 2). Regression lines were fitted by the least squares method. The average motility of all samples was 3.37 and the average percentage of live sperm was 55.86. Using P = 0.01 as the level of significance, the correlation between initial motility and initial percentage of live sperm was highly significant with a coefficient of correlation (r) of .69. There was a wide variation found in the percentage of live sperm in each motility classification and the plotted linear relationship has an Sy (scatter about the line) of ± 15.9%.

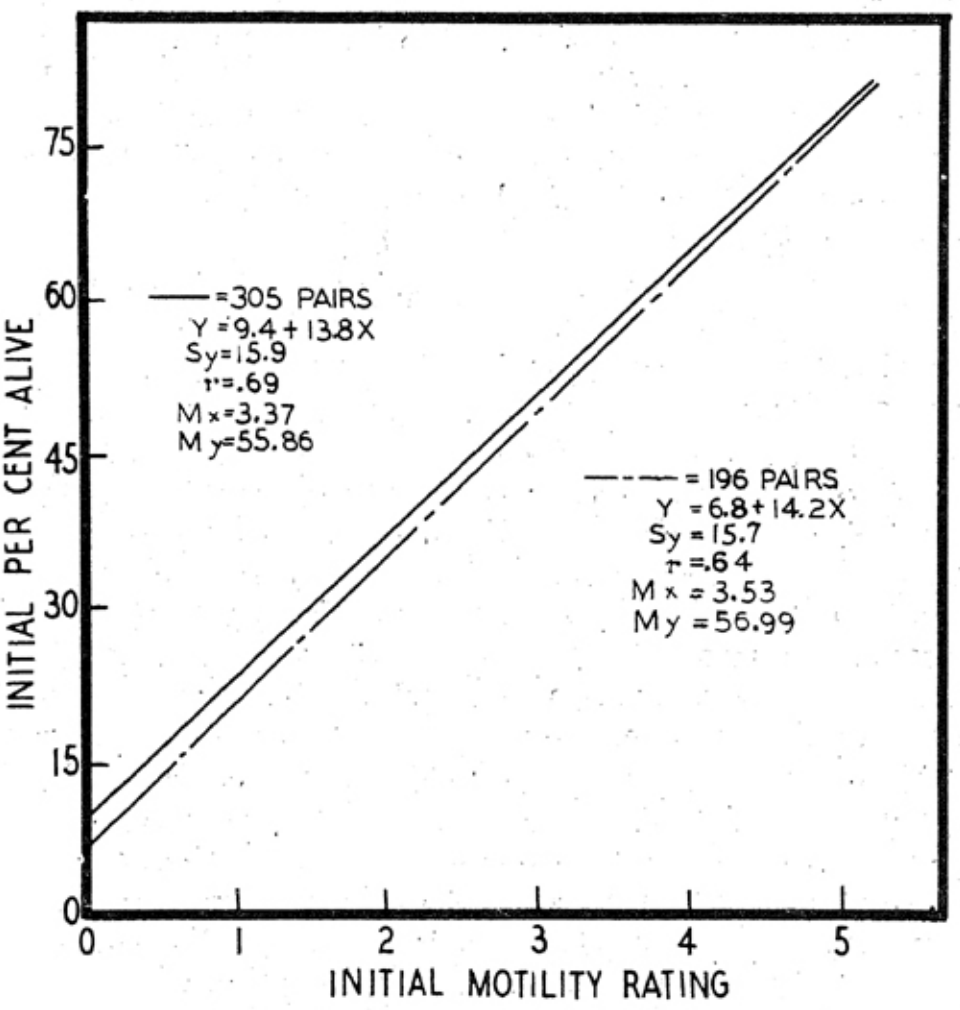


Figure 2.—Correlation Between Initial Motility and Initial Percentage Live Spermatozoa.

One hundred and ninety-six samples were used in comparing the percentage of live sperm with longevity; therefore, these 196 pairs were also compared for initial motility and initial percentage of live sperm (broken line in Fig. 2). There were only slight differences between the means of the 196 pairs and the means of the 305 pairs. The coefficient of correlation of these pairs was also highly significant.

A total of 1,116 motility ratings and differential stains were made on these 196 samples. A comparison of the average values of live sperm for each motility rating is given in Table 1. These values include all examinations during storage as well as the initial ratings.

TABLE 1.—AVERAGE PERCENTAGE LIVE SPERM IN DIFFERENT MOTILITY RATINGS AND VARIATIONS BETWEEN RATINGS

| Motility Rating | Number of Samples | Average % Live Sperm | Average Decrease in % Live Sperm | Range % Live Sperm |
|---|---|---|---|---|
| 5 | 70 | 74.27 | ..... | 41-93 |
| 4 | 152 | 67.91 | — 6.36 | 34-88 |
| 3 | 247 | 53.44 | —14.47 | 28-81 |
| 2 | 293 | 37.42 | —16.02 | 12-68 |
| 1 | 320 | 27.19 | —10.23 | 5-60 |
| 0 | 34 | 20.35 | — 6.84 | 0-50 |

The linear relationship (Figure 2) between initial motility and initial percentage of live sperm shows an increase of about 14% for each unit increase in motility rating whereas the actual averages of live sperm percentages in each motility classification show a roughly symmetrical parabolic variation. The general relationship between motility and percent live sperm using all observations is fairly similar to the values found with initial observations alone, but there were generally four to ten percent fewer live sperm within each motility class.

### Percentage Live Sperm and Motility Compared with Longevity in Storage

The initial percentage of live sperm was compared with the length of time the semen maintained motility rating of 2 or better in storage. The initial motility ratings were also compared with the storage time so that a comparison could be made of the value of the two tests as a means of predicting the length of time a semen sample will maintain a 2 or better motility. These results are shown in Figure 3.

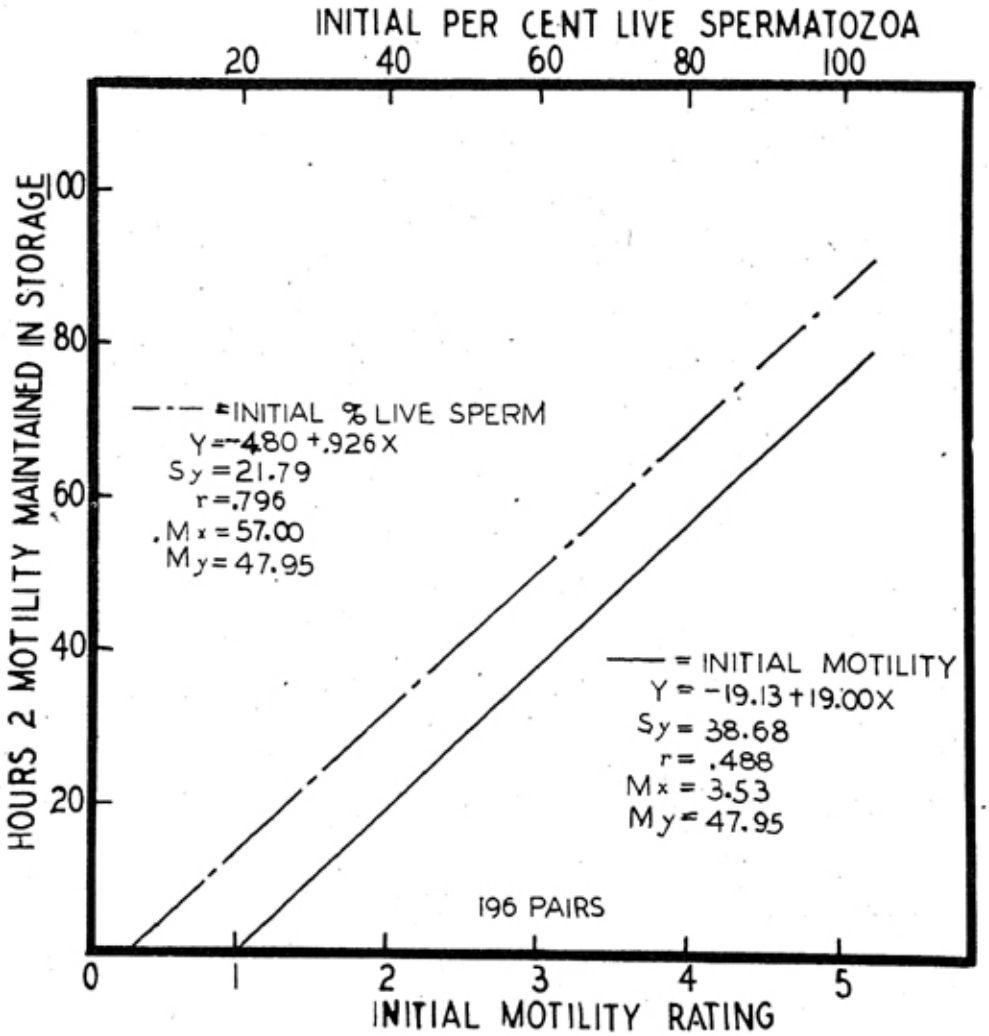


Figure 3.—Correlation between Initial Percentage Live Sperm and Hours 2 Motility Maintained in Storage. Correlation between Initial Motility and Hours 2 Motility Maintained in Storage.

The initial percentage live sperm was very significantly correlated with the length of time a 2 motility was maintained with an r of .796. A motility of 2 or better was maintained about .9 of an hour longer for each one percent increase in initial live sperm. A deviation of ± 21.8 hours (Sy) showed that there was a fairly wide variation in the relationship, however. The correlation between initial motility ratings and hours of 2 or better motility in storage was also highly significant with r = .488 but was not as closely correlated with storage time as was the initial percentage of live sperm. This relationship showed a 2 motility maintained for an average of 19 hours longer in storage for each unit increase in motility rating. The com-

parison of motility rating and storage time showed a wide variation with an Sy of 38.68 hours above or below the line of relationship.

The average motility and average percentage of live sperm were plotted for each 24 hour period to show the rate of decrease in motility as compared to the rate of decrease in live sperm (Figure 4). Sixty-eight different collections were used in this comparison since only these samples had complete data for the entire 96 hour period

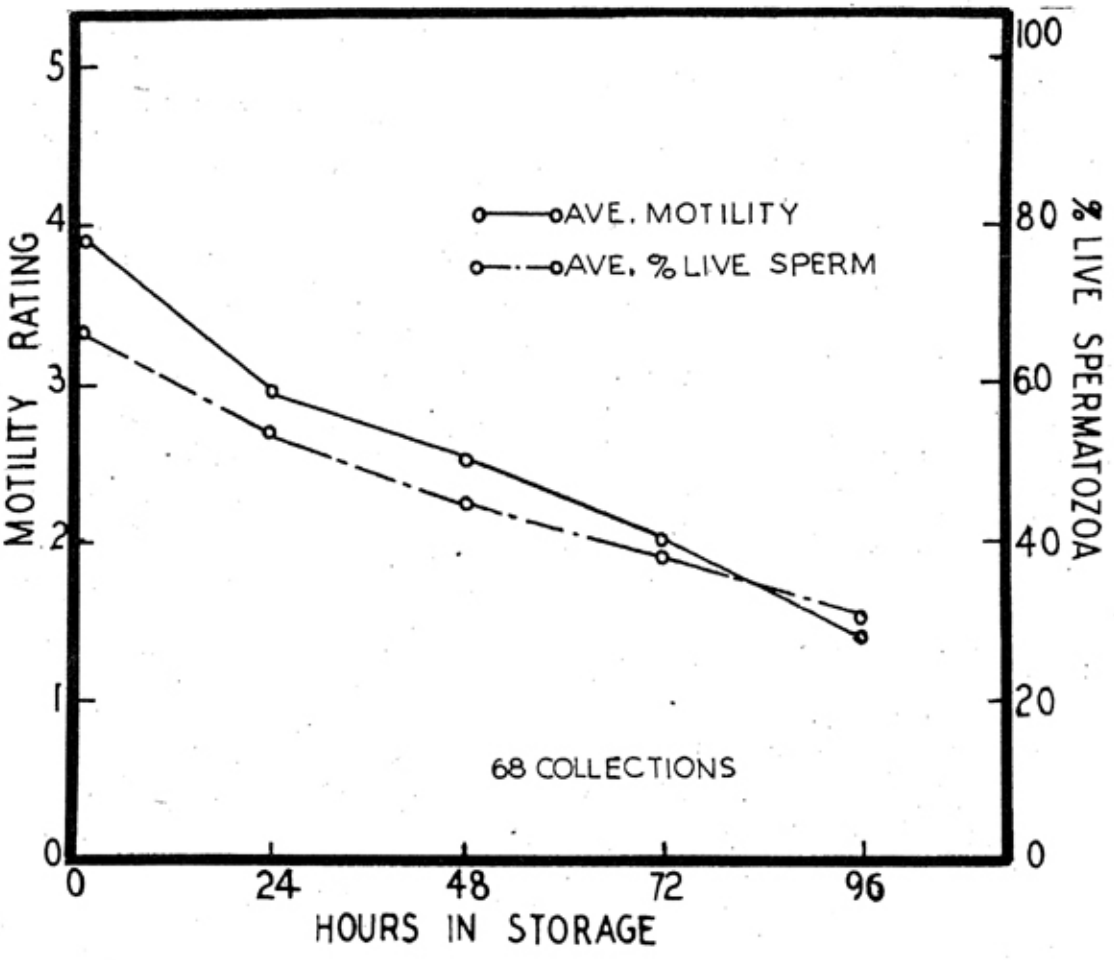


Figure 4.—Decrease in Motility and Percentage Live Spermatozoa During Storage

plotted. These collections were fairly representative of all collections, however, and the relationship between each motility rating and the average percentage of live sperm found with that rating was fairly constant with the motility rating and live percentage comparisons made. The rate of motility decline is about the same as found earlier at this station (5). The rate of decrease of live sperm was roughly parallel but showed a somewhat less rapid rate of decline.

While comparing the decrease in the percentage of live sperm with storage time, it was noted that a large percentage of the long-lived sperm had coiled tails. Twenty-one observations were made of semen samples from 15 different sires and are presented in Table 2. All of these samples had less than 10% live sperm when these morphology counts were made, except one which had 10.6% live sperm. After an average storage time of 296 hours, the live sperm were 61.9% coiled-tailed sperm, whereas only 17% were coiled-tailed in the same semen initially. This was true in most of the few cases observed.

Table 2. Percentage Coiled Tail Sperm in Semen after Storage until 10% or Less Remained Alive.

| Bull | Age Semen in Hours | % Live Sperm | % Live Sperm with Coiled Tails | % Live Sperm in Fresh Semen | % Coiled Tails in Fresh Semen |
|---|---|---|---|---|---|
| Freedom | 360 | 2.6 | 76.9 | 82.0 | 11.1 |
| Lad | 360 | 3.8 | 5.3 | 56.2 | 9.9 |
| 20 | 168 | 10.6 | 92.5 | 65.8 | 27.3 |
| " | 336 | 1.6 | 87.5 | 65.8 | 27.3 |
| 10 | 384 | 1.0 | 80.0 | 91.2 | 62.7 |
| 1 | 384 | 1.2 | 0 | 63.4 | 6.3 |
| 9 | 288 | 1.0 | 80.0 | 80.0 | 10.8 |
| 15 | 312 | 4.6 | 100.0 | 52.6 | 55.8 |
| " | 336 | 2.0 | 100.0 | 52.6 | 55.8 |
| 6 | 312 | 2.6 | 23.1 | 81.8 | 13.8 |
| " | 336 | 1.0 | 0 | 81.8 | 13.8 |
| Remus | 240 | 4.4 | 31.8 | 77.0 | 13.5 |
| 5 | 388 | 0.4 | 50.0 | 79.4 | 4.5 |
| 2 | 216 | 3.4 | 58.8 | 87.6 | 4.5 |
| FSHP | 312 | 1.4 | 100.0 | 82.6 | 6.6 |
| CCF | 240 | 2.4 | 7.6 | 73.0 | 2.1 |
| AMBAM | 144 | 4.0 | 70.0 | 67.0 | 2.7 |
| " | 168 | 2.2 | 54.5 | 67.0 | 2.7 |
| " | 240 | 1.6 | 60.0 | 67.0 | 2.7 |
| Design | 480 | 6.8 | 58.8 | 61.6 | 15.9 |
| Design | 312 | 4.6 | 69.6 | 63.2 | 14.7 |
| Average | 296 | 2.98 | 61.9 | 71.36 | 17.36 |

## Percentage Live Sperm and Motility and Storage Time Compared with Conception Rate

During the period included in this study 119 inseminations were made in the University dairy herd and these are included in the fertility study. Included are all services on which complete data as to initial motility rating and initial percentage of live sperm are available. Inseminations were made with fresh semen from eight different bulls. Only two cases were excluded from the study. In one case a cow died 12 days after being inseminated, thus making it im-

Table 3. Analysis of Semen used for Inseminations and Comparisons with All Samples from Each Bull.

| Bull | Service Record 1945 & 1946 | No. of Ejaculates Included | | | No. of inseminations | No./Ratio Conceptions | Conceptions | | | | | | Non-Conceptions | | | | | | All Samples Collected | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total services<br>Total conc.<br>Conc. ratio | Conceptions | Non-conceptions | Both | | | Ave. Motility | Range Motility | Ave. % Live Sperm | Range % Live Sperm | Ave. Hrs. 2 Mot. in Storage | Range 2 Mot. in Storage | Ave. Motility | Range Motility | Ave. % Live Sperm | Range % Live Sperm | Ave. Hrs. 2 Mot. in Storage | Range 2 Mot. in Storage | No. of Samples | Ave. Motility | Ave. % Live Sperm | Ave. Hrs. 2 Mot. in Storage |
| 69th (H) | 59<br>25<br>1:2.36 | 5 | 5 | 2 | 16 | 7<br>1:2.29 | 3.43 | 1-5 | 59.7 | 23-85 | 82.7 | 30-132 | 3.44 | 2-4 | 71.1 | 31-87 | 83.2 | 30-168 | 14 | 3.29 | 61.1 | 67.8 |
| Chieftan (H) | 39<br>23<br>1:1.70 | 7 | 6 | 2 | 20 | 10<br>1:2.00 | 3.90 | 3-4 | 67.9 | 55-86 | 40.5 | 15-84 | 3.35 | 2-5 | 57.3 | 22-86 | 30.9 | 9-90 | 32 | 3.47 | 53.3 | 34.7 |
| Zev (H) | 45<br>26<br>1:1.73 | 4 | 2 | 1 | 9 | 5<br>1:1.80 | 4.00 | 2-5 | 62.8 | 34-84 | - | - | 4.75 | 4-5 | 72.2 | 59-82 | 39.0 | 36-42 | 34 | 3.76 | 53.2 | 49.1 |
| Philidora (J) | 34<br>23<br>1:1.48 | 15 | 6 | 3 | 30 | 21<br>1:1.43 | 4.10 | 2-5 | 72.4 | 41-87 | 54.8 | 16-90 | 4.22 | 1-5 | 71.4 | 18-88 | 69.4 | 18-126 | 28 | 3.93 | 70.7 | 62.9 |
| Country-man (G) | 39<br>24<br>1:1.62 | 7 | 4 | 0 | 12 | 8<br>1:1.50 | 3.25 | 3-4 | 56.3 | 28-80 | 34.3 | 12-56 | 2.75 | 1-4 | 45.8 | 28-76 | - | - | 55 | 2.82 | 46.9 | 36.4 |
| Zirc (H) | 38<br>20<br>1:1.90 | 4 | 0 | 2 | 8 | 6<br>1:1.33 | 4.50 | 4-5 | 85.3 | 77-93 | 86.4 | 30-132 | 4.50 | 4-5 | 86.5 | 86-87 | - | - | 6 | 4.50 | 85.3 | 86.4 |
| Pan (H) | 35<br>22<br>1:1.59 | 6 | 2 | 1 | 12 | 8<br>1:1.50 | 3.75 | 3-5 | 58.5 | 31-76 | 66.4 | 24-98 | 4.00 | - | 70.0 | 59-74 | - | - | 39 | 3.18 | 49.3 | 47.4 |
| Boast (H) | 12<br>8<br>1:1.50 | 5 | 2 | 2 | 12 | 8<br>1:1.50 | 3.63 | 2-4 | 74.8 | 64-88 | - | - | 3.75 | 2-5 | 77.8 | 74-82 | - | - | 20 | 3.45 | 72.5 | 63.0 |
| All Bulls Total & Ave. | 299<br>169<br>1:1.77 | 53 | 27 | 13 | 119 | 73<br>1:1.63 | 3.85 | 1-5 | 67.9 | 23-93 | 53.1 | 12-132 | 3.75 | 1-5 | 67.2 | 18-88 | 64.0 * | 9-168 | 228 | 3.38 | 56.2 | 47.6 |

*Includes storage time on only 72% of cases.

possible to classify the service as to conception or non-conception. The other case involved 8 inseminations with semen from four different bulls and the cow was eventually sold as a non-breeder and was removed from the study. Of the 119 inseminations included in the comparisons, 73 resulted in conceptions for a ratio of one conception for each 1.63 services. This efficiency ratio is slightly better than the average for the herd over a period of time but may be because only good semen was used since only that which had been examined and accepted prior to use was included in these fertility comparisons. Each bull's service record may be compared with his record for the previous year to determine the accuracy of the representative samples used. The analysis of the semen resulting in conceptions or non-conceptions may be compared with the averages of all samples studied from each particular bull in order to compare the semen used for inseminations with a larger number of samples. The complete results of this study are presented in Table 3.

Very little difference was found between the semen which resulted in conceptions and that which did not. Wide variations were found in each of the three semen characteristics considered, both among samples and among bulls. It would appear that in this study where cows failed to settle on the first service that the semen used was not at fault. This observation has been repeatedly observed in other studies at this station.

## DISCUSSION

The percentage of live spermatozoa in semen, as determined by means of a differential stain, was compared with some "standard" quality tests for semen. These tests used as comparisons were motility and longevity in storage because these two quality tests have been correlated with actual fertility and because they are widely used as quality indices. The percentage of live sperm, motility rating, and length of time a 2 or better motility was maintained in storage have all been compared in semen which resulted in conceptions and semen which did not give conceptions.

By comparing the percentage of live sperm with motility ratings of the semen there was found to be approximately 75% live sperm for semen with a 5 motility, 63-68% in 4 motility semen, 50-53% in 3 motility classes, 35-38% in 2 motility, 21-27% in 1 motility, and less than 20% in 0 motility semen. Although these two characteristics were significantly correlated there were wide variations in the percentage of live sperm found in each motility classifications. The greatest decrease in average percentage of live sperm was found between the 2 and 3 motility classifications. This is logical since the

curvilinear relationship between motility and conception rate (10) has shown 3, 4, and 5 motilities to give little difference in conception rates. Low motility ratings might be given a sample with a high percentage of live sperm where the sperm, although alive, are weak, slowing moving, oscillatory, or non-motile. In the same manner a high motility rating might be given a sample of semen containing a relatively low percentage of live sperm if the sperm that are alive are vigorous, rapidly moving, and show strong progressive motility. Motility ratings may be criticized as being only a rough classification based upon estimates of number and strength of motile spermatozoa, although it is a rapid, easy means of estimating semen quality. The live sperm percentage may also be criticized as not necessarily being indicative of sperm resistance and motility. A low percentage of vigorous sperm may easily give better conception rates and maintain a satisfactory motility in storage longer than semen containing a much higher percentage of live sperm that may be weak or non-motile even though alive. These considerations will aid in accounting for many of the apparent discrepancies between the motility rating and the percentage of live sperm in individual samples. These two tests might be used together to supplement the inadequacies of each.

In the comparison of the two tests, motility and percentage of live sperm, with the length of time a 2 motility rating or better was maintained in storage the two were very similar. The percentage of live sperm present in semen might serve as a more accurate index of the probable storage time since it was more highly correlated with actual storage time and there was less variation found in this correlation than was present in the motility—storage time correlation. Part of this superior relationship might also be attributed to the greater range of possible live-percentage values, thus making each rating more exactly fitted to the individual sample considered. The motility ratings used offered a fairly non-versatile range of values and might be affected by the variations and extremes possible within each motility classification.

The rate of decrease of both motility rating and the percentage of live sperm seem to indicate that either might be used as an index of semen quality with fairly comparable results. A combination of the two may, again, give a better picture of longevity and the retention of viable, vigorous sperm during storage. In some cases the loss of progressive motility seemed to be the limiting factor during or after storage. In other cases the relatively low percentage of live sperm caused the semen to lose motility and give gross microscopic appearance of being unsatisfactory for insemination use.

The comparison of semen resulting in pregnancies with semen which resulted in non-conceptions shows little difference in average motility, percentage of live sperm, and longevity in storage. These results should be expected where only semen which shows good degree of motility and live spermatozoa is used for insemination. Since only one spermatozoon is actually required for fertilization, a greater number of strong live sperm will not necessarily give any different results. The necessity of classifying the semen into only two groups, either resulting in conceptions or in non-conceptions, automatically decreases the probability of the average comparisons showing any decided differences where good quality semen is used in both cases.

## SUMMARY

The differential staining technique for determining the percentage of live sperm in semen has been used with dairy bull semen. The value of this stain as a quality test for semen has been studied by correlating it with motility rating and longevity of sperm in storage, two quality tests which have previously been shown to be significantly correlated with conception rate.

The initial percentage of live sperm in semen was significantly correlated with the initial motility of the semen. However, this relationship was fairly variable and neither test necessarily gave an accurate reflection of the other. The reason for these variations have been suggested. The two tests may be used to supplement each other, and may provide a more accurate analysis of the semen.

The percentage of live sperm was compared with motility rating during storage and both values decrease at a fairly uniform rate with the percentage of live sperm showing a slightly more uniform rate of decline than did motility.

No significant differences were noted in motility, longevity, or the percentage of live sperm between semen which resulted in conception and semen which did not give conceptions. Except in extreme cases the percentage of live sperm in semen did not seem to be a worthwhile test for estimating the value of semen to be used for inseminating purposes.

Determination of the percentage of live sperm by differential staining did not appear to be a more accurate quality test than was motility rating or longevity in storage. The percentage of live sperm may be obtained by this method for comparisons in research work but would not generally warrant its use as a routine test in artificial breeding organizations. It is, however, a test which would logically be used in appraising the semen of bulls of low fertility, or for bulls under consideration for purchase.

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