CHARACTERISTICS OF SEMEN OF YOUNG DAIRY BULLS MAINTAINED ON TWO LEVELS OF PHOSPHORUS INTAKE

By

ROBERT L. MONTGOMERY

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Thesis Approved:

Stanley D. Musgrave
Thesis Adviser

O. L. Horton
Faculty Representative

Coleman Rankin
Dean of the Graduate School

361559

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TABLE OF CONTENTS

INTRODUCTION

Literature dealing with bovine semen has been based almost exclusively on that produced by bulls that may be generally classified as mature. In instances where experimental data have been obtained on the semen of young dairy bulls, the semen was evaluated on standards developed from mature bull semen (3, 21, 41, 52, 54, 77, and 85). The validity of such procedures and interpretations based thereon may be seriously questioned.

A primary purpose of the data reported here has been to formulate standards by which the semen of young bulls might be evaluated, as well as to study the semen of young dairy bulls that were maintained on known but varying intakes of phosphorus. Another objective was to obtain semen data on young dairy bulls raised under climatic conditions existing in the Southwest.

REVIEW OF LITERATURE

Experimental data concerning dairy bulls and their semen is voluminous. In order that these data may be readily comprehended the subject matter has been subdivided as follows: storage time; volume; motility; percent of abnormal spermatozoa; concentration of spermatozoa (both alive and dead); pH; effect of age; effect of exercise; effect of ration; effect of climate; effect of collection procedure; effect of diluter; effect of antibacterial agents; and the effect of cooling and storage on semen quality.

Storage Time

The number of days that spermatozoa exhibit the property of movement has been defined as "storage time". This definition has been arbitrarily modified in various ways by some workers in the field. Herman and Swanson (32) found that time of survival with good motility was a good index of fertility. This observation was based on 342 ejaculates of semen produced by 55 dairy bulls. Swanson and Herman (76) were able to show a linear relationship between viability or livability of spermatozoa and conception rate. A 2.6% increase in conception rate per 20 hour increase in storage time of undiluted semen was shown. Weeth and Herman (80) found that a 20 hour increase in storage time was associated with a 1% increase in per cent non-return rate. Swanson and Herman (74) had shown earlier that storage time had a higher correlation with fertility than other characteristics studied. Weatherby et al. (79), working with 108 paired first and

second ejaculates, found that the livability of spermatozoa in second ejaculates exceeded that of first ejaculate spermatozoa for all bulls studied.

Volume

Lagerlof (32) found the average ejaculate to contain 3 c.c. of semen with a range of 2 to 8 c.c., whereas, Herman and Ragsdale (31) reported an average volume of 4.2 c.c. with a range of 2.1 to 6.1 c.c. Their data were based on 161 ejaculates from 12 bulls.

Davis and Williams (19), working with 190 to 224 ejaculates from 11 fertile bulls, found correlations of 0.51 or less when the volume of the ejaculate was related to pH, concentration and motility. In this study 3 ejaculates were collected from each bull on each day of collection. Swanson and Herman (74) found no relationship between the volume of semen produced by a bull and his fertility level. VanDemark and associates (78) obtained a highly significant correlation between volume of semen and the methylene blue reduction time of the spermatozoa.

Magill and associates (48), working with one pair of Jersey identical twin bulls found that the volume of semen per ejaculate was essentially the same when both animals were handled in the same way prior to collection. However, their results with one pair of crossbred monozygotic bulls showed significantly ($P<0.01$) that teasing for five minutes resulted in a superior volume per ejaculate. Both pairs of bulls were less than 13 months of age. During the period of 13 to 16 months of age one bull of each twin pair mounted a live animal while the other bull of each pair mounted a dummy. With this procedure there was no difference in volume. Later when the bulls were 17 to 20 months of age, one bull of each twin pair was teased for five minutes prior to

collection. There was a significantly superior volume ($P<0.01$) per ejaculate in favor of the teased Jersey bull over his twin. Between the crossbred pair there was no difference due to treatment.

Patrick and associates (55) found no significant difference in volume per ejaculate where one group of bulls was ejaculated once each four days; a second group was ejaculated twice every eighth day; or in the third group which was ejaculated three times every twelfth day.

Weatherby and associates (79), working with 108 paired first and second ejaculates from three mature bulls, found the second ejaculates to be almost twice as large as the first.

Davis, Kuhne, and Miller, as reviewed by Herman and Swanson (32), found that volume per ejaculate was affected by the method of collection. Rectal massage produced larger volumes per ejaculate than did other collection methods.

Lasley and Bogart (43), working with normal range bulls found volume per ejaculate to be positively correlated with fertility.

Wiggins and associates (81) found a slight correlation between volume per ejaculate and per cent of ewes lambing. Rambouillett, Columbia, and Targhee rams were used.

Motility

Branton and associates (10) stated that the per cent of motile spermatozoa in an ejaculate should be at least 50%. Davis and Williams (19) suggested that per cent of motile spermatozoa in combination with pH would give a better appraisal of semen quality than any individual observation.

Swanson and Herman (74) could distinguish between bulls of high or low fertility on the basis of the ability of the spermatozoa

to exhibit vigorous motility. They suggested that semen of good fertility should maintain a high degree of motility for 30 hours or more at 40° F.

Beck and Salisbury (5) found that the decrease in per cent of motility in a water bath for one hour at 46.5° C. was highly correlated with the decrease in motility in storage for 10 days at 5° C. ($r = 0.9088$). VanDemark and associates (78) obtained a highly significant ($P<0.01$) correlation between initial motility and methylene blue reduction time. They recommended that initial motility, spermatozoan concentration and methylene blue reduction time should be utilized for routine prediction of semen quality.

Madden and associates (47), working with 13 dairy bulls that were not on any definite collection schedule, studied the relationship of rate of initial motility to per cent of motile spermatozoa as well as the effect of each on conception rate. The comparison of semen resulting in conceptions with semen resulting in non-conceptions showed little difference in average rate of motility or per cent of live spermatozoa. They found a wide range in per cent of live spermatozoa in each motility classification. A comparison of initial per cent of live spermatozoa with the hours that a rate of 2 in motility was maintained also showed a wide variation of values. Similarly, a comparison of rate of initial motility with the hours that a rate of 2 was maintained gave a wide variation. The variability of the data reduces the emphasis that could otherwise have been placed on the fact that all three of these comparisons yielded highly significant ($P<0.01$) correlations.

Swanson and Herman (76) found a curvilinear relationship between motility of spermatozoa and conception rate. They observed that there was little difference in the conception rate resulting from ejaculates rated 3 to 5 with over 45% progressively motile spermatozoa. Weeth and Herman (80) were able to show that the hours duration of a motility rate of 1 in diluted semen was significantly correlated with per cent non-returns ($r = 0.198$). Therefore, routine determination of this value should be of value to artificial breeding associations.

Ehlers and Erb (23) compared initial motility and motility in storage (up to 6 days) with 60-90 day non-returns for 7,421 first and second inseminations from 275 ejaculates by 18 bulls. The relationship between initial motility and non-return rate was low ($r = 0.138$). However, the r values between motility during storage and non-return rate were highly significant ($P<0.01$) and were higher: 1 day $r = 0.205$; 2 days $r = 0.335$; 3 days $r = 0.335$; 4 days $r = 0.317$; 5 days $r = 0.306$; and 6 days $r = 0.291$. An analysis of variance of their data showed a significant difference ($P<0.05$) between bulls and between ejaculates. The ability of semen to withstand storage up to 6 days was deemed to be a better criterion of fertilizing capacity than initial motility.

Stone and associates (72), working with 236 ejaculates from 19 dairy bulls, found highly significant ($P<0.01$) differences among bulls with regard to initial motility, duration of time in hours that a rate of 2 was maintained and duration of time that any motility was maintained in undiluted semen. Likewise, they found highly significant ($P<0.01$) differences among bulls for: initial per cent of live spermatozoa; per cent of live spermatozoa following cold shock; and fertility. A multiple correlation coefficient of 0.69 ($P<0.05$) was obtained for per cent of

motile spermatozoa following cold shock, spermatozoan concentration and fertility. Harvey and Hulet (30), in studying reproductive efficiency of young Jersey and Holstein bulls, found considerable variation within bulls of the same age and breed with respect to initial motility and the rate of decrease per day in motility. They found initial motility to increase with age up to 13 months. At that point this characteristic tended to level off. With the increase in initial motility they obtained an increased rate of decline of motility with storage. The age of the bull was not related directly, at least to decline of motility with storage.

Johnston and associates (33) presented data from 350 ejaculates from 14 bulls that were used on 15,029 first service cows. They found that initial motility and per cent of live spermatozoa were highly significantly ($P<0.01$) correlated with 60-90 day non-return rate. Other criteria utilized failed to show significant correlation to fertility.

Magill and associates (48), working with Jersey and crossbred twin bulls, found a significant difference ($P<0.05$) between bulls in per cent of motile spermatozoa after 168 hours of storage.

Herman and Swanson (32) observed that the length of time that spermatozoa survived in storage was a good index of fertility while initial motility was related to fertility to a very limited extent.

Lasley and Bogart (43), working with range bulls, found per cent of live spermatozoa to be positively correlated with fertility. They were not able to show significant correlations between fertility and per cent of spermatozoa surviving cold shock or rate of spermatozoan motility in undiluted semen. They found that as the interval between

collections increased, the per cent of spermatozoa retaining motility after cold shock decreased. During the breeding season the per cent of live spermatozoa per ejaculate showed definite variation.

Lasley (42), observed 78 ejaculates from 7 Hereford bulls and found the average per cent of motile spermatozoa to be 64.1 with an average progressive motility of 51.6%. After 4 days storage in egg yolk-phosphate buffer these same ejaculates averaged 34.9% motility. A highly significant ($P<0.01$) correlation was found between per cent of live spermatozoa and progressively motile spermatozoa in fresh semen. The correlation ($r = 0.314$) between per cent of motile spermatozoa and fertility was significant ($P<0.05$), but low. However, the correlation ($r = 0.167$) between progressively motile spermatozoa and fertility was not significant.

Comstock and Brady (16) found a curvilinear relationship between per cent of motile spermatozoa after storage for 2 and 5 days and head length of the spermatozoa. Wiggins and associates (81) observed that rate of motility, per cent of motile spermatozoa, and estimated motile spermatozoan concentration were significantly correlated ($P<0.05$) with the per cent of ewes lambing.

Abnormals

The abnormality of a spermatozoa is recognized by its shape. Lagerlof (38) has categorized them into types as follows: not normal size; twin heads or twin tails; abnormal shape of head; abnormalities on connecting piece or tail; deficient staining capacity; and loose heads. Normal bull semen has been found to contain 10-12% of abnormal spermatozoa. Lagerlof (39) also reported that sterile bulls and old bulls with reduced fertility produced semen with 33-35% abnormal

spermatozoa per ejaculate. Green and Comstock (29) suggest that an abnormality concerning the head of the spermatozoon is indicative of a more fundamental physiological injury than is an abnormality of the tail. They state that the type of abnormality should be considered along with the per cent.

The method of preparing smears for making abnormal counts may affect the number of abnormals according to Salisbury and associates (68). Frequency of ejaculation was also pointed out as a factor influencing the percentage of abnormal spermatozoa by McKenzie and Phillips (49). They found that a sexual rest of several weeks or more, materially increased the abnormal count. Differences between bulls have been shown in this respect by Magill and associates (48).

Attempts to associate the percentage of abnormal spermatozoa per ejaculate with fertility have not yielded significant correlations (32, 33, 43, 74, and 76). VanDemark and associates (78) did not obtain significant correlations between per cent of abnormal spermatozoa and methylene blue reduction time, initial ascorbic acid content, initial glucose level, or glucose loss in diluted semen after 10 days storage at 5° C.

Concentration and Dilution Rates

Evaluation of spermatozoan density, or concentration, was originally determined by use of the hemacytometer (63). All other methods have been based on the density of the ejaculate. Visual comparison with opacity standards, based on hemacytometer counts, has enjoyed but limited use (63). The most common method of determining the number of spermatozoa per unit volume has been the use of the photolometer. The validity of the use of this technique was established by Salisbury

and associates (63), Willett and Buckner (83), and Emick and Sidwell (24).

The number of spermatozoa per ml. has been reported by many workers (8, 32, 38, 39, and 79). The average concentration reported has ranged from 800 million to 1,150 million with a range of 300 million to over 2,500 million spermatozoa per ml.

Attempts to correlate concentration of spermatozoa with fertility have been conducted on one of two bases: (a) semen diluted to an arbitrary volume regardless of spermatozoa present; and (b) semen diluted to a constant number of live spermatozoa per ml. There were no significant correlations between concentration and fertility as long as each volume used per insemination contained 12 million or more spermatozoa (12, 33, 61, 62, 65, and 76), or where semen was diluted to contain a constant number of spermatozoa. However, when semen was diluted to such an extent that it contained less than 12 million live spermatozoa per ml. there was significant relationship between concentration and fertility (11, 64, and 82). Lasley and Bogart (43) working with range bulls found a relationship between number of spermatozoa resistant to cold shock and fertility that was higher than the relationship of number of spermatozoa inseminated and fertility.

pH

Experimental data regarding the pH of freshly ejaculated bull semen indicate a range of values from 5 to more than 7 (21, 32, and 69). The average pH of bull semen was described by Shergin (70) as 6.74. He noted that the pH of semen of a given species is usually lower than the blood pH for that species.

The method of semen collection has been found to have an effect on the pH of a given ejaculate. Semen collected by rectal massage is almost always alkaline, whereas that semen collected via the artificial vagina is acid unless contaminated with urine or other contaminants or is produced by very young bulls (18, 21, and 32).

Ejaculates having a pH greater than 7.0 are usually thin and discolored, whereas those of 6.8 or lower are associated with high concentration and motility (32, 69). Schneerson (69) presented evidence that as the pH of the ejaculate rises the number of spermatozoa, survival of spermatozoa at room temperature and resistance to salt solution all decrease. Others (19, 32, 76, and 81) have found no significant relationship between conception rate and pH. Dubincik (20) found that motility of spermatozoa of various farm animals stopped at a pH of 4.2 but resumed if a pH shift toward alkalinity was made. He demonstrated irreversible immotility when the pH was allowed to drop to 3.4-3.5.

VanDemark and associates (78) found a highly significant correlation between pH and methylene blue reduction time.

Age

The age at which sexual interest was first shown and the age at which semen was first collected from young experimental dairy bulls has been reported by several workers (3, 21, 30, 52, and 77). Baker and VanDemark (3) using nine Holstein male calves, found that sexual interest was first exhibited at 25-45 weeks of age. A teaser female was used in this study. The same bulls produced first ejaculates at 36-46 weeks of age. These findings support data presented by Dunn (21) and Musgrave (52).

Harvey and Hulet (30), using young Jersey and Holstein bulls, reported semen production from these bulls began at 8 or 9 months of age. Considerable variation existed in initial motility and in average daily drop in motility between bulls of the same age within a breed. The authors suggested that some bulls may reach their peak at an earlier age than others. Initial motility was found to increase with age up to 13 months, where it began to level off. As initial motility increased, the rate of decline under storage also increased. Age, however, was not directly associated with decline under storage.

Dunn (21) and Musgrave (52) have shown that the age at which the first ejaculate is produced can be altered considerably by the level of nutrition. They were able to collect ejaculates at 28 and 30 weeks of age from two bulls. Using a Holstein bull calf, VanDemark and associates (77) were able to obtain the first ejaculate when the calf was 38 weeks of age.

Williams (85) states that mature bulls produce a much greater volume of semen per ejaculate than young bulls.

Exercise

The effect of exercise on semen production by dairy bulls has been the subject of some controversy. Bartlett and Perry (4) reporting on three bulls in an artificial breeding stud, state that regular systematic exercising of the bulls yielded as much as 51% increase in semen produced. Total volume for three bulls for one month (August) when no exercise was given was 147 c.c. The next month when the bulls were walked at a rate of two and one-half miles per hour, semen production totaled 223 c.c. for the month. This increase was attributed to the forced exercise given, since the rest of the management program

remained fairly constant.

However, Snyder and Ralston (71) conducted a study with eight Holstein and eight Guernsey bulls. These bulls were forced to exercise six days a week for six months. A comparable group of bulls was given no exercise. No significant differences were found between the groups in semen quality and in non-return rates.

Ration

The effect of ration on semen production of young dairy bulls was studied by Lardy and associates (41). They reported low spermatozoan count, small volume, poor motility and short storage periods for three bulls reared on a low manganese ration. In contrast, semen from three bulls fed the same ration of corn, corn gluten, timothy hay and minerals plus a supplement of manganese was found to have normal volume, high spermatozoa count, excellent motility, and long livability of spermatozoa. Three other bulls fed a supplement of ground oats produced semen comparable to that of the bulls on the manganese supplement.

Branton and associates (8), in comparing the effects of protein of plant and animal origin on semen production and fertility of dairy bulls, fed three rations to 18 bulls. A "double change over" experiment with three 120-day periods was used. The two protein supplements of plant source fed were corn gluten feed and soybean oil meal. The animal protein was skim milk powder. Timothy hay was fed to all groups. The three feeds appeared about equal, based on semen data. However, the per cent non-returns showed the soybean oil meal significantly better than the skim milk powder. Per cent non-returns for the skim milk powder, corn gluten feed and soybean oil meal, respectively, were

found to be 61.6%, 63.5% and 65.7%. In a study of 17 young Jersey and Holstein bulls, Harvey and Hulet (30) found that bulls fed a regular dairy ration plus a fish oil (vitamin A) supplement produced semen averaging 1.1 points higher in initial motility rate than a control group that received no vitamin A supplement. Motility was estimated on a basis of 0-10. They found that the ration had no apparent effect on average daily drop in motility of semen under storage for six days.

Musgrave (52) conducted a study using 36 Holstein male calves. Three groups were fed rations varying in TDN, protein, mineral and vitamin content. The levels of nutrition for each group were 75, 100, and 140% of the upper limit of Morrison's TDN standard for growing dairy heifers. Semen data were obtained on six of the calves in each group with the exception that one bull in the lower group failed to produce any semen in the 48 week trial. The other five bulls in this group produced first ejaculates from 38 to 44 weeks of age. Bulls fed the normal ration produced first ejaculates from 32 to 42 weeks of age, while the bulls receiving the 140% ration began semen production from 30 to 38 weeks of age.

Ejaculates containing motile spermatozoa were first produced from 44 to 46 weeks by the lower group; from 34 to 44 weeks by the normal group; and from 34 to 38 weeks by the upper group. Motility rate and percent in semen of the normal group was found to be low until 42 to 46 weeks, while the group receiving the 140% ration averaged 58% motility with an average rate of 3.0 at 38 weeks. The first ejaculates with sufficient concentration to be measured by routine laboratory procedures for the lower, normal and upper groups were produced at 46, 42 and 36 weeks respectively.

In a further study of the effect of three levels of nutrient intake, Dunn (21) modified the nutrient levels used by Musgrave (52), and used 60, 100, and 160% of the upper limit of Morrison's recommended TDN allowances for growing dairy heifers. Ages at which the lower, normal and upper group first produced ejaculates were reported to be 44 to 46, 42 to 46, and 28 to 38 weeks, respectively. Three bulls in the 60% and one bull in the normal group were reported to have produced no semen at 48 weeks of age when the trial ended. Motile spermatozoa were first produced at 44, 42 and 34 weeks of age by the lower, normal and upper groups, respectively. These samples were the first of which a measurement was made for spermatozoan concentration. This work was in agreement with that done by Musgrave (52).

Climate

Knowledge of the extent to which climatic conditions affect semen quality and fertility is relatively limited. However, definite variations in semen quality and in fertility have been related to season of of the year by some workers (25, 50, 56, and 59). Swanson and Herman (75) found significant differences ($P<0.05$) between months regarding initial motility and storage time. The fact that poorer quality semen was produced in the winter was attributed partly, however, to the old age of the bulls and to the influence of inadequate housing facilities.

In comparing non-return rates from 93,113 first and second services for Guernsey, Jersey and Holstein bulls over a six year period, Erb and Waldo (25) found a highly significant ($P<0.01$) variation between months. Non-returns were lowest in January and highest in September. The period of July through December averaged 3.4 per cent higher than January through June in non-return rates. This difference between six

month periods was found to be highly significant also ($P<0.01$). Monthly correlations were made of initial motility, spermatozoan concentration, motility after 30 minutes incubation at 45° C. and drop in motility during incubation with non-return rates. These relationships were reported to be quite variable from one period of the year to another. Therefore, the usefulness of the quality tests mentioned might well vary with the season of the year.

Results of the analysis of 448 ejaculates from 10 bulls for initial motility were reported by Mercier and Salisbury (50). Highly significant ($P<0.01$) variation was found between months. The highest mean per cent motility was found in March and the lowest in December. Phillips and associates (59) reported on work done with six bulls collected every two weeks. No significant difference was found between motility score and seasons. From 1135 matings made from 1935 to 1942, the highest fertility results were obtained in April (59.6%) and the lowest in August (40.8%).

A study was made of two bulls in a controlled chamber with the temperature at 80° F. and the humidity at 19 mm. of Hg. as compared with two bulls under ordinary environmental conditions by Patrick and associates (56). The trial lasted from June through August. Ninety-one per cent of the ejaculates from bulls in the chamber was usable, while only 47% of ejaculates from the control bulls was usable. Breeding efficiency for the bulls kept at 80° F. was 73.6%, while for the control bulls was 71.9%. During July and August, semen collected from the two bulls in the latter group was found to be very poor in quality.

Collection

Kust (37), using Russian techniques, found sperm collected with an artificial vagina better than that collected by the rectal massage method. He stated that besides being slow, the rectal massage method produced contaminated spermatozoa.

Branton and associates (9), conducted an 18 week study using six mature bulls to determine the relationship between sexual excitement, semen production, fructose content, and fertility. Three levels of restraint were used, namely, no restraint, one false mount, and two false mounts. Criteria of response were volume, initial motility per cent, number of spermatozoa per ml. ($x10^6$), number of spermatozoa per ejaculate ($x10^6$), number of motile spermatozoa per ejaculate, mg. of fructose per ml., per cent usable ejaculates, and per cent 60-90 day non-returns. All of these criteria were found to be influenced markedly by the level of restraint. The first level (no restraint) gave poorer average results than the other two methods. The authors recommended one false mount for routine practice by bull studs.

Using two sets of monozygotic twin bulls, Magill, et al. (48) found that bulls showed decreased libido when dummy mounts were used instead of live mounts.

In a study to determine the effects of various time intervals between collections upon semen production and fertility, Patrick and associates (55) used six bulls for 180 days. Three treatments were applied, using two 3X3 latin square designs and three sixty-day periods. The treatments were one ejaculate every fourth day; two ejaculates every eighth day; and three ejaculates every twelfth day. No significant differences were found between treatments in comparing

volume per ejaculate, per cent motility, methylene blue reduction time and per cent of shippable ejaculates. Fertility results, as measured by 30-60 days non-returns, showed no significant differences.

In an effort to determine the effect of frequency of ejaculation upon semen characteristics and libido, Baker and VanDemark (3) made a study of nine Holstein male calves. Calves were assigned at random to three groups, with collections made once, twice, or thrice weekly. The semen characteristics were highly variable. Total spermatozoa, per cent motility, per cent normal spermatozoa, and rate of motility generally increased in all three groups in the first eight weeks of collection. Two bulls, however, in the thrice-weekly collection group showed a reduction in libido by the sixteenth week of collection.

In comparing first and second ejaculates, Weatherby et al. (79) made a study of 108 paired ejaculates from three mature bulls. Using volume, concentration and livability as the criteria, the second ejaculates were superior in average volume and longevity. Two bulls showed higher average concentration in the second ejaculates while the third bull had a higher average concentration in the first ejaculates. An average volume from the three bulls for the first ejaculates was 3.4 ml., while the second ejaculates had an average volume of 6.2 ml. Likewise, average livability for the first ejaculates was 9.3 days, and for the second ejaculates, 12.3 days. Concentration averaged 1,269 million spermatozoa for the first ejaculates and 1,247 million spermatozoa for the second ejaculates.

VanDemark and associates (77) reported on semen production of one Holstein bull ejaculated three times per week for three consecutive years after puberty. During the first year the 153 ejaculates averaged 2.8 ml.,

with a concentration of 1,089 X 10^6 spermatozoa per ml. and an average initial motility of 56%. The second year the 151 ejaculates averaged 3.8 ml. in volume, with a concentration of 1,530 X 10^6 spermatozoa per ml., and an average initial motility of 59%. The 143 ejaculates collected during the third year had an average volume of 5.0 ml., an average concentration of 1,513 X 10^6 spermatozoa per ml. and an average motility of 57%. During the third year the bull was proven fertile, with no indication of a reduction in libido or semen quality.

Diluters

In work done with diluters for bull semen, Phillips (58) showed that an egg yolk-phosphate solution served as a metabolite as well as a diluter for the semen.

Lardy and Phillips (40) compared the hours that motility of spermatozoa was maintained in an egg yolk-buffer diluter with that of semen alone, using semen from two bulls, a stallion, a ram and a turkey. In every case spermatozoan livability was considerably greater when the semen was diluted. In only one instance did undiluted spermatozoa survive up to 24 hours, while diluted spermatozoa from the same ejaculate maintained motility for 388 hours.

In order to determine the differences in extending semen before cooling as compared to extending after cooling, Foote and Bratton (26) compared 11 split ejaculates on the basis of mean per cent of motile spermatozoa during six days of storage in yolk-citrate-sulfanilamide extender. A comparison between slow and rapid cooling was made with the same ejaculates. All samples were extended at a rate of 1:100. Mean per cent of motile spermatozoa during storage for semen diluted after cooling was 29% and 47% for the rapid and slow-cooled samples,

respectively. Using the same criterion, semen cooled after dilution showed a mean per cent motile spermatozoa count of 57% and 62% for the rapid and slow-cooled groups, respectively.

Sixty-four split ejaculates were cooled with and without extender and stored after dilution at 5° C. Per cent motility estimates were made at 0 and 2 days of storage. Average readings for semen cooled after dilution were 63% and 51%, while semen cooled before dilution averaged 48% and 34% motility at 0 and 2 days, respectively. Comparison of per cent of 28-35 day non-returns of semen cooled before dilution with that cooled after dilution was made with 32 of the 64 ejaculates, using 3,067 first service cows. The results of 71% non-returns for semen cooled before dilution as compared to 76% for semen cooled after dilution showed a statistically significant difference ($P<0.05$).

Similar work reported later by Foote and Bratton (27) gave essentially the same fertility results, in comparing pre-extended semen (diluted with yolk-citrate-sulfanilamide extender before cooling) with post extended semen (semen diluted after cooling). Sixty four ejaculates from 31 Holstein bulls were divided into two equal portions, with one portion being pre-extended, while the other was post-extended. Comparison of the two cooling procedures was based on the percentage of 60-90-day non-returns with inseminations to 8,518 first and second service cows. Semen in both treatments was cooled from 30° C. to 5° C. in 75 minutes. Fertility level of the two groups were: pre-extended semen, 59.3% and post-extended semen 52.8%. The difference between treatments was statistically highly significant ($P<0.01$).

In a study made to establish the best concentration of sodium citrate to use in egg yolk sodium citrate buffer diluters, Swanson (73)

concluded that 3.0% sodium citrate was superior when compared to solutions of 1, 2, 4, and 5% sodium citrate. Reducing the ratio of egg yolk to 20% gave motility and livability as good as the commonly used 50% level. Reducing the level of egg yolk to 10% gave good protection from cold shock, but motility was slightly impaired.

Bratton and associates (14) made a comparison of six different diluents regarding their effect on spermatozoan livability and fertility. Sixty semen samples from 10 Holstein bulls were diluted at a rate of 1:200. Spermatozoan livability at 5° C. was satisfactory for all six diluents. Fertility data were obtained from about 1,850 first service cows for each diluter. Based on the percentage of cows not returning for service in 60 to 90 days, the mean fertility levels for the respective diluents were: phosphate-yolk, 50.5; 3.6% citrate-yolk, 50.5; 3.6% citrate-sulfanilamide-yolk, 55.3; 2.9% citrate-sulfanilamide-yolk, 56.5; Ortho tablet-yolk, 56.4; and Ortho liquid, 55.0%. The last four diluents mentioned contained sulfonamides. The sulfonamide-containing liquids averaged 5 percentage units higher than the other diluents. This difference was significant statistically ($P<0.01$).

Almquist (2) conducted two experiments comparing heated milk with yolk-citrate-penicillin-streptomycin diluters with respect to their influence on fertility. In the first experiment 12 Guernsey and Holstein bulls were used. Six were of relatively high fertility, while the other six were of relatively low fertility. Fertility data were collected on inseminations for 6,670 first service cows. Milk was used with and without penicillin and streptomycin. Per cent non-returns for the three diluters were: yolk-citrate-penicillin-

streptomycin, 64.1%; heated homogenized milk, 66.2%; and heated milk-penicillin-streptomycin, 72.1%. A difference between milk with antibiotics and yolk-citrate with antibiotics of 8% was highly significant. An increase of 5.9% in milk with over milk without antibiotics was significant at the 5% level.

In the second experiment, 240 ejaculates from 30 Guernsey and Holstein bulls were used to inseminate 8,400 first service cows. Results with milk and yolk-citrate diluters both containing antibiotics showed a significant difference in favor of the boiled milk-penicillin-streptomycin. Egg yolk-citrate-antibiotics showed a fertility level of 63.5% (60-90 day non-returns), while the milk diluter with antibiotics had a non-return rate of 71.0%. All bulls were classified either as relatively high or relatively low in fertility. The difference in favor of milk over yolk-citrate diluter for the high fertility bulls in experiments one and two was 4.2 and 3.4% fertility respectively. The advantage for the milk over the yolk-citrate diluter for the low-fertility bulls was 11.5% in experiment one and 12.4% in experiment two.

Perkins and associates (57), using fertility data of semen collected from Jersey, Guernsey, Holstein and Brown Swiss bulls, made a comparison of the value of boiled homogenized milk and egg yolk-citrate as diluters. Alternate collections of semen were diluted 1:50 in homogenized milk heated to 97° C. for 10 minutes or in 1:4 yolk-citrate diluter. To each diluter was added 500 units each of penicillin and streptomycin per ml. During the four-month experimental period, fertility results, based on 60-90-day non-returns, were taken from 19,939 first services. No significant difference was

found between diluters when total non-returns were compared. Approximately half the inseminations were made with 2-day-old semen and half with 3-day-old semen. Milk diluter gave better results than yolk-citrate diluter when used at two days after collection. However, yolk-citrate diluter was considerably better than milk when both were used as 3-day-old semen. Differences between fertility level for 2-day-old and 3-day-old semen were found to be highly significant ($P<0.01$) by analysis of variance. Interaction of age of semen and diluters was also highly significant ($P<0.01$). Lower fertility bulls in the study tended to improve in fertility when milk diluter was used. This was in agreement with work done by Almquist (2).

Antibacterial agents

Considerable work has been done recently regarding the use of antibacterial agents in semen diluters. Sulfanilamide has been most commonly used in diluted semen, along with penicillin, streptomycin and others. Certain organisms that have been isolated from bull semen were shown by Edmondson and associates (22) to reduce substantially the livability of the semen in storage. Willett and Larson (84) have shown a significant improvement in breeding efficiency by the use of antibiotics. Using 300 mg. of sulfanilamide in 100 ml. of 3.6% citrate buffer, Salisbury and Knodt (67) reported an increase in fertility when the buffer solution was protected from direct light rays. An increase of 6.1% non-returns was found in one experiment and 4.5% in another. The authors concluded that the beneficial effect of sulfanilamide on fertility were largely due to metabolic influences, rather than to bacterial control.

A study was made by Knodt and Salisbury (35) as to the effect of

sulfanilamide on livability and metabolism of bovine spermatozoa. A level of 300 mg. per 100 ml. of diluter gave a highly significant increase in storage time ($P<0.01$). Bacterial growth was prevented in the diluted semen. Also, a depression of glucose and oxygen utilization in the presence of sulfanilamide was noted. Lactic acid accumulation was increased in the presence of sulfanilamide. The amount of lactic acid accumulation was also increased in relation to glucose utilization. The 300 mg. per cent level of sulfanilamide in buffer solution was suggested as optimal.

A study was made by Mixner (51) to determine the effects of sulfanilamide and penicillin used singly and in combination on the fertility of semen of nine relatively fertile bulls. A comparison of diluters was made, using 1,000 units of penicillin per ml. of diluter in one diluter, three mg. of sulfanilamide per ml. of diluter in the second, and a combination of penicillin and sulfanilamide at the same rates in the third. Fertility data were based on 60-90-day non-returns from 520 first and second services. Analysis of variance showed no significant difference between diluters.

In studying the influence of antibiotics on delayed returns, Foote and Bratton (28) compared non-return rates on 112,312 first services made with semen diluted in citrate-sulfanilamide-yolk extender, with non-return rates on 233,354 first services using the same diluter plus antibiotics. The addition of 500 units each of penicillin and streptomycin was made to each ml. of the second diluter. Without penicillin and streptomycin, the percentages of 28-35-day, 60-90-day and 150-180 day non-returns were 79.1, 64.1 and 60.3%, respectively. The corresponding values for insemination with penicillin and

streptomycin were 82.5, 73.0 and 69.7%. Improvement in delayed returns by the second treatment was considered indirect evidence of a marked decrease in embryonic mortalities connected with control of infectious organisms in the semen.

Branton and Prather (13) have shown evidence that sulfanilamide may reduce viability as well as depress metabolism. Fructose loss, lactic acid gain and per cent motility of bovine spermatozoa stored at 4.5° C. was determined at four and ten days of storage. A combination of egg yolk-citrate-penicillin-streptomycin gave the best motility results. Spermatozoa in the egg yolk-citrate-sulfanilamide diluter showed less motility than either egg yolk-citrate alone or egg yolk-citrate plus streptomycin or penicillin or both. Except for the control (egg yolk-citrate) these differences with the sulfanilamide containing diluent were highly significant. ($P<0.01$).

Almquist (1) studied the effects of various levels of penicillin on the semen of five bulls of relatively low fertility. Five treatments were applied to the semen of these bulls. Penicillin was added to 3.6% sodium citrate dihydrate buffer at rates of 250, 500, 750 and 1,000 units per ml. of buffer solution. This solution was mixed with equal parts of egg yolk. A control group contained no penicillin. A total of 3,576 first and second inseminations were made for the trial with approximately the same number of services used with each treatment. Fertility results were based on 6-month non-returns to service. Improvement of the 500 and the 1,000 unit treatments over the control was highly significant ($P<0.01$). An increase that was noted in the other two treatments (250 and 750 units) showed no significance statistically. Considerable variation was found in individual

response, some bulls showing a much greater increase with the penicillin than others.

Cooling and storage

Conflicting reports are found in the literature on methods of cooling semen for storage. Much of the change in cooling procedure has accompanied the development of new diluters.

In discussing storage, Walton, as cited by Herman and Swanson (32) states that the cooling of semen is perhaps the most important factor for maintaining the fertility of spermatozoa outside the body. Komarov and Gladcinova (36) found the optimum temperature for survival of spermatozoa in storage to be 8° to 12° C. although fertility was somewhat higher for spermatozoa kept at 15° to 25° C. for a short time. The authors stated that spermatozoa deteriorated within 12 hours when kept at 0° to 5° C. According to Bernstein (6) the reduction of the temperature of spermatozoa to 12° C. caused a decrease in glycolysis which corresponded to a loss of motility. However, immotile spermatozoa appeared to maintain some glycolysis.

Davis (18) recommended an hour's time for cooling freshly collected semen to 50° F. After storing semen at 35°, 42° and 50° F., he considered 50° F. the maximum temperature for proper storage of semen. (This is equivalent to 10° C.). Working with bull and ram semen, Birillo and Puhaljskii (7) found a rapid decrease of resistance and a loss of the ability to be reactivated when semen was cooled from 40° to 0° C. in 30 to 60 minutes. It was further noted that spermatozoa cooled gradually for three to four hours were readily reactivated. In a report by Miller, as cited by Herman and Swanson (32), it was suggested that semen should be cooled gradually to 45° F. in preparation

for storage. Use of a normally operating household refrigerator was found sufficient for gradual cooling.

Steensma, as cited by Herman and Swanson (32), found that cooling of semen to 5° C. within 15 minutes gave better motility than that of semen cooled slowly. Foote and Bratton (26), using 11 split ejaculates, compared per cent motility results of four different cooling and extending procedures. All semen was extended either before or after cooling at the rate of 1:100 in citrate-sulfanilamide-yolk diluter. The four methods used were: (a) rapid cooling (from 30° to 5° C. in 5 minutes) before extension; (b) slow cooling (from 30° to 5° C. in 75 minutes) before extension; (c) rapid cooling (30° to 5° C. in 5 minutes) after extension; and (d) slow cooling (30° to 5° C. in 75 minutes) after extension. The mean per cent motility during six days of storage for each group was found to be (a) 29; (b) 47; (c) 57; and (d) 62%. A significant difference ($P<0.05$) was found between all treatments except (c) and (d). These results seem to indicate that rapid cooling of undiluted semen has a very detrimental effect, while rapid cooling of diluted semen gives essentially the same motility results as slow cooling.

EXPERIMENTAL PROCEDURE

An experiment was established to determine, by a laboratory study, the characteristics of semen of young dairy bulls. The data reported here are in conjunction with another experiment designed to characterize semen of young bulls on known levels of phosphorus intake. All ejaculates of four bulls of each of two experimental groups were evaluated for the following characteristics: (a) total volume of ejaculate; (b) per cent of initial motility; (c) rate of initial motility; (d) pH of the fresh ejaculate; (e) per cent of abnormal spermatozoa; (f) concentration of spermatozoa; and (g) days of time that motility could be observed in a subsample that was diluted 1 to 50 with egg yolk-citrate-sulfanilamide and stored at 5° C. The data obtained in categories "a" through "f" were related to "g" by a multiple correlation technique (46). In this procedure data from categories "a", "b" and "f" were combined, so that the relationship between total live spermatozoa per ejaculate and livability might be obtained.

All bulls were offered similar amounts of total protein and TDN as recommended by the National Research Council (N.R.C.) sub-committee on nutrition of dairy cattle for growing dairy heifers (53). Group I, consisting of one Guernsey and three Holstein bulls (numbers 3, 4, 15 and 21), received essentially two-thirds of the recommended phosphorus allowance. The four Ayrshire bulls (34, 35, 36, and 37) making up Group II were offered approximately one-third of the recommended phosphorus allowance. All eight experimental animals were changed from

a conventional type ration to the experimental rations at an age range of four to six months by Poitras (60).

The animals were housed in the main section of the Oklahoma Agricultural and Mechanical College Dairy Barn. Adequate protection was provided against weather extremes. Water was available at all time. Conventional swinging steel stanchions limited movement of the bulls. Essentially no exercise was given the animals.

Collection of semen was accomplished by the use of artificial vaginas. Collections were made at weekly intervals, with a few exceptions. At these times the schedule varied only a few days. Semen was collected from the animals as soon as they would ejaculate. One of the bulls in the group served as a dummy for the other bulls to mount. All collections were made outdoors in a chute constructed for that purpose. Only one collection per bull was made each week except in cases where the first ejaculate was not considered a true ejaculate. At each collection, bulls were restrained and given at least one false mount before serving the artificial vagina.

Freshly collected semen samples were evaluated immediately for volume, initial rate of motility, per cent of motile spermatozoa, concentration of spermatozoa, and pH. A subsample of semen was diluted and stored at 5° C. to determine days livability and motility in storage. Abnormal spermatozoan counts were made at a later date, using stained slides prepared on the day of collection.

Volume of each sample was easily determined, since all samples were collected in graduated tubes.

Thorough mixing of semen samples was accomplished by gently inverting the test tube ten times. Care was taken not to mechanically

damage the spermatozoa by vigorous motion (32). Motility estimates were made by use of the microscope. A drop of raw semen was diluted in a drop of 3% sodium citrate solution. Per cent motility was estimated on the basis of the per cent of spermatozoa that exhibited motion. An interval of ten per cent was used for all motility of ten per cent or over. Rate of motility was estimated on the basis of 0-4. Spermatozoa that were difficult to follow because of their rapid motion were given a rate of four. A rate of three was given spermatozoa that were easily seen, yet moved rapidly across the microscopic field. Those cells with a rate of two showed forward motion, but moved rather slowly. A rate of one indicated movement other than forward motion. All cells that were immotile were rated zero. It is possible that some of these spermatozoa were alive, although motionless. Several fields were observed for the rate and per cent motility estimates for each sample in order to decrease the bias of the test.

A pH reading for each semen sample was obtained by use of an electric Beckman H-2 pH meter.

Concentration of spermatozoa was determined by use of an Evelyn photoelectric colorimeter and by the standard hemacytometer method of counting red blood cells. Hemacytometer readings were discontinued on February 16, 1955. Prior to this date, both hemacytometer and colorimeter readings were made on nearly all ejaculates.

Colorimeter readings were made with a one ml. subsample of semen diluted in nine ml. of a solution of 3% sodium citrate and 0.6% sulfanilamide. A one in ten dilution was used because the semen of young bulls has a lower optical density than that of older bulls. Percentage of light transmission of the diluted semen was determined

with a no. 620 filter.

Previous work reported on standards for determining approximate spermatozoan numbers by measurement of optical density of the ejaculate has been done with mature bulls (17, 63). In order to develop a standard for estimating spermatozoan numbers for young bulls, the regression of colorimeter reading on hemacytometer count was calculated, using information from 127 ejaculates.

A small drop of semen from each sample was used to prepare a stained slide, using rose-bengal dye. Approximate percentage of abnormal spermatozoa was later determined for each ejaculate by examining a total of 333 spermatozoa on each slide.

A 0.05 ml. subsample from each freshly collected ejaculate was extended in 2.5 ml. of diluter for storage. The diluter was composed of 50% egg yolk and 50% of a buffer solution containing 3% sodium citrate and 0.6% sulfanilamide. Distilled water was used in preparing the diluter. The sodium citrate-sulfanilamide solution was never stored longer than two weeks after preparation. The buffer solution was mixed with the egg yolk on the day of collection. Test tubes containing diluted semen were placed in beakers of water at room temperature. Cooling and storage at 5° C. were accomplished by placing the beakers in an ordinary electric refrigerator.

Rate and per cent motility during storage were estimated every other day for the first week and every day thereafter until motility ceased. These estimates were made on the same basis as were the initial motility readings.

In an effort to determine the best time to make motility readings to predict storage time, an experiment was set up using 28 ejaculates

from seven bulls during one four-week period. Estimates of motility rate and per cent were made on all ejaculates at various intervals during the first 24 hours following collection. Each sample was inspected cold as well as warm (100° F.). Initial readings were made before and after dilution in egg yolk-citrate diluter, followed by readings made after 2,4,6,8,12,20 and 24 hours of storage at 5°C. All motility estimates in this study were made by one technician in order to minimize the amount of variation in technique. Code numbers were used, so that the technician did not know which bull's semen he was examining.

All statistical analyses of data were made according to Love (46).

RESULTS AND DISCUSSION

Data for 344 ejaculates used for analysis in this study are presented in Appendix tables I through VII. Every ejaculate was used on which information concerning total volume, rate and per cent of motility, pH, per cent of abnormal spermatozoa, total live spermatozoa, live spermatozoa per ml. and days livability was complete. The first ejaculates used in the study were collected on February 22, 1954, and the last on July 28, 1955. Seven of the bulls on the experiment produced semen. Bull no. 36 failed to produce an ejaculate and exhibited very little libido, although given an opportunity to serve the artificial vagina each week.

Bulls no. 3, 4, 15, and 21 produced semen at 55, 47, 45 and 56 weeks of age, respectively. In Group II, bulls no. 34, 35, and 37 produced ejaculates at 71, 68 and 77 weeks of age, respectively. Therefore, Group I bulls produced semen approximately 21 weeks of age earlier than the three bulls which produced semen in Group II. This difference between groups has been attributed to the difference in ration. This compares somewhat with work done by Dunn (21) and Musgrave (52) with bulls on low and normal levels of nutrition. A gross study of data presented in tables I through VIII reveals that the semen produced by these bulls was of comparable quality and quantity to that reported for other young bulls elsewhere (21 and 52).

No direct comparison has been made between Group I and II regarding the quality of semen produced. Appendix table VIII gives a summary of the totals and means by bulls of the volume, rate and per

cent of motility, pH, per cent of abnormal spermatozoa, days livability, and total spermatozoa per ejaculate for all ejaculates analyzed. However, a direct comparison of the means in table VIII would be biased, since each bull in Group I had a considerably larger number of ejaculates in this summary than any of the bulls in Group II. Since Group I bulls were approximately seven months older, the average quality of semen they produced should be superior to that of bulls of Group II because of their greater maturity.

Fertility data are not available on these bulls at this time, although their actual ability to reproduce is now being established. Therefore, it was assumed here that spermatozoan livability in storage would give the best indication of fertility that was available (32, 74, 76, and 80).

Multiple correlation

Values derived from the multiple correlation of motility rate, per cent abnormals, pH, and live spermatozoa per ejaculate on livability are presented in table 1. The correlations between rate and pH ($r=-0.3107$) rate and days livability ($r = 0.3059$), and pH and days livability ($r =-0.1975$) were highly significant ($P<0.01$) for semen of Group I bulls. The other correlations between variables were not significant for this group. The multiple correlation coefficient ($R = 0.3300$) was statistically significant ($P<0.01$). Storage time was found to be dependent on the other four variables to the extent of 5.6%.

Table 2 shows the correlation coefficient for rate and pH ($r =-0.1553$) was not significant. On the other hand, pH and live spermatozoa per ejaculate were significantly correlated ($P<0.01$), with a coefficient of $r =-0.5448$. The correlation coefficients of

Table 1

Summary of the multiple correlation of semen characteristics of bulls of Group I

	Values with livability	Abn. % (r)	pH (r)	Live sperm/$_7$ ejac. (x 10^7) (r)	Livability[1] (days) (r)
Rate	0.2694	0.0426	**-0.3107	0.0108	**0.3059
Abn. %	0.0166		0.0859	0.0696	0.0223
pH	-0.1134			-0.0327	**-0.1975
Live sperm/$_7$ ejac. (x 10^7)	0.0575				0.0652
N = 274		R = **0.3300			

[1] Livability was the dependent variable, the other four characteristics independent variables.

Table 2

Summary of the multiple correlation of semen characteristics of bulls of Group II

	Values with livability	Abn. % (r)	pH (r)	Live sperm/$_7$ ejac. (x 10^7) (r)	Livability[1] (days) (r)
Rate	0.3405	0.0373	-0.1552	0.1418	**0.3996
Abn. %	-0.1359		-0.0110	0.1900	-0.1684
pH	-0.3965			**-0.5448	**-0.4237
Live sperm/$_7$ ejac. (x 10^7)	-0.0532				0.1853
N = 70		R = **0.5182			

[1] Livability was the dependent variable, the other four characteristics independent variables.

rate and storage time ($r = 0.3996$), and pH and storage time ($r = -0.4237$) were statistically significant ($P<0.01$). The multiple correlation coefficient ($R = 0.5182$) was found to be highly significant ($P<0.01$). Storage time was found to be dependent upon the independent variables to the extent of 14.47%. It is evident that other factors not included in the two above multiple correlations had an influence on livability.

Storage time and per cent motility were correlated, using data from Group I and II separately. The correlation coefficients determined from Group I data ($r = 0.3997$) and Group II data ($r = 0.3364$) were found to be significant ($P<0.01$).

The results of these analyses would indicate that rate and per cent motility and pH are fairly good measures of the potential livability of an ejaculate of semen of young bulls in storage. Further analyses, including a partial correlation, would possibly give a better indication of the relationship of these four variables. In this study, per cent of abnormals and live spermatozoa per ejaculate had no real value in predicting storage time. The significant negative correlation between pH and live spermatozoa found with Group II data is in agreement with the results of Kuhne as reviewed by Herman and Swanson (32) and Schneerson (69) in their work with pH and total spermatozoa. The correlation coefficient ($r = -0.0327$) of pH and live spermatozoa per ejaculate in Group I was not significant.

0-24 Hour semen characteristics and livability

Semen data (livability, pH, total spermatozoa, and motility) for the 28 ejaculates collected from seven bulls in Group I and II are recorded in table IX. Correlation coefficients between motility (rate and per cent) readings and days livability, initial pH, and

total spermatozoa are presented in table 3. Motility readings were made at nine different times after collection.

The results shown in table 3 would seem to indicate that initial pH had considerable influence upon initial motility, using the cold stage readings. The correlation coefficients between initial pH and initial rate, undiluted ($r = -0.5887$) ($P < 0.01$) and pH and initial per cent, undiluted, ($r = -0.4709$) ($P < 0.05$) for cold stage readings were significant. Similar motility per cent and rate values obtained with the use of a microscope stage warmer were correlated with the initial pH of the ejaculates. These values approached significance but were not significant. Similar work was done by Davis and Williams (19). They found a correlation of $r = -0.5900$ between pH and motility.

Motility and total spermatozoa were not significantly related. In correlating total spermatozoa with initial rate undiluted and per cent motility readings, coefficients of correlation for cold stage readings were positive, while those for warm stage readings were slightly negative. No significance was found. Similarly, tables 1 and 2 show only a slight relationship between rate of motility and total live spermatozoa. Davis and Williams (19) reported a correlation of 0.3095 between per cent motility and concentration.

A comparison of the initial warm and cold stage motility readings (before dilution) and livability shows a higher correlation between cold stage readings and livability. Although no significance was found, the results indicate that the cold stage readings were of greater value. In correlating these motility readings with pH and with total spermatozoa, cold stage values were again higher. Apparently when fresh undiluted semen is at room temperature it is disadvantageous

Table 3

Correlation coefficients and standard deviations of warm and cold stage motility readings (rate and %) with days livability, initial pH and total spermatozoa ($x10^7$) at various times after collection. (N = 28)

Hours after collection	Warm stage				Cold stage			
	Rate		%		Rate		%	
	r	S.D.	r	S.D.	r	S.D.	r	S.D.
	(r of motility and livability)							
0_1	0.0597	0.9371	0.1004	19.38	0.2675	0.6342	0.2186	13.97
0_2	0.0096	0.6506	0.1008	12.72	-0.1006	0.7201	0.0284	12.01
2	-0.0658	0.5040	0.0265	11.33	-0.1346	0.7381	0.2301	10.66
4	0.2677	0.4757	0.3248	9.81	-0.0388	0.5077	0.1586	9.79
6	0.1150	0.4409	0.2380	11.00	0.2791	0.5040	0.1914	9.95
8	0.2086	0.4757	0.3136	12.47	0.0388	0.5077	0.1082	9.57
12	0.3420	0.3901	0.2888	11.00	0.1612	0.4880	0.0287	9.37
20	0.2671	0.3564	0.2002	10.44	0.1654	0.4880	0.1156	9.94
24	**0.4439	0.5481	0.1071	12.17	-0.0559	0.7229	0.0405	8.70
	(r of motility and pH)							
0_1	-0.3490		-0.3559		**-0.5887		*-0.4709	
0_2	-0.0022		-0.2128		-0.0078		-0.2020	
	(r of motility and total spermatozoa)							
0_1	-0.0207		-0.1405		0.2998		0.2291	
0_2	-0.2256		0.1319		0.2078		0.0310	

* level of significance ($P<0.05$)
** level of significance ($P<0.01$)
1 before dilution in egg yolk citrate
2 after dilution in egg yolk citrate

to incubate it before making a motility estimate.

All livability-motility correlation coefficients were low for initial readings made after dilution. This may have been due to the change of environment made by dilution. Correlation coefficients for the 2 hour readings were very low except for per cent motility estimated on the cold stage.

Beginning with the 4 hour readings, with the exception of the 6 hour readings, warm stage rate and per cent correlations with livability are higher than corresponding correlations for cold stage readings and livability. The correlation between rate of motility, warm stage, at 24 hours after collection with livability was significant ($P<0.05$). It is likely that the readings made on the cold stage after storage varied to some extent in temperature at the time they were examined, while all samples examined on the warm stage were approximately 100° F. Warming of samples of semen after 4 hours storage increased the rate over that of samples on the cold stage. By increasing the rate of those spermatozoa that were potentially more active, some real differences may have been brought out. Per cent of motility was increased in some cases and decreased in others by incubation. The results of this trial indicate that the only justification for using a microscope stage warmer is that the procedure is thereby further standardized to that extent. Further work with the semen of young bulls will possibly clarify this situation.

A significant ($P<0.05$) negative correlation ($r = -0.4266$) was found between total spermatozoa and the initial pH value of the ejaculates. This is not in accord with the data of Davis and Williams (19) in which they reported a positive correlation ($r = 0.6300$) between these

factors. It should be pointed out that an inspection of the published data of Davis and Williams (19) does not demonstrate the positive nature of this correlation coefficient. In fact, a careful study of the summary data as published would lead the investigator to suspect a strong negative correlation.

The relationship between total spermatozoa and livability was not significant ($r = 0.1264$) but was positive. This is in agreement with values shown in tables 1 and 2 where the relationship between total live spermatozoa and livability was determined.

In this trial the correlation between initial pH and livability ($r = -0.3190$) approached significance but was not significant. This relationship is in the same direction as the correlations shown in tables 1 and 2. It is thought that added numbers in this trial would also have produced a significant relationship between these factors.

Calibration of Colorimeter

An Evelyn colorimeter was calibrated, according to procedures used by Salisbury and associates (63), to determine concentration of spermatozoa by measuring the optical density of the ejaculate. Data from 127 ejaculates (Appendix table X) were used for this purpose. A significant correlation coefficient ($r = 0.85$) ($P<0.01$) was obtained for the mean of two hemacytometer counts and the Log $\frac{100}{\text{reading of sample}}$. Therefore, a total of 72% of the variance between samples was accounted for by the calculated regression equation $E = 1211.938X - 58.76$ (where $X = \text{Log}\frac{100}{\text{reading of sample}}$, and E = number of spermatozoa per ml. $\times 10^6$). The results of the application of this regression equation to most of the colorimeter scale are presented in table X.

Eighty-four ejaculates produced by these bulls at 18 to 24 months

of age were selected from the group of 127 ejaculates. This was done to determine if this refinement would reduce the variation between ejaculates as measured by hemacytometer count. A significant correlation ($r = 0.85$) ($P<0.01$) between the hemacytometer counts and the $\text{Log}\frac{100}{\text{reading of sample}}$ was found. Again, 72% of the variance between ejaculates was accounted for by this relationship.

It appears, therefore, that there was little difference in variability of optical density that was due to spermatozoan concentration within the ages during which the 127 ejaculates were produced (ten months to two years) as compared to the ages at which the 84 ejaculates were produces (18 months to two years).

This leads, therefore, to the hypothesis that variability between ejaculates during the entire period was due to something other than numbers of spermatozoa. Based on observations made during hemacytometer counts and routine microscopic examinations it is thought that this variability is due primarily to the amount of epithelial-like cells present in the ejaculates. Although there are no data presented here to substantiate this, ejaculates varied considerably both within and between bulls as to the amounts of these cells observed.

These correlation coefficients are not as high as those reported by Salisbury and associates (63) for mature bulls, where a correlation of 0.9820 accounting for 96% of the variance between ejaculates was obtained.

SUMMARY AND CONCLUSIONS

A laboratory study was conducted involving 344 ejaculates from 7 young dairy bulls (1 Guernsey, 3 Holsteins, and 3 Ayrshires) that were maintained on two levels of phosphorus intake. Livability was used as the dependent criterion of semen quality in this study since fertility data were not available. Semen quality was acceptable for bulls of this age.

Two multiple correlations coefficients were calculated (one for each phosphorus level group). A highly significant ($P<0.01$) correlation was found in each case between livability and rate, % of abnormals, pH, and live spermatozoa per ejaculate. Simple correlations showed initial rate and pH; initial rate and livability; and pH and livability to be significantly correlated ($P<0.01$) for semen of Group I bulls, whereas initial rate and livability; pH and live spermatozoa per ejaculate; and pH and livability were significantly ($P<0.01$) correlated for Group II bulls. Apparently the percentage of abnormal spermatozoa was of little importance in the determination of livability for the ejaculates of Group II bulls.

Simple correlations between livability and per cent motility for each phosphorus group were both found highly significant ($P<0.01$).

A short time study involving 28 ejaculates from seven bulls producing semen was run to determine the time after collection that livability could best be predicted from microscopic evaluation. A micriscope stage incubator and an unincubated or cold stage microscope

were used to make evaluations at 0 hours (both before and after dilution with egg yolk citrate) 2, 4, 6, 8, 12, 20, and 24 hours after collection. Highest correlation between 0 hour motility and rate (before dilution) and livability was with the cold stage. However, this was not significant. After four hours storage the highest correlations were found between warm stage readings and livability with the only significant ($P<0.01$) correlation being between rate of motility at 24 hours of storage and livability. Initial rate of motility (undiluted) was significantly ($P<0.01$) correlated with initial pH, and initial per cent of motile spermatozoa (cold stage, undiluted) was significantly ($P<0.05$) correlated with pH. All other relationships studied did not show significant correlation coefficients.

Results obtained in the calibration of a colorimeter to determine spermatozoan concentration by optical density were not as precise as has been reported for semen of mature bulls. It is felt that this decrease in precision is largely due to the presence of considerable numbers of extraneous epithelial-like cells in the ejaculates of these young bulls. Whether this is characteristic of young bulls in general cannot be definitely stated at this time.

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A P P E N D I X

TABLE I

Semen data for bull No. 3 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
Feb.								
22	2.3	1	5	20.0	7.3	6	1	.69
Mar.								
11	5.0	3	70	30.0	7.0	14	10	4980.50
19	2.5	3	80	30.3	6.8	14	12	2846.00
25	4.0	3	50	17.1	7.2	12	10	2466.00
Apr.								
6	2.0	0	0	20.1	6.8	32	0	0.00
9	2.4	3	70	37.5	6.9	4	17	67.20
May								
4	2.7	1	10	17.7	6.7	49	6	1333.26
4*	2.3	2	40	18.6	6.7	40	16	3659.76
11	3.0	4	70	22.8	6.6	49	19	102.90
June								
11	2.0	4	70	20.0	5.8	104	18	145.60
18	3.0	4	50	10.0	5.9	147	16	220.50
25	2.2	4	40	10.0	6.8	70	14	61.60
July								
2	3.1	4	60	20.0	6.3	65	18	120.90
9	1.5	3	60	30.9	6.6	38	20	34.20
23	2.7	4	50	20.0	5.8	114	7	153.90
28	2.2	4	70	10.0	6.3	31	8	47.74
Aug.								
6	3.0	4	70	10.0	6.3	14	3	29.40
20	3.3	4	50	43.8	6.1	130	18	214.50
26	2.4	4	50	20.0	7.0	27	1	32.40
Sept.								
7	3.2	2	10	10.0	6.6	79	8	25.28
7*	2.0	4	90	10.0	6.4	55	13	99.00
10	2.0	4	60	20.0	6.6	24	18	28.80
15	1.6	4	50	10.0	6.2	77	17	61.60
22	0.7	4	30	20.0	6.7	18	11	3.78
22*	1.5	4	60	10.0	6.6	135	13	121.50
29	1.6	0	0	20.0	7.9	17	0	0.00

* Denotes 2nd collection and taken only where first collection was not a true ejaculate. All other data represent a true ejaculate on first collection.

TABLE I (cont'd.)

Semen data for bull No. 3

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		(x10^7)	(days)	(x10^7)
Oct.								
6	0.3	0	0	0.0	7.5	0	0	
13	2.5	4	70	10.0	6.2	116	18	31.50
20	1.5	4	50	10.0	6.8	64	21	48.00
27	1.7	4	60	49.5	6.7	94	15	95.88
Nov.								
3	3.7	4	60	34.5	6.8	124	15	275.28
10	2.8	4	70	14.4	6.4	141	14	276.36
17	3.0	4	60	18.3	6.5	92	17	165.60
24	4.0	4	60	13.8	6.7	54	14	129.60
Dec.								
1	2.4	4	90	20.7	6.6	80	18	172.80
8	2.5	4	70	23.1	6.6	84	14	147.00
15	3.7	3	40	25.2	6.8	86	12	127.28
21	3.0	3	40	30.3	6.6	40	17	48.00
30	4.4	3	50	27.9	6.6	76	14	167.20
(1955)								
Jan.								
5	2.6	3	40	24.0	6.8	42	5	43.68
12	3.5	3	40	23.0	6.5	72	16	100.80
21	5.0	3	30	6.0	7.2	21	11	31.50
26	1.5	4	40	24.9	6.7	59	12	35.40
Feb.								
2	4.0	3	40	31.5	6.6	82	17	131.20
10	3.3	3	30	27.0	6.8	62	5	61.38
16	4.0	3	50	20.4	6.6	115	17	230.00
26	6.0	4	50	27.9	6.7	98	14	294.00
Mar.								
1	1.6	4	60	11.4	6.4	64	13	61.44
8	2.4	3	50	15.3	6.6	106	15	127.20
15	3.1	3	40	20.4	6.5	87	18	107.88
23	2.4	3	70	13.8	6.1	95	27	159.60
29	3.8	3	60	24.6	6.4	125	14	285.00
Apr.								
4	4.5	3	50	16.2	6.5	79	17	177.75
12	3.5	3	70	13.8	6.5	65	16	159.25
19	3.5	3	70	26.1	6.5	69	14	169.05
28	2.8	3	70	12.9	6.4	87	12	170.52

TABLE I (cont'd.)

Semen data for bull No. 3 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1955)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
May								
3	5.8	3	50	17.7	6.6	62	14	179.80
12	3.6	4	50	28.2	6.6	67	13	120.60
17	4.1	2	60	28.2	6.4	100	19	246.00
27	2.7	2	70	15.0	6.3	98	19	185.22
June								
1	4.9	3	40	19.2	6.4	117	13	229.32
9	3.8	3	70	7.5	6.5	91	15	243.12
16	6.4	3	60	30.0	6.4	90	13	344.68
23	3.1	4	70	34.2	6.6	106	15	229.41
30	4.2	2	50	36.0	6.5	96	20	201.10
July								
6	5.3	4	40	30.0	6.2	87	22	185.25
15	4.5	4	80	30.6	6.3	104	13	372.85
21	7.2	3	40	30.0	6.5	84	7	243.01
28	4.1	4	50	28.8	6.2	108	16	221.32

TABLE II

Semen data for bull No. 4 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		(x10^7)	(days)	(x10^7)
Feb.								
22	6.5	2	30	20.0	6.8	37	4	72.1
Mar.								
3	5.0	2	70	20.0	6.9	49	5	171.5
11	6.0	3	70	40.0	7.2	62	13	260.4
19	3.2	3	70	20.4	6.8	165	15	369.6
25	5.6	3	70	27.0	6.8	112	8	439.0
Apr.								
6	2.3	2	60	15.0	6.5	82	20	113.6
9	2.0	2	40	21.6	6.9	32	10	25.6
9	3.3	2	40	37.2	6.9	77	19	101.6
20	2.0	4	80	10.0	6.8	89	25	142.4
May								
4	4.6	2	50	12.3	6.5	128	22	294.4
4	4.8	3	70	19.8	6.5	74	24	248.6
11	8.0	4	80	10.0	6.4	94	28	601.6
June								
11	5.1	4	90	20.4	5.5	187	19	858.3
18	5.5	4	60	18.3	5.4	182	19	600.6
25	6.5	4	60	21.6	5.6	190	36	741.0
July								
2	3.0	4	70	30.0	5.5	176	23	369.6
16	4.5	4	60	33.0	6.0	100	14	270.0
23	3.5	4	80	37.8	6.2	62	4	173.6
28	4.0	4	70	21.3	6.2	76	3	212.8
Aug.								
6	3.5	4	80	10.0	6.2	76	5	212.8
13	10.0	3	50	20.0	6.5	92	5	460.0
13*	3.1	4	70	10.0	6.9	33	12	71.6
20	4.3	4	70	16.2	5.9	208	17	626.1
26	4.2	4	90	29.1	5.8	140	14	529.2

* Denotes 2nd collection and taken only where first collection was not a true ejaculate. All other data represent a true ejaculate on first collection.

TABLE II (cont'd.)

Semen data for bull No. 4 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
Sept.								
7	4.5	4	60	19.2	6.1	133	12	359.1
10	3.5	4	60	24.0	6.7	83	9	174.3
15	3.7	4	70	20.7	6.2	126	18	326.3
22	3.4	4	70	8.4	6.6	83	13	197.5
29	1.6	4	70	21.6	6.8	27	5	30.2
Oct.								
6	3.0	4	90	20.1	6.7	84	13	226.8
13	3.9	4	90	8.7	6.2	134	20	470.3
20	5.2	4	80	18.0	6.2	147	24	611.5
27	2.0	4	70	18.3	6.7	166	15	232.4
Nov.								
3	4.5	4	80	17.7	6.7	129	12	464.4
10	5.3	4	80	13.2	6.0	153	14	648.7
17	3.9	4	70	18.0	6.2	135	17	368.5
24	2.0	4	70	13.5	6.4	144	15	201.6
Dec.								
1	3.6	4	90	17.4	6.6	58	13	187.9
8	7.5	4	80	41.7	6.6	99	18	594.0
15	3.5	4	60	9.6	6.4	177	17	371.7
21	5.3	4	80	20.4	5.8	120	22	508.8
30	4.2	0	0	30.0	7.5	28	0	0.0
(1955)								
Jan.								
5	9.0	4	80	26.4	6.9	105	11	756.0
12	3.0	4	80	16.0	6.4	107	15	256.8
21	5.2	4	70	28.5	6.6	119	10	433.2
26	2.5	4	70	26.0	6.7	63	5	110.2
Feb.								
2	2.0	4	60	13.0	6.4	123	14	147.6
16	6.4	4	80	13.8	6.3	152	15	778.2
26	3.4	4	70	15.0	6.7	69	9	164.2
Mar.								
1	5.3	4	60	11.7	6.4	114	16	362.5
8	2.6	4	70	18.3	6.6	104	15	189.3
15	7.6	4	70	51.0	6.7	127	16	675.6
23	5.3	4	70	9.9	6.4	142	19	526.8
29	4.0	4	70	16.2	6.4	142	21	397.6

TABLE II (cont'd.)

Semen data for bull No. 4 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1955)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
Apr.								
5	5.5	4	60	20.4	6.4	132	22	435.6
12	7.4	4	70	9.6	6.4	140	17	725.2
19	9.3	4	70	9.0	6.5	129	18	839.8
28	5.4	4	80	6.3	6.4	114	19	492.5
May								
3	6.1	4	60	11.7	6.2	134	20	490.4
12	7.1	4	50	33.0	6.1	130	15	461.5
17	6.2	4	80	16.8	6.4	125	19	620.0
27	7.4	4	70	11.4	6.4	102	30	528.4
June								
1	4.5	2	50	13.8	7.0	60	8	135.0
9	8.0	4	60	13.2	6.4	101	12	484.8
16	8.4	4	60	17.7	6.2	134	11	675.4
23	6.0	4	80	8.1	6.4	99	12	475.2
30	6.5	4	50	18.3	6.4	125	14	406.2
July								
6	10.5	3	80	9.6	6.1	118	16	991.2
15	5.1	3	30	9.6	7.1	90	8	137.7
21	8.3	2	10	15.0	6.8	82	3	68.1
28	8.8	3	20	29.0	6.5	36	5	63.4

TABLE III

Semen data for bull No. 15 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		(x10^7)	(days)	(x10^7)
Mar.								
11	4.5	3	80	30.6	6.8	62	17	223.2
19	2.8	3	60	44.4	7.3	123	21	206.6
25	6.4	3	80	14.4	6.8	69	18	353.3
Apr.								
6	2.9	2	60	25.8	6.6	72	24	125.3
9	2.2	3	50	18.9	6.9	94	34	103.4
May								
4	4.0	3	50	17.7	6.5	119	28	238.0
4	4.0	1	60	33.0	6.5	132	8	316.8
11	2.4	4	90	45.9	6.7	166	27	358.6
June								
11	2.7	4	70	20.1	5.9	90	22	170.1
18	3.5	4	50	27.3	5.5	162	21	283.5
25	3.2	4	50	34.5	6.3	85	19	136.0
July								
2	2.8	4	80	25.8	6.0	124	23	277.8
16	4.5	4	70	12.0	6.2	94	11	296.1
23	5.5	4	60	22.5	5.7	135	21	445.5
28	2.1	4	70	38.7	6.3	67	5	98.5
Aug.								
6	6.0	4	60	10.0	5.9	143	14	514.8
13	5.1	4	50	10.0	5.8	152	24	387.6
20	3.0	4	90	21.6	6.2	153	20	413.1
26	2.6	4	80	31.2	6.2	88	12	176.0
Sept.								
7	2.8	4	80	50.4	6.4	52	22	116.5
10	2.0	4	80	26.1	6.2	105	16	168.0
15	2.5	4	60	49.8	6.2	103	19	154.5
22	1.9	4	90	29.1	6.6	105	14	179.5
29	2.2	4	80	24.0	6.3	99	38	174.2
Oct.								
6	3.2	3	40	30.9	6.6	78	15	99.8
13	2.9	4	60	10.0	6.2	121	29	210.5
20	4.5	4	60	45.0	6.3	106	21	286.2
27	4.9	3	50	27.6	6.7	142	21	347.9
Nov.								
3	5.0	3	50	27.6	6.7	121	15	302.5
10	2.3	4	90	25.5	6.3	123	14	254.6
17	4.9	4	70	31.8	6.4	119	26	408.2
24	4.5	4	60	24.0	6.5	105	16	283.5

TABLE III (cont'd.)

Semen data for bull No. 15 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
Dec.								
1	4.0	4	90	27.6	6.7	83	19	298.8
8	4.5	4	80	26.1	6.9	78	14	280.8
15	3.5	4	70	18.0	6.6	89	23	218.0
21	6.0	3	60	33.9	6.8	117	22	421.2
30	6.4	4	90	20.1	6.5	103	32	593.3
(1955) Jan.								
5	6.8	3	60	29.4	6.9	103	20	420.2
12	4.5	4	80	70.0	6.6	97	19	349.2
21	4.0	3	70	19.8	6.6	103	26	288.4
26	3.2	3	30	13.5	6.6	111	17	106.6
26*	4.7	4	60	87.2	6.5	154	24	434.3
Feb.								
2	2.3	3	60	27.0	6.7	103	16	142.1
10	6.2	3	60	18.6	6.8	160	22	595.2
16	4.3	4	70	23.7	6.6	110	36	331.1
26	5.3	4	70	22.5	6.5	102	20	378.4
Mar.								
1	1.8	3	40	27.0	6.8	76	13	54.7
8	4.5	3	70	27.3	6.8	91	29	286.6
15	4.5	3	50	29.4	6.8	118	27	265.5
23	5.0	4	80	21.6	6.3	118	26	472.0
29	4.0	4	60	24.6	6.6	132	26	316.8
Apr.								
5	1.9	3	60	11.1	6.6	104	26	118.6
12	5.0	4	70	30.6	6.4	124	27	434.0
19	1.7	4	70	37.8	6.6	106	27	126.1
28	5.2	4	70	24.3	6.4	118	20	429.5
May								
3	3.3	4	80	11.1	6.3	125	28	330.0
12	6.7	4	70	49.8	6.3	119	19	558.1
17	6.0	4	80	27.9	6.3	114	32	547.2
27	8.5	3	70	26.7	6.6	101	23	600.9

* Denotes 2nd collection and taken only where first collection was not a true ejaculate. All other data represent a true ejaculate on first collection.

TABLE III (cont'd.)

Semen data for bull No. 15 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1955)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
June								
1	6.7	4	90	25.8	5.9	130	16	621.1
9	8.2	3	60	27.9	6.5	127	16	624.8
23	5.7	4	80	13.5	6.4	119	14	542.6
30	6.2	4	60	40.2	6.3	117	12	435.2
July								
6	6.8	4	80	35.4	6.2	121	21	658.2
15	7.0	4	80	12.6	6.5	107	15	599.2
21	6.1	3	80	17.4	6.3	121	13	590.5
28	7.1	4	60	25.5	6.2	132	19	562.3

TABLE IV

Semen data for bull No. 21 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
Mar.								
25	3.5	3	60	13.5	6.9	45	17	94.5
Apr.								
6	3.5	3	90	24.6	6.3	84	23	264.6
9	2.0	3	60	24.3	6.8	73	25	87.6
9	2.2	3	60	31.8	6.8	64	17	84.5
20	4.0	4	90	10.0	6.8	36	22	129.6
May								
4	1.2	1	10	25.8	7.2	25	4	3.0
4*	1.3	3	90	20.1	7.1	43	15	50.3
11	2.8	4	80	19.2	6.6	77	17	172.5
June								
11	1.5	3	40	16.5	6.2	68	11	40.8
11*	3.0	4	80	13.8	5.8	106	17	254.4
18	3.0	4	50	12.9	6.0	105	12	157.5
25	3.0	4	60	19.5	5.7	126	15	226.8
July								
2	2.2	4	70	26.1	6.3	77	10	118.6
9	3.2	4	80	60.0	6.2	118	17	302.1
28	3.1	4	60	13.5	6.3	90	5	167.4
Aug.								
6	5.2	4	60	10.0	6.1	136	12	424.3
13	3.7	4	40	20.0	6.6	59	12	87.3
20	4.8	4	60	24.6	6.2	181	14	521.3
26	4.9	4	90	19.8	6.3	92	12	405.7
Sept.								
7	1.7	3	90	10.2	6.4	127	10	194.3
10	3.3	4	60	32.1	6.5	106	11	209.9
15	3.0	4	60	3.0	6.2	143	18	257.4
22	3.5	4	50	26.4	6.6	128	13	224.0
29	4.4	4	90	8.4	6.6	153	24	605.9
Oct.								
6	1.9	4	60	31.5	6.6	79	14	90.1
13	4.0	4	80	17.7	6.1	129	15	412.8
20	2.5	4	80	21.3	6.4	122	22	244.0
27	2.0	4	70	27.3	6.7	95	17	133.0

* Denotes 2nd collection and taken only where first collection was not a true ejaculate. All other data represent a true ejaculate on first collection.

TABLE IV (cont'd.)

Semen data for bull No. 21 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
Nov.								
3	3.0	4	70	18.9	6.8	94	14	197.4
10	2.7	4	80	12.3	6.3	122	11	263.5
17	2.0	4	80	14.4	6.6	80	17	128.0
24	3.7	4	60	21.0	6.8	132	11	293.0
Dec.								
1	3.0	4	50	7.2	6.8	117	17	175.5
8	3.5	4	70	31.8	6.9	66	12	161.7
15	3.0	3	50	7.8	6.6	144	14	216.0
21	3.2	3	80	20.7	6.8	133	17	340.5
30	5.5	4	80	5.4	6.5	113	18	497.2
(1955)								
Jan.								
5	4.0	4	60	19.5	6.6	118	14	283.2
12	4.5	4	90	36.3	6.3	113	19	457.6
21	5.0	3	60	15.9	6.5	114	16	342.0
26	4.5	3	90	42.0	6.5	125	14	506.2
Feb.								
2	5.5	4	60	40.8	6.5	119	9	392.7
10	5.4	3	60	48.9	6.6	121	11	392.0
16	6.4	4	50	26.1	6.7	118	15	453.1
26	1.7	4	60	45.0	6.7	109	13	111.2
Mar.								
1	2.3	4	90	18.0	6.8	76	11	139.8
8	7.4	3	60	29.7	6.3	124	17	550.6
15	1.7	3	50	30.0	7.2	79	9	67.1
23	1.7	4	50	38.7	6.8	69	9	58.6
29	7.7	4	70	34.2	6.5	140	17	754.6
Apr.								
5	5.1	4	90	31.2	6.5	98	25	449.8
12	4.8	4	70	28.2	7.3	56	8	188.2
19	4.6	4	80	17.4	6.3	132	24	485.8
28	5.4	4	80	15.9	6.5	105	21	453.6
May								
3	3.8	4	80	9.6	6.2	130	27	395.2
12	5.8	4	70	44.3	6.3	125	27	507.5
17	5.6	4	70	21.0	6.5	114	19	446.9
27	5.3	3	60	16.8	6.2	121	24	384.8

TABLE IV (cont'd.)

Semen data for bull No. 21 (Group I)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1955)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
June								
1	6.6	4	90	11.1	5.9	122	17	724.7
9	7.1	4	70	27.6	6.3	121	11	601.4
16	4.5	4	80	27.0	6.4	114	13	410.4
23	6.5	4	80	43.5	6.5	114	17	592.8
30	6.3	4	60	19.2	6.4	115	14	434.7
July								
6	8.7	4	80	27.0	6.2	117	17	814.3
15	6.2	4	80	30.9	6.3	108	13	535.7
21	6.8	4	90	18.3	6.2	119	9	728.3
28	6.2	4	80	38.7	6.3	134	16	664.6

TABLE V

Semen data for bull No. 34 (Group II)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1955)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
Jan.								
26	2.7	4	50	27.3	6.9	75	12	101.25
Feb.								
2	4.2	4	60	16.2	7.0	67	14	168.84
26	2.2	4	70	36.0	6.7	69	19	106.26
Mar.								
1	1.5	4	60	17.4	7.1	23	12	20.70
8	2.1	4	50	26.1	6.6	76	12	79.80
29	2.2	4	50	14.7	6.6	96	11	105.60
Apr.								
5	2.2	4	50	23.1	6.8	68	15	74.80
12	2.3	4	40	16.5	6.7	63	18	57.96
19	3.7	4	80	24.6	6.5	102	24	301.92
28	1.5	4	70	16.2	6.6	111	15	116.55
May								
3	2.1	1	40	24.9	6.8	39	7	32.76
12	3.5	2	60	9.0	6.9	43	11	90.30
17	3.3	3	80	11.0	6.5	100	18	264.00
27	1.3	2	70	7.8	6.7	66	7	60.06
June								
1	5.2	3	50	12.9	6.5	76	15	197.60
9	3.0	4	60	18.9	6.3	81	19	145.80
16	1.1	3	60	18.6	6.7	33	15	21.78
23	3.2	4	80	16.8	6.6	65	14	166.40
30	1.8	3	50	15.6	6.6	57	17	51.30
July								
6	2.6	4	60	18.3	6.0	94	23	146.64
15	10.5	2	2	17.4	7.0	37	0	7.77
21	3.9	3	40	10.8	6.5	91	12	141.96
28	1.8	4	70	14.7	6.8	28	14	35.28

TABLE VI

Semen data for bull No. 35 (Group II)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1954)	(ml.)	(rate)	(%)	(%)		(x10^7)	(days)	(x10^7)
Dec.								
30	2.2	3	60	29.4	6.6	118	11	155.76
(1955)								
Jan.								
12	1.5	3	40	38.0	6.8	103	16	61.80
21	1.0	3	40	29.5	6.9	134	11	53.60
26	1.3	3	30	15.6	6.8	58	10	22.62
Feb.								
2	2.2	4	80	8.4	6.7	113	19	198.88
10	2.4	2	20	40.5	6.6	69	8	33.12
16	2.7	3	50	27.6	6.5	121	17	163.35
26	2.2	2	60	24.9	6.4	118	16	155.76
Mar.								
1	2.5	2	60	15.9	7.6	28	5	42.00
8	3.0	3	60	19.8	6.5	82	14	147.60
29	3.3	3	40	16.5	7.7	15	11	19.80
Apr.								
5	2.6	4	60	11.7	6.6	100	25	156.00
12	1.7	4	50	15.3	6.7	134	21	113.90
19	3.4	4	60	13.2	6.4	105	22	214.20
28	3.2	4	40	20.1	6.5	125	15	160.00
May								
3	3.4	3	50	30.6	6.7	125	11	212.50
12	3.8	3	70	25.5	6.5	114	24	303.24
17	2.7	2	90	20.1	6.5	105	19	255.15
27	4.1	2	70	20.1	6.3	129	14	370.23
June								
1	4.2	4	80	21.9	6.2	122	16	409.92
9	3.4	3	60	10.2	6.6	96	13	195.84
16	3.9	4	40	8.4	6.4	130	15	202.80
23	3.8	4	80	28.8	6.4	124	14	376.96
30	3.9	4	50	17.1	6.5	108	13	210.60
July								
6	4.4	2	40	15.3	6.2	110	16	193.60
15	7.2	3	70	36.6	6.3	152	8	766.08
21	4.2	3	40	14.4	6.4	134	11	225.12
28	4.0	3	40	22.5	6.6	87	10	139.20

TABLE VII

Semen data for bull No. 37 (Group II)

Date	Vol.	Motility		Abn.	pH	Sperm/ ml.	Stor. time	Live sperm/ ejac.
(1955)	(ml.)	(rate)	(%)	(%)		($x10^7$)	(days)	($x10^7$)
Mar.								
15	2.2	3	50	23.1	6.8	17	8	18.70
23	3.6	2	40	29.1	6.9	18	9	25.92
29	3.5	3	40	37.2	6.8	104	10	145.60
Apr.								
5	4.6	4	40	22.5	6.7	87	20	160.08
12	2.7	4	60	28.2	6.6	67	10	108.54
19	3.8	4	70	22.2	6.7	60	18	159.60
28	3.7	3	20	24.6	6.5	109	11	80.66
May								
3	4.6	3	80	19.2	6.7	70	12	257.60
12	2.6	3	90	34.5	6.6	67	13	156.78
17	3.4	2	70	17.1	6.4	73	18	173.74
27	4.4	3	70	22.8	6.5	99	8	304.92
June								
1	3.2	3	60	16.2	6.6	87	12	167.04
9	3.6	2	50	22.8	6.8	63	12	113.40
16	3.9	4	50	28.5	6.6	88	15	171.60
23	3.7	4	70	27.9	6.7	67	8	173.53
30	6.2	4	40	17.7	6.5	91	14	225.68
July								
6	3.5	4	50	16.2	6.6	63	8	110.25
15	6.8	4	50	40.8	6.6	124	11	421.60
21	4.7	4	50	18.9	6.6	91	13	213.85

TABLE VIII

Summary of totals and means of all semen data by bulls

	Group I				Group II		
Bull no.	3	4	15	21	34	35	37
N=	69	71	67	67	23	28	19
Volume							
(tot.)	216.9	357.9	291.4	271.1	67.9	88.2	74.7
(avg.)	3.1	5.0	4.3	4.0	3.0	3.2	3.9
Mot. rate							
(tot.)	220.0	256.0	242.0	250.0	78.0	87.0	63.0
(avg.)	3.2	3.6	3.6	3.7	3.4	3.1	3.3
Mot. %							
(tot.)	3575.0	4630.0	4540.0	4650.0	1302.0	1530.0	1050.0
(avg.)	51.1	65.2	67.8	69.4	56.6	54.6	55.3
pH							
(tot.)	454.0	453.3	431.9	434.7	153.4	184.9	126.2
(avg.)	6.5	6.4	6.4	6.5	6.7	6.6	6.6
Stor. time							
(tot.)	915.0	1036.0	1394.0	1028.0	324.0	405.0	230.0
(avg.)	13.1	14.6	20.8	15.3	14.1	14.5	12.1
Live sperm							
(tot.)	23888.8	26768.4	22141.6	21564.4	2495.3	5559.6	3189.1
(avg.)	341.3	377.0	330.5	321.7	108.5	198.6	167.8
Abn. %							
(tot.)	1461.1	1359.8	1871.1	1576.2	414.8	597.9	469.5
(avg.)	21.2	19.1	27.9	23.51	18.0	21.4	24.7

TABLE IX

Warm and cold stage semen motility estimates

for 24 hours after collection

		0 Hours (before dil.)				0 Hours (after dil.)			
		Warm		Cold		Warm		Cold	
Date 1955 June	Bull	(rate)	(%)	(rate)	(%)	(rate)	(%)	(rate)	(%)
1	3	3	60	3	40	2	80	2	80
	15	4	90	4	90	2	90	2	90
	21	4	90	4	90	3	90	3	90
	4	2	60	2	50	2	90	2	90
	4*	4	90	4	70	3	70	3	70
	34	3	50	3	50	3	70	3	70
	35	4	70	4	80	2	90	2	90
	37	3	60	3	60	3	80	2	80
9	3	2	60	3	70	3	80	3	80
	15	1	30	3	60	3	60	3	70
	21	1	30	4	70	4	80	4	70
	4	4	50	4	60	4	70	3	60
	34	3	60	4	60	3	70	3	60
	35	2	50	3	60	3	50	3	70
	37	3	40	2	50	3	50	2	50
16	3	4	50	3	60	3	60	3	60
	21	4	60	4	80	4	80	4	80
	4	4	40	4	60	4	50	4	50
	34	3	60	3	60	3	60	3	60
	35	3	40	4	40	4	60	3	50
	37	4	50	4	50	3	80	3	60
23	15	4	90	4	80	3	80	2	70
	3	3	40	4	70	4	60	3	60
	21	4	90	4	80	4	80	4	70
	4	4	80	4	80	3	80	4	70
	34	4	90	4	80	3	90	3	80
	35	4	60	4	80	4	70	4	70
	37	4	80	4	70	3	70	4	70
Total		92	1,720	100	1,850	88	2,040	84	1,970
Corr. S^2		23.7	10,143	10.9	5,268	11.4	4,371	14.0	3,896

* Second ejaculate for bull 4 on June 1, 1955. Same for following pages of TABLE IX.

TABLE IX (cont'd.)

Warm and cold stage semen motility estimates

for 24 hours after collection

		2 Hours				4 Hours			
		Warm		Cold		Warm		Cold	
Date 1955 June	Bull	(rate)	(%)	(rate)	(%)	(rate)	(%)	(rate)	(%)
1	3	3	60	2	60	3	80	3	70
	15	3	90	3	80	4	70	3	90
	21	3	90	4	80	3	80	3	70
	4	3	80	3	70	3	70	3	70
	4*	4	80	3	80	3	80	3	80
	34	4	80	3	70	4	90	3	60
	35	3	70	3	80	4	70	3	70
	37	3	70	3	80	3	60	3	60
9	3	3	80	1	60	3	70	3	70
	15	4	80	2	80	4	80	3	70
	21	4	80	4	70	4	80	4	80
	4	4	80	3	70	4	70	4	70
	34	3	70	3	70	4	80	4	70
	35	4	60	3	60	4	60	4	70
	37	3	60	3	60	4	60	3	50
16	3	3	50	3	50	3	60	3	50
	21	4	80	3	80	4	70	4	70
	4	3	50	4	50	3	50	4	60
	34	4	70	3	50	4	60	4	90
	35	4	50	4	60	4	60	3	60
	37	4	60	4	80	4	60	4	70
23	15	4	80	3	70	4	80	4	80
	3	4	60	3	60	4	60	3	70
	21	4	70	4	80	4	80	4	80
	4	4	70	4	80	4	70	3	80
	34	4	70	4	80	4	80	4	80
	35	3	70	4	60	3	70	4	70
	37	4	80	4	60	4	60	4	70
Total		100	1,990	90	1,930	103	1,960	97	1,980
Corr. S^2		6.9	3,468	14.7	3,068	6.1	2,600	10.0	2,586

TABLE IX (cont'd.)

Warm and cold stage semen motility estimates

for 24 hours after collection

		6 Hours				8 Hours			
		Warm		Cold		Warm		Cold	
Date 1955 June	Bull	(rate)	(%)	(rate)	(%)	(rate)	(%)	(rate)	(%)
1	3	3	50	3	50	3	60	3	60
	15	4	70	4	70	4	90	3	80
	21	4	80	4	90	4	90	4	90
	4	3	80	3	70	3	60	3	80
	4*	4	80	3	90	4	80	4	70
	34	3	90	4	80	3	70	4	70
	35	4	80	4	80	3	80	3	70
	37	3	70	4	70	3	70	3	70
9	3	3	70	3	70	3	60	3	80
	15	4	80	3	70	4	70	3	70
	21	4	80	4	80	4	80	4	80
	4	4	80	4	70	4	70	4	70
	34	4	80	4	70	4	80	4	80
	35	4	70	4	80	4	60	3	60
	37	4	60	3	60	3	50	3	50
16	3	4	50	3	60	4	50	3	60
	21	4	70	4	80	4	70	4	80
	4	4	50	3	60	3	40	4	60
	34	4	80	4	80	4	80	3	80
	35	4	60	3	60	4	60	3	70
	37	4	60	4	70	4	60	4	60
23	15	4	70	3	70	4	80	4	80
	3	3	60	4	60	4	70	4	60
	21	4	80	4	90	4	80	4	70
	4	4	80	3	70	4	80	3	80
	34	3	80	3	80	4	80	4	80
	35	4	70	4	70	3	60	4	80
	37	4	60	4	70	4	80	4	80
Total		105	1,990	100	2,020	103	1,960	99	2,020
Corr.	S^2	5.2	3,268	6.9	2,671	6.1	4,200	7.0	2,471

TABLE IX (cont'd.)

Warm and cold stage semen motility estimates

for 24 hours after collection

		12 Hours				20 Hours			
		Warm		Cold		Warm		Cold	
Date 1955 June	Bull	(rate)	(%)	(rate)	(%)	(rate)	(%)	(rate)	(%)
1	3	3	50	3	60	3	60	3	60
	15	4	90	3	90	4	80	4	80
	21	4	90	4	90	4	90	4	90
	4	3	70	3	80	4	80	3	80
	4*	3	70	3	70	4	70	3	70
	34	4	90	4	60	4	80	4	80
	35	4	80	3	70	4	80	3	70
	37	4	70	3	70	3	70	3	70
9	3	3	70	3	60	4	80	3	60
	15	4	70	4	70	4	70	3	70
	21	4	80	4	80	4	70	4	70
	4	4	80	4	80	4	80	3	70
	34	4	80	4	80	4	80	4	80
	35	4	70	4	70	4	70	4	60
	37	3	60	3	60	4	50	3	50
16	3	4	60	3	70	4	60	3	60
	21	4	90	4	80	4	70	4	80
	4	4	50	4	60	4	50	4	50
	34	4	80	4	80	4	70	4	60
	35	4	60	3	60	4	60	3	60
	37	4	70	4	80	4	60	3	60
23	15	4	80	4	80	4	60	3	70
	3	4	70	4	60	3	60	3	60
	21	4	80	4	70	4	80	3	70
	4	4	80	4	80	4	80	4	70
	34	4	80	4	80	4	80	3	80
	35	4	70	4	70	4	80	3	70
	37	4	80	4	80	3	80	3	80
Total		107	2070	102	2040	108	2000	94	1930
Corr.	S^2	4.1	3268.	6.4	2371.	3.4	2943.	6.4	2668

TABLE IX (cont'd.)

Warm and cold stage semen motility estimates

for 24 hours after collection

		24 Hours						
		Warm		Cold		days storage	total sperm	pH
Date 1955 June	Bull	(rate)	(%)	(rate)	(%)		($x10^7$)	
1	3	3	50	3	50	13	573.3	6.4
	15	4	80	3	70	16	871.0	5.9
	21	4	90	4	80	17	805.2	5.9
	4	3	60	3	70	8	270.0	7.0
	4*	4	80	4	70	11	115.0	5.8
	34	4	80	4	70	15	395.2	6.5
	35	4	70	4	70	16	512.4	6.2
	37	3	80	4	60	12	278.4	6.6
9	3	4	60	2	60	15	347.3	6.5
	15	4	70	2	50	16	1041.4	6.5
	21	4	70	4	70	11	859.1	6.3
	4	4	80	3	70	12	808.0	6.4
	34	4	70	3	70	19	243.0	6.3
	35	3	60	2	60	13	326.4	6.6
	37	2	40	2	60	12	226.8	6.8
16	3	3	60	3	60	13	574.5	6.4
	21	4	70	4	70	13	513.0	6.4
	4	4	50	3	50	11	1125.6	6.2
	34	4	80	4	60	15	36.3	6.7
	35	4	60	4	60	15	507.0	6.4
	37	3	70	4	60	15	343.2	6.6
23	15	4	80	3	70	14	678.3	6.4
	3	4	70	3	70	15	327.7	6.6
	21	4	70	3	80	17	741.0	6.5
	4	4	80	3	70	12	594.0	6.4
	34	4	80	4	80	14	208.0	6.6
	35	4	60	4	70	14	471.2	6.4
	37	3	90	4	80	8	247.9	6.7
Total		103	1960	93	1860	382	14040.2	180.0
Corr. S^2		8.1	4000	14.1	2043	176.4	2155974.4	1.9

TABLE X

Spermatozoa per milliliter of undiluted semen

Colorimeter reading	Log 100/reading of sample	Sperm no. ($x10^6$)	Colorimeter reading	Log 100/reading of sample	Sperm no. ($x10^6$)
0.25			10.50	0.97881	1,127.50
0.50			.75	0.96859	1,115.11
0.75			11.00	0.95861	1,103.02
1.00	2.00000	2,365.12	.25	0.94885	1,091.19
.25	1.90309	2,247.67	.50	0.93930	1,079.61
.50	1.82391	2,151.71	.75	0.92996	1,068.29
.75	1.75696	2,070.57	12.00	0.92082	1,057.22
2.00	1.69897	2,000.29	.25	0.91186	1,046.36
.25	1.64782	1,938.30	.50	0.90309	1,035.73
.50	1.60206	1,882.84	.75	0.89449	1,025.31
.75	1.56067	1,832.67	13.00	0.88606	1,015.09
3.00	1.52288	1,786.88	.25	0.87778	1,005.05
.25	1.48812	1,744.75	.50	0.86967	995.23
.50	1.45593	1,705.74	.75	0.86170	985.57
.75	1.42597	1,669.43	14.00	0.85387	976.08
4.00	1.39794	1,635.46	.25	0.84619	966.77
.25	1.37161	1,603.55	.50	0.83863	957.61
.50	1.34679	1,573.47	.75	0.83121	948.61
.75	1.32331	1,545.01	15.00	0.82391	939.77
5.00	1.30103	1,518.01	.25	0.81673	931.07
.25	1.27984	1,492.33	.50	0.80967	922.51
.50	1.25964	1,467.85	.75	0.80272	914.09
.75	1.24033	1,444.44	16.00	0.79588	905.80
6.00	1.22185	1,422.05	.25	0.78915	897.64
.25	1.20412	1,400.56	.50	0.78252	889.61
.50	1.18709	1,379.92	.75	0.77599	881.69
.75	1.17070	1,360.06	17.00	0.76955	873.89
7.00	1.15490	1,340.91	.25	0.76321	866.20
.25	1.13966	1,322.43	.50	0.75696	858.63
.50	1.12494	1,304.60	.75	0.75080	851.16
.75	1.11070	1,287.34	18.00	0.74473	843.81
8.00	1.09691	1,270.63	.25	0.73874	836.55
.25	1.08355	1,254.44	.50	0.73283	829.38
.50	1.07058	1,238.72	.75	0.72700	822.32
.75	1.05799	1,223.46	19.00	0.72125	816.05
9.00	1.04576	1,208.64	.25	0.71557	808.47
.25	1.03386	1,194.21	.50	0.70997	801.68
.50	1.02228	1,180.18	.75	0.70443	794.96
.75	1.01100	1,166.51	20.00	0.69897	788.35
10.00	1.00000	1,153.32	.25	0.69357	781.80
.25	0.98928	1,140.19	.50	0.68825	775.36

TABLE X (cont'd.)

Colorimeter reading	Log 100/reading of sample	Sperm no. ($\times 10^6$)	Colorimeter reading	Log 100/reading of sample	Sperm no. ($\times 10^6$)
20.75	0.68298	768.97	32.00	0.49485	540.97
21.00	0.67778	762.67	.25	0.49147	536.87
.25	0.67264	756.44	.50	0.48812	532.81
.50	0.66756	750.28	.75	0.48479	528.77
.75	0.66254	744.20	33.00	0.48149	524.78
22.00	0.65758	738.19	.25	0.47821	520.80
.25	0.65267	732.24	.50	0.47496	516.86
.50	0.64782	726.36	.75	0.47173	512.95
.75	0.64302	720.54	34.00	0.46852	509.06
23.00	0.63827	714.78	.25	0.46534	505.20
.25	0.63358	709.10	.50	0.46218	501.37
.50	0.62893	703.46	.75	0.45905	497.58
.75	0.62434	697.90	35.00	0.45593	493.80
24.00	0.61979	692.39	.25	0.45284	490.05
.25	0.61529	686.93	.50	0.44977	486.33
.50	0.61083	681.53	.75	0.44672	482.64
.75	0.60642	676.18	36.00	0.44370	478.98
25.00	0.60206	670.90	.25	0.44069	475.33
.25	0.59774	665.66	.50	0.43771	471.72
.50	0.59346	660.48	.75	0.43474	468.12
.75	0.58922	655.34	37.00	0.43180	464.55
26.00	0.58503	650.26	.25	0.42887	461.00
.25	0.58087	645.22	.50	0.42597	457.49
.50	0.57675	640.22	.75	0.42308	453.99
.75	0.57268	635.29	38.00	0.42022	450.52
27.00	0.56864	630.40	.25	0.41737	447.07
.25	0.56463	625.54	.50	0.41454	443.64
.50	0.56067	620.74	.75	0.41173	440.23
.75	0.55674	615.97	39.00	0.40894	436.85
28.00	0.55284	611.25	.25	0.40616	433.48
.25	0.54898	606.57	.50	0.40340	430.14
.50	0.54516	601.94	.75	0.40066	426.81
.75	0.54136	597.33	40.00	0.39794	423.52
29.00	0.53760	592.78	.25	0.39523	420.23
.25	0.53387	588.26	.50	0.39254	416.97
.50	0.53018	584.55	.75	0.38987	413.74
.75	0.52651	579.34	41.00	0.38722	410.53
30.00	0.52288	574.94	.25	0.38458	407.33
.25	0.51927	570.56	.50	0.38195	404.14
.50	0.51570	566.24	.75	0.37934	400.98
.75	0.51215	561.93	42.00	0.37675	397.84
31.00	0.50864	557.68	.25	0.37417	394.71
.25	0.50515	553.45	.50	0.37161	391.61
.50	0.50169	549.26	.75	0.36906	388.52
.75	0.49826	545.10	43.00	0.36653	385.45

TABLE X (cont'd.)

Colorimeter reading	Log 100/reading of sample	Sperm no. ($x10^6$)	Colorimeter reading	Log 100/reading of sample	Sperm no. ($x10^6$)
43.25	0.36401	382.40	55.00	0.25964	255.91
.50	0.36151	379.37	.25	0.25767	253.52
.75	0.35902	376.35	.50	0.25571	251.14
44.00	0.35655	373.36	.75	0.25376	248.78
.25	0.35409	370.37	56.00	0.25181	246.42
.50	0.35164	367.41	.25	0.24988	244.08
.75	0.34921	364.46	.50	0.24795	241.74
45.00	0.34679	361.53	.75	0.24603	239.41
.25	0.34438	358.61	57.00	0.24413	237.11
.50	0.34199	355.71	.25	0.24222	234.80
.75	0.33961	352.83	.50	0.24033	232.50
46.00	0.33724	349.95	.75	0.23845	230.23
.25	0.33489	347.11	58.00	0.23657	227.95
.50	0.33255	344.27	.25	0.23470	225.68
.75	0.33022	341.47	.50	0.23284	223.43
47.00	0.32790	338.63	.75	0.23099	221.19
.25	0.32560	335.85	59.00	0.22915	218.96
.50	0.32331	333.07	.25	0.22731	216.73
.75	0.32103	330.31	.50	0.22548	214.51
48.00	0.31876	327.56	.75	0.22366	212.30
.25	0.31650	324.82	60.00	0.22185	210.11
.50	0.31426	322.10	.25	0.22004	207.91
.75	0.31203	319.40	.50	0.21824	205.73
49.00	0.30980	316.70	.75	0.21645	203.56
.25	0.30759	314.02	61.00	0.21467	201.41
.50	0.30539	311.35	.25	0.21289	199.25
.75	0.30321	308.71	.50	0.21112	197.10
50.00	0.30103	306.07	.75	0.20936	194.97
.25	0.29886	303.44	62.00	0.20761	192.85
.50	0.29671	300.83	.25	0.20516	189.88
.75	0.29456	298.23	.50	0.20412	188.62
51.00	0.29243	295.65	.75	0.20239	186.52
.25	0.29031	293.08	63.00	0.20066	184.43
.50	0.28819	290.51	.25	0.19894	182.34
.75	0.28609	287.96	.50	0.19723	180.27
52.00	0.28400	285.43	.75	0.19552	178.20
.25	0.28191	282.90	64.00	0.19382	176.14
.50	0.27984	280.39	.25	0.19213	174.09
.75	0.27778	277.89	.50	0.19044	172.04
53.00	0.27572	275.40	.75	0.18876	170.00
.25	0.27368	272.92	65.00	0.18709	167.98
.50	0.27165	270.46	.25	0.18542	165.96
.75	0.26962	268.00	.50	0.18376	163.95
54.00	0.26761	265.57	.75	0.18210	161.93
.25	0.26560	263.13	66.00	0.18046	159.95
.50	0.26360	260.71	.25	0.17881	157.95
.75	0.26162	258.31	.50	0.17718	155.97

TABLE X (cont'd.)

Colorimeter reading	100 reading of sample	Sperm no. ($x10^6$)	Colorimeter reading	100 reading of sample	Sperm no. ($x10^6$)
66.75	0.17555	154.00	78.25	0.10652	70.34
67.00	0.17393	152.03	.50	0.10513	68.65
.25	0.17231	150.07	.75	0.10375	66.98
.50	0.17070	148.12	79.00	0.10237	65.31
.75	0.16909	146.17	.25	0.10100	63.65
68.00	0.16749	144.23	.50	0.09963	61.98
.25	0.16590	142.30	.75	0.09827	60.34
.50	0.16431	140.37	80.00	0.09691	58.69
.75	0.16273	138.46	.25	0.09555	57.04
69.00	0.16115	136.54	.50	0.09420	55.40
.25	0.15958	134.64	.75	0.09286	53.78
.50	0.15802	132.75	81.00	0.09151	52.14
.75	0.15646	130.86	.25	0.09018	50.53
70.00	0.15490	128.97	.50	0.08884	48.91
.25	0.15335	127.09	.75	0.08751	47.30
.50	0.15181	125.22	82.00	0.08619	45.70
.75	0.15027	123.36	.25	0.08486	44.08
71.00	0.14874	121.50	.50	0.08355	42.50
.25	0.14722	119.66	.75	0.08223	40.90
.50	0.14569	117.81	83.00	0.08092	39.31
.75	0.14418	115.98	.25	0.07962	37.73
72.00	0.14267	114.15	.50	0.07831	36.15
.25	0.14116	112.32	.75	0.07702	34.58
.50	0.13966	110.50	84.00	0.07572	33.01
.75	0.13817	108.69	.25	0.07443	31.44
73.00	0.13668	106.89	.50	0.07314	29.88
.25	0.13519	105.08	.75	0.07186	28.33
.50	0.13371	103.29	85.00	0.07058	26.78
.75	0.13224	101.51	.25	0.06931	25.24
74.00	0.13077	99.72	.50	0.06803	23.69
.25	0.12930	97.94	.75	0.06677	22.16
.50	0.12784	96.17	86.00	0.06550	20.62
.75	0.12639	94.42	.25	0.06424	19.09
75.00	0.12494	92.66	.50	0.06298	17.57
.25	0.12349	90.90	.75	0.06173	16.05
.50	0.12205	89.16	87.00	0.06048	14.54
.75	0.12062	87.42	.25	0.05923	13.02
76.00	0.11919	85.69	.50	0.05799	11.52
.25	0.11776	83.96	.75	0.05675	10.02
.50	0.11634	82.24	88.00	0.05552	8.53
.75	0.11492	80.52	.25	0.05429	7.04
77.00	0.11351	78.81	.50	0.05306	5.54
.25	0.11210	77.10	.75	0.05183	4.05
.50	0.11070	75.40	89.00	0.05061	2.58
.75	0.10930	73.70	.25	0.04988	1.69
78.00	0.10791	72.02			

VITA

Robert L. Montgomery
candidate for the degree of
Master of Science

Thesis: CHARACTERISTICS OF SEMEN OF YOUNG DAIRY BULLS MAINTAINED ON TWO LEVELS OF PHOSPHORUS INTAKE

Major: Dairy Production

Biographical:

Born: December 24, 1930. Wetumka, Oklahoma

Undergraduate Study: Oklahoma A & M College
Stillwater, Oklahoma
1948-50. 1952-54.

Graduate Study: Oklahoma A & M College
1954-55.

Experiences: U. S. Army, 1950-51.

Member of American Dairy Science Association, Farm House Fraternity, Red Red Rose.

Date of Final Examination: December 29, 1955.

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AUTHOR: Robert L. Montgomery

THESIS ADVISER: Dr. Stanley D. Musgrave

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TYPIST: Mrs. Carol Janssen
Mrs. Twyla Milligan