EFFECT OF FORCED EXERCISE UPON REPRODUCTION

OF THE DAIRY BULL

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OF THE DAIRY BULL

by

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INTRODUCTION

The number of cows enrolled in artificial breeding associations approached the three million mark in 1949 which indicates that one cow in nine in the United States probably will be bred artificially in 1950. Compared with January, 1939, this is an increase of 372,322 or 576.3 percent in number of herds and 2,819,991 or 2,651.6 percent in number of cows.

This method of mass improvement for dairy cattle has directed attention toward the many factors affecting fertility. Fundamental research on artificial insemination procedures and their practical applications have increased the average number of cows served by each bull in the associations.

In artificial breeding, extending the use of older bulls with creditable breeding records has increased the average service age and made it imperative that management practices be evolved which will prolong the useful life of desirable sires.

Up to the present, increased dilution rates probably have contributed most to the wide spread use of good sires. While the experience of those concerned with the reproductive efficiency of bulls suggests that exercise is beneficial to their breeding abilities and fertility, the reports in the literature give only limited quantitative data on the quantity and quality of semen and on fertility.

Various investigators have indicated that proper exercise contributes to the physical well-being of the bull and increases spermatogenic activity. Bartlett and Perry (19) exercised three bulls at the rate of two and one-half miles per hour for one hour each day for one month. The volume of semen increased 51 percent as compared to the previous month. Woodward (160) reported that exercise at two and one-quarter miles per hour for 30 minutes daily greatly diminished the time of service with a slow bull. It was found that exercise increased motility and longevity of the spermatozoa. Hamilton and Symington (65) observed that daily exercise increased the volume of the ejaculate, sperm concentration and motility. The lower quality of semen obtained during February and March by Weatherby and associates (151) was believed to be due to lack of exercise during these months. Lepard and co-workers (87) noted no significant differences between exercised and non-exercised sires in the factors of morphology, concentration of the sperm, and viability of the sperm at 4.5° C. The volume of semen showed a slight but insignificant advantage to bulls receiving exercise. Conception rates of the two groups followed the same general trend. This study only shows the value of exercise on the amount and quality of semen as determined by semen characteristics for a sixteen weeks' trial.

Most of the above mentioned workers measured the effect of exercise on bulls only by laboratory tests presumably designed to indicate the quality of semen. In spite of the findings favorable to exercise, many of the larger bull studs have operated over a period of years in which the bulls have received limited exercise (movement in stalls). This question of the value of exercise for bulls to produce good quantities of semen of acceptable quality has become one of widespread interest.

The conception rates measured as "non-returns" for 60-90 days have been increasing due to improvement in collection, processing, storage and shipment of semen. Therefore, any procedure that will further increase the rates are diligently pursued. An experiment was designed to investigate the influence of forced exercise of dairy bulls on semen characteristics, general well being of the animals, and primarily on conception rates, "non-returns" for 60-90 days.

REVIEW OF LITERATURE

The reproductive processes of the dairy bull are intricate functions affected by many interrelated forces. To entirely separate and discuss them as separate entities is impossible. However, in this thesis, the review has been limited to spermatogenesis and related factors from an artificial insemination viewpoint.

Unless otherwise indicated, this review of literature pertains to the reproduction of the dairy bull. Herman and Swanson (69) and Anderson (13) have made extensive reviews concerning the variations in dairy bull semen with respect to its use in artificial insemination.

Characteristics of Spermatozoa

Normal Morphology

The normal spermatozoon of the bull is a slender motile flagellate cell having a head, neck, body and tail. When stained, it is found to consist mainly of a compact structureless mass of deeply staining chromatin carrying the hereditary factors.

Anderson (14) gave the following measurements for the various parts of bull spermatozoon: head length -- 5 u., head width -- 2 u., and tail length -- 32 u.. He states that it contains little or no cytoplasm. The head of the spermatozoon of the bull, which is somewhat similar to the sperm of most domestic animals, is oblong or broadly elliptical and appears blunt at the end.

Reed and Reed (113) confirmed the work done by Clark on the observations of bull spermatozoa made by the electron microscope. These workers found the existence of a protoplasmic cap and a brush-branched tail tip. In addition, a certain number of organisms found in every field revealed heavy granulation throughout the protoplasmic cap. The granules were approximately 0.25 u.. in diameter and when photographed, gave the appearance of enlarged micro-photographs of intestinal villi. Since the granules appeared to encroach on the spermatozoon head, these authors believed this was due to the cap overlying the crescentic anterior border of the head. They suggested that these granules may represent age changes in the cells.

Savage and associates (125) investigated the relationship between the head length of bull spermatozoa and fertility. They found that in bulls of good fertility that the head length showed normal or almost normal distribution, and these workers maintained that the coefficient of variation of head length provides a good indication of fertility. In normal bulls, the coefficient should not exceed four according to Lagerlof (84). He considered bulls with a coefficient of four and under as fertile, and those with a coefficient 4.5 and over as of reduced fertility.

Abnormal Morphology

Abnormal dairy bull spermatozoa have been placed into various classifications by investigators in this field. Addis (1) recognized the following types of abnormal spermatozoa, (1) those differing from normal in size, that is, giant and dwarf forms, (2) those with two heads or two tails, (3) those with abnormally shaped heads, such as general tapering of the head, (4) those with loose heads, (5) those with abnormalities in the middle pieces and (6) those with disturbed staining capacity (staining too strongly or too faintly).

Herman and Swanson (69) classified abnormal dairy bull spermatozoa into the following groups in the descending order of their occurrence: (1) coiled tails, (2) tailless, (3) pyriform heads, (4) other head abnormalities such as damaged heads, tapering or pointed heads, small and large heads, and phantom heads, and (5) body abnormalities including damaged, enlarged, filiform, beaded or shrunken middle pieces, double tails, broken necks, and protoplasmic drops in the neck. Bulls with poor breeding records had a relatively greater number of coiled tails and tailless spermatozoa than bulls having a high conception rate. The percentage of abnormal spermatozoa varied widely within and between bulls at each fertility level. The semen of dairy bulls of good, questionable and poor fertility averaged 14.2, 24.0 and 45.8 percent respectively. No particular type of abnormality seemed to be

associated with reduced fertility. These authors indicated that the upper limit of percentage of abnormal spermatozoa compatible with good fertility is approximately 30 percent.

Salisbury and associates (123) classified spermatozoa into the following groups: (1) morphologically normal, (2) true abnormals, which included such types as those possessing any abnormality with respect to the shape or size of the head, those with enlarged, abaxial, beaded or filiform middle pieces, and those with thickened or double tails, (3) tailless spermatozoa, (4) spermatozoa with broken necks, and (5) spermatozoa with coiled tails. Lagerlof (84) studied the semen of good fertility bulls. He found that the percentage of abnormal spermatozoa did not exceed 18 percent (average 10 to 12 percent). One-hundred and fifty bulls of lessened or arrested fertility showed 20 percent or more abnormal spermatozoa.

Addis (1) reported that three sterile bulls which he examined were normal as far as volume, appearance, concentration and motility of the semen were concerned, but that 40 to 50 percent of the spermatozoa were abnormal. Generales (55) stated that good fertility could be expected if the percentage of abnormal spermatozoa was not over 25 percent. When it reached 60 percent, the animal was sterile. Similar results were reported by Sciuchetti (127).

Anderson (11) found abnormal spermatozoa averages of 8.1, 13.1 and 17.6 percent respectively in fertile bulls, bulls of reduced fertility, and in sterile bulls. In a further study, Anderson (13) showed that six bulls of high fertility gave an average of 10.5 percent. Morphological characteristics of 24 dairy bulls of the Holstein, Guernsey, Jersey, and Ayrshire breeds used in the University of Nebraska's Dairy Herd were observed monthly for a period of years by Trimberger and Davis (143). They found that the bulls averaged 790.5 normal sperm cells per 1,000, with a range from 276 to 968 for individual samples, and from 373 to 904.4 from the lowest to the highest average for a bull. The breeding efficiency was 57.76 percent conception in the 483 cows bred. Bulls with 900 normal sperm cells per 1,000 spermatozoa had significantly better breeding records. Whereas, bulls with less than 500 normal sperm cells per 1,000 spermatozoa had very poor breeding records.

Davis et al., (40) found that the percentage of atypical sperm in the semen from 11 fertile bulls was relatively constant, average 18 percent or less. Erb and associates (51) observed that bulls produced a greater number of abnormal sperm during the summer months than during any other season. Swanson and Herman (137) showed that for some bulls, good fertility was maintained in spite of concentrations of abnormal spermatozoa as high as 30 percent. They concluded that some pathological condition must be present when the percentage of abnormal sperm approaches 50 percent.

In conclusion, the percentage of abnormal spermatozoa that appears to be compatible with good fertility in the dairy bull varies between 10 to 30 percent. However, it is not always possible to determine the exact fertility level of the dairy bull by using the percentage of abnormal spermatozoa.

Characteristics of Semen

Anderson (13) defines the ejaculate of a normal bull of good fertility as an opaque, whitish yellow fluid of milky or milky-creamy consistency. The measurements of semen which characterize it are empirical and cannot be discussed as separate entities. An attempt will be made to treat each characteristic, but to do so means that these measurements will be relative to associated factors of semen on its production.

Volume

The volume varies considerably in the same bull and in different bulls from time to time. Herman and Swanson (69) noted that in one series of 334 ejaculates collected from 50 bulls, the volume ranged from 2.5 to 5.5 ml. with a mean of 4.38 ± 1.02 ml. Anderson (13) gave the average volume of the ejaculate as 4 ml. with a range of 5 ml. to 12 ml.

It was early observed by Anderson (11), that the volume of semen collected from sterile bulls is less than that obtained from bulls of high fertility. He indicated that the volume is usually smaller in young bulls than in adult bulls.

However, Swanson and Herman (69) found that the volume of semen did not vary directly with the age of the bulls. In general, the volume of the ejaculate varied as the size of the bull within any one breed. A few old bulls that were small gave much less semen per ejaculate than younger, larger bulls. Anderson (9) noted that the volume is maintained fairly constant over a period of five to six years, but it may decrease as the bulls become older.

A second ejaculate collected shortly after the first is usually larger as shown by Anderson (11). Davis and Williams (41) and Mercier and Salisbury (94) noted a slight increase in the volume of the second ejaculate, with little difference between the second and third ejaculates. Lasley and Bogart (86) observed that the volume of semen in range beef bulls increased as the interval between collections increased.

There are indications of possible breed differences. Anderson(9) observed that Holstein bulls gave larger ejaculates than Ayrshires. Lewis (88) found a highly significant difference between bulls of the same breed, and also an indication that Guernsey bulls gave larger amounts of semen per ejaculate than did Holstein bulls.

Concentration

The total number of spermatozoa in an ejaculate (volume X concentration) is a highly important factor in determining the usefulness of bulls in artificial insemination.

A wide range of variation in concentration occurs in both bulls of low and high fertility. Erb and co-workers (51) noted highly significant differences between bulls for concentration of spermatozoa per cu. mm. Anderson (13) gives the average number of spermatozoa as 600,000 to 1,000,000 per cu. mm. Davis and Williams (41) noted a range from 8,000 to nearly 2,000,000 with an average of 734,000/cu. mm.

Walton and Edwards (146) showed by "exhaustion tests" that the total number of spermatozoa for 10 collections from 14 bulls ranged from 2,880 million to 32,140 million with an average of 14,000 million per collection. Herman and Swanson (69) found that the concentrations of spermatozoa per cu. mm. rank in order as follows: first ejaculate, 826,000; second ejaculate, 635,000; third ejaculate, 357,000. Anderson (9) reported that spermatozoa are few or absent in epididymites, in atrophied cases and in hypoplasia of the testes.

It would be of considerable assistance to artificial breeding stud managers to know the approximate number of spermatozoa required for conception in order to make maximum use of semen. Herman and Swanson (69) found that the conception rate was practically independent of the amount of semen used in inseminations when .4 ml. or more of fresh semen was used per insemination. Salisbury and associates (117) in cooperation with the New York Artificial Breeder's Co-operative diluted semen at a rate varying from 1:2 to 1:14. Comparable rates of conception were obtained. Later, work by Salisbury and Bratton (118) showed in an experiment involving 7,343 inseminations, that when sulfanilamide was added to yolk-citrate diluter, there was no difference in fertility level for dilution rates of one part of semen to 100, 150, 200, 300 and 400 parts of the yolk-citrate-sulfanilamide diluter. Branton and co-workers (32) found that diluting bull semen with egg-yolk-citrate-sulfanilamide containing 16, 12, 6, and 4 million spermatozoa per ml. resulted in 62.8, 64.0, 58.0 and 63.5 percent non-returns respectively on 30 - 60 day basis. Statistically, these fertility levels were not significantly different. Not less than o.3 ml. of diluted semen should be used for insemination according to a study by Herman and Swanson (69).

Four controlled experiments were conducted by Willett (153) to study dilution levels above 1:100. A total of 11,372 services from 69 collections from bulls selected for high fertility showed a downward trend with an increase in dilution level, but none of the differences were significant. As the number of spermatozoa decreased from approximately 12 million to six million spermatozoa per ml., there was a drop of about 2.6 percent in non-returns per million of spermatozoa used per insemination. There was a marked decrease in livability of spermatozoa with increased dilution rates. Chang et al. (36) studied the effect of dilution upon motility of bull spermatozoa on six semen samples from each of five bulls. The semen was diluted 1:10 and also successively in eight different dilutions from 1:100 to 1:12,800. Two different diluents were used, 0.9 percent sodium chloride and 0.08 M. sodium

citrate. The percentage of motile spermatozoa decreased markedly in the higher dilutions.

It appears to the author that further investigations are needed to determine the optimum dilution rate for fertile bull semen to insure the highest possible conception rates.

Motility

Motility of spermatozoa varies considerably. However, in general, high motility ratings signifies good conception rate. It is not an infallible index, but it has been the one criterion most commonly used by successful artificial breeding associations.

The motility of spermatozoa from normal, fertile bulls is usually 70 percent and above as reported by Anderson (9). He noted that in one series of 456 ejaculates, only three percent had a motility of less than 70 percent. In another series of 254 ejaculates, 11 percent had motility of less than 70 percent.

Herman and Swanson (137) examined 256 ejaculates from 10 bulls. They noted that initial motility varied less than any other semen characteristic. There were striking differences in motility ratings between bulls of high and low fertility. Bulls with questionable breeding records were occasionally observed to give high motility ratings, but on the other hand, not all bulls which produced semen of good initial motility were of high fertility. These workers stated that semen very high in abnormal spermatozoa usually does not have good

motility or viability, but if it does have good motility and viability, it will probably give good breeding efficiency. They concluded that semen used for insemination should show an initial motility rating of three (75 - 85 motile spermatozoa). Although semen with a low motility rating was of lower fertility, they indicated that semen showing a progressive motion of 45 percent gave nearly as high a conception rate as semen with a higher motility rating. These data showed that a curvilinear relationship existed between conception rate and motility rating of the semen at time of insemination.

A study by Anderson (12) of 1,049 ejaculates during a 27 month experiment showed highly significant individual bull and monthly differences in motility of spermatozoa. Lewis (88) found that the average semen motility was least in July, August and September. Anderson (13) states that occasionally motility in the first ejaculation is poorer than in the second or third ejaculates. Davis and Williams (41) found that in motility, the ejaculates ranked as follows: first, with a mean of 73 percent; second, with a mean of 75 percent; and third, with a mean of 69 percent. Mercier and Salisbury (94) found that in general, the second ejaculate contained more motile sperm than the first ejaculate.

In a study of some factors affecting spermatogenesis and fertility of dairy bulls, Lewis (88) observed a breed difference in initial motility, the Holstein breed showing a higher initial motility than the Guernsey breed. This was especially true in the winter and spring, but not as pronounced in the

fall and summer.

Several workers have tried to classify the various types of spermatozoa motility. Walton (147) recognized three types of motility: (1) progressive, (2) rotary, and (3) oscillatory. In addition to this classification, other workers have used different terms to describe various other types of movements. Rao and Hart (111) classified the motility of bovine sperm into three main types, depending on the vigor and direction of movement, (1) maximal motility, (2) circular motility, and (3) convulsive motility. They stated that under routine artificial insemination, maximal motility is desired.

In summary, one may say that a motility of 70 percent or more is necessary for practical use in artificial insemination. However, semen relatively low in motility may be effective in producing conception.

Hydrogen-Ion Concentration

The hydrogen-ion concentration of semen would appear to be of general value for the appraisal of fertility in the dairy bull according to Anderson (13). One of the earlier estimations of the pH of bull semen was made by Webster (150) who found that almost all specimens ranged between 7.0 and 7.5. Davis (39) found that the pH varied widely between semen collected by massage of the seminal vesicles, the ampullae of the vasa deferentia and by the artificial vagina. Semen obtained by the former method yielded a relatively greater amount of accessory secretions and a pH of 7.5 to 8.0 was not uncommon, while for the latter method, the pH was ordinarily below 7.0.

Davis and Williams (41) using a quinhydrone gold electrode, obtained a pH which ranged from 6.18 to 8.31 with a mean of 6.99. Anderson (10) found a pH of $6.73 \pm .020$ for 221 ejaculates from clinically normal bulls. Herman and Swanson (69) observed little difference between the pH values of the semen of bulls of good and poor fertility. The pH was practically the same for the more fertile bulls (pH of 6.47) and the questionable bulls pH of 6.50 but was slightly higher for the poorer breeders; this being mainly due to two bulls which produced poor quality semen. They noted that the pH of bull semen showed the least absolute variation next to the initial motility.

Erb and co-workers (51) secured highly significant differences between bulls and between months for all semen characteristics except the pH. The pH of the semen of abnormal bulls was investigated by Anderson (13). An alkaline reaction was characteristic in typical cases of epididymitis and bulls with small testes. The alkalinity was associated with a decrease in concentration or absence of spermatozoa.

Anderson (10) noted that when two ejaculates are collected, one after the other, from fertile bulls, the second ejaculate usually has more acid than the first. In general, this change in reaction is associated with greater concentration and higher motility of the second ejaculate. Swanson and Herman (135) in their study of the correlation between some characteristics of dairy bull semen and conception rate found little practical value in making routine pH determinations. The correlation between conception rate and pH of the semen was not significant. The pH of the semen was significantly lower in the summer than in the fall, as reported by Swanson and Herman (136).

Semen characteristics presented by the foregoing authors are summarized in Table I.

Hyaluronidase

Spermatozoa movements have been credited with partially erroding the cumulus oophorous and penetrating the corona radiata, zona pellucida and the vitelline membrane of the ovum. Recent work has indicated that an enzyme, hyaluronidase, present in sperm aids the process of fertilization by liquefying the gel, thus helping to disperse the cumulus cells surrounding the ovum.

It has been shown by McClean (90) that some species of pathogenic bacteria produce a spreading factor which markedly increases the permeability of the skin to injected fluids. A similar action is accomplished by a heat labile extract of mammalian testes, McClean (91) and Pincus and Enzmann (110). Swyer (138) found a close correlation between hyaluronidase content of the semen and number of spermatozoa in rabbits, bulls, boars and men. No correlation was evident in the case of dogs and fowls. The fact that semen from cryptochids con-

TABLE I

SUMMARY OF SEMEN CHARACTERISTICS

Author	Volume ml.	Concentration cu. mm.	Motility Percent	Abnormals Percent	pH
Anderson (13)	4.00	648,540	73 ± 1.8		
Anderson (9)				10.5	
Anderson (10)					6.73
Herman (69)	4.38	826,000	75 - 85	30.0	6.50
Erb (51)		914,000		12.7	
Lagerlof (84)	3.00	800,000		10.7	
Generales (55)				25.0	
Walton (147)		14,000 million per ejaculate			
Williams (156)				17.0	
Herman (70)	4.20				7.7
Webster (150)					7.5
Davis (39)					6.99
Davis (40)		734,000	73.7		

tains no hyaluronidase indicates that it is secreted by the seminiferous epithelium rather than by the accessory glands. A study by McClean (91) and Rowlands (116) showed that the hyaluronidase content of the semen was related to the total number of spermatozoa in the sample. Thus, these authors suggest that it is reasonable to assume that some cases of sterility or low breeding efficiency could be due to insufficient spermatogenesis, with the result that the hyaluronidase present was reduced to such an extent that the cumulus cells surrounding the ovum were not dispersed and the relatively few sperm present were unable to penetrate to the ovum.

In line with this suggestion, Rowlands (116) conducted an experiment in which hyaluronidase was added to various concentrations of rabbit spermatozoa. In these studies the treated semen required only one sixth as many spermatozoa to produce a 50 percent response when compared to the semen to which no hyaluronidase was added. Chang (37) has also studied the effect of adding hyaluronidase to rabbit semen containing the minimal number of sperm necessary for fertilization. Under his conditions, the addition of the hyaluronidase did not increase the fertilizing capacity of the sperm. Kurzrok (82) found that in normal human semen the hyaluronidase content increases with sperm concentration up to 100 million sperm cells per cc. The hyluronidase content did not appear to be related to the morphology, motility or percent motility of the sperm in this study.

The source of hyaluronidase in semen has been studied by several investigators. Bergenstal and Scott (23) have reported that hyaluronidase is probably derived from the sperm cell itself or is very closely associated with them. No hyaluronidase was found in prostatic and seminal fluids of humans and dogs although it was recovered from the testes. The hyaluronidase content of homogenized semen was at its peak at or shortly after ejaculation. However, in whole semen, the content gradually increases with time.

The question of whether hyaluronidase is released into the semen by live sperm or is diffused only by moribund spermatozoa has been studied by Swyer (138), Kurzrok and associates (83) and Johnston and co-workers (75). The evidence appears to indicate that hyaluronidase is released into the seminal fluid by dead spermatozoa. Swyer (138) observed that freezing or otherwise mistreating sperm increased the amount of hyaluronidase present in the seminal fluid. Johnston and associates (75) found the concentration of hyaluronidase increased at a slower rate when the semen was stored at 5'C. than under storage at 37'C. The concentration of hyaluronidase in spermatic fluid is in direct proportion to the number of spermatozoa found in the particular specimen as reported by Greenburg and Gargill (60).

Kurzrok (82) and Johnston and associates (75) have reported a lack of correlation between hyaluronidase titre and one or more of the following: morphology, motility, concentration, total sperm and volume. Johnston et al. (75) have reported significant negative correlation between hyaluronidase content, the initial percentage of live sperm and the percentage of live sperm surviving cold shock.

Sallman and Berkeland (124) were the first workers to study the relation of hyaluronidase to bull fertility. The percent of fertile services increased with increasing amounts of hyaluronidase up to a certain point beyond which fertility decreased. From these findings, the authors suggest that in some cases marked by oligospermia the addition of hyaluronidase might increase the conception rate. On the other hand, the fertilizing capacity of some semen, in which the hyaluronidase titre is high, might be improved by removing some of the hyaluronidase.

Tobin and associates (142) have reported that the hyaluronidase activity of testicular homogenate is decreased in rats by a protein deficient diet. Johnston and Mixner (76) secured no statistical correlations between the hyaluronidase content and fertility levels of semen from bulls of relatively high fertility when the semen is diluted at a rate of 1:100 or less.

Preservation of Semen

Storage of Semen

Many investigators working on artificial insemination problems have shown that a satisfactory rate of conception can be obtained when semen is stored for varying lengths of time.

Herman and Ragsdale (70) noted that one-third more services per conception was required when fresh semen was used four to five hours after collection than when used one to two hours after collection. Using an egg-yolk phosphate buffer as a diluter, Phillips (106) and Phillips and Lardy (107) noted that diluted semen which had been stored for 100 hours at 10° C maintained its fertilizing capacity.

Breeding results obtained from 13 artificial breeding associations receiving semen from the University of Nebraska bull stud was analyzed by Schultze et al. (128) to determine the effect of day to day storage on semen fertility. They found that fertility declines an average of 4.61 percentage units with each day of storage up to four days. Other investigators, Anderson (13) and Willett and associates (154), have reported that no significant decrease in fertility occurred in semen stored up to four days when the semen was of good quality, properly diluted, and quickly cooled.

Patrick (102) found in his study of factors affecting semen storage and handling, that the quality of the semen is lowered slightly by shipment. This slight reduction in the quality of semen was believed to result from the lack of a low uniform temperature in transit.

Anderson (7) studied the keeping qualities of bull semen by determining the amount of time required to collect semen, length of time before dilution, and time required to cool the semen. He compared the results by diluting immediately and cooling as soon as possible after collection, diluting immediately and delaying cooling for 45 minutes, and delaying diluting and cooling for 45 minutes after collection. The differences found in motility ratings 72 hours after collection, in incubation tests 72 hours after collection, and in methylene blue reduction time 24 hours after collection, proved that significant differences existed, and immediate dilution and quick cooling was the best procedure.

Foote and Bratton (52) found that based on 60 to 90 days non-returns from 8,518 first and second service cows, the fertility level of semen cooled in extender was 59.3 percent and that cooled without extender was 52.8. The difference between treatments of 6.5 percentage units was highly significant statistically. Motility estimates made after 3, 24, 48, 72 and 96 hours of storage indicated that the samples cooled without extender had a definitely lower percentage of motile spermatozoa.

Diluters

Considerable difference of opinion exists as to the relative merits of the available diluents for bovine semen used in artificial breeding. Since the development in this country of the phosphate-egg-yolk diluents by Phillips (106) and Phillips and Lardy (107) and the citrate-yolk formula by Salisbury et al. (120) various diluents have been used to preserve the semen of the dairy bull.

Phillips and Lardy (107) found that egg-yolk buffered with monobasic potassium phosphate and dibasic sodium phosphate to a pH of 6.75 preserved motility of dairy bull semen for periods in excess of 100 hours stored at 10° C. Salisbury and associates (120) observed that an M/7.5 solution of sodium citrate dihydrate mixed in equal amounts with fresh egg-yolk produced a diluent which was superior to the yolk-phosphate diluent for the preservation of motility when semen was stored for six days or more. Later work by this author and associates (121) showed that 2.9 percent of the sodium citrate dihydrate per 100 ml. of distilled water gave an isotonic diluter.

By means of a 6 x 6 latin square design, Bratton and co-workers (33) compared six bovine semen diluents. Based on the percent non-returns, the mean fertility level for each diluent was as follows: phosphate-yolk, 50.5; 3.6 percent citrate-yolk, 50.5; 3.6 percent citrate-sulfanilamide-yolk, 55.3; 2.9 percent citrate-sulfanilamide-yolk, 56.5; ortho tablet-yolk, 56.4; and ortho liquid, 55.0. The average non-returns for the sulfanilamide-containing diluents was 5 percentage units higher than for those diluents not containing sulfanilamides. This difference was significant at the one percent level of probability.

Gravers and associates (58) found that the following treatments of fresh semen had no significant effect upon conception rate: (1) no treatment (control), (2) diluted with fresh egg-yolk buffer, (3) diluted with stored egg-yolk buffer, and (4) diluted with autoclaved milk.

Weeth and Herman (151) found that a synthetic pabulum (Phillips) maintained a grade one motility in dairy bull semen significantly longer than did egg-yolk citrate diluent, however, conception rate in the field with pabulum was significantly less. Bayley and co-workers (20) furthered the work of Weeth and Herman (151) by studying a synthetic pabulum vs. yolk-citrate buffer as diluter of bull semen. In a trial involving 1,284 field inseminations, the fertility was 15 percent higher with semen of 19 bulls diluted with egg-yolk citrate buffer than with other portions of the same semen diluted with a synthetic pabulum.

Anti-Biotics

Practically all workers in the rapidly expanding field of artificial insemination have stressed the bacteriological control of semen. Gunsalus et al. (63) first discussed the undesirable effect of large numbers of certain bacteria in semen. They collected 43 ejaculates from 19 bulls using an artificial vagina. The bacterial count of these ejaculates ranged from 1,000 to 22,000 per cc. Gunsalus and associates (62) observed that when sanitary precautions were practiced, the number of bacteria found in freshly drawn semen or freshly prepared yolk-citrate diluent was not sufficient to interfere with the methylene blue reduction test for semen quality.

In a study of the genital tract of bulls inspected shortly after slaughter, Gilman (56) reported finding Pseudomonas pyrocyaneus along with unidentified rods, micrococci, streptococci and coliform organisms. In reproductive tracts of normal bulls, his findings on bacterial content agreed with those of Williams and Kingsbury (155) as to normal bulls being relatively free of bacteria, whereas impotent bulls harbor large numbers of bacteria. These authors reported finding micrococci, hemolytic and non-hemolytic streptococci, coliform and Brucella abortus in semen recovered from the vagina of cows immediately after service.

Gunsalus et al. (63) recorded plate counts of semen from bulls with unclean sheaths from 10 to 100 fold higher than those which previously had been cleaned. They found pseudomonas, coliform, diptheroids, bacilli, staphylococci and, in one case, hemolytic diptheroids present in the semen. A high percentage of the bulls diminished in fertility within six months after the isolation of Pseudomonas pyocyaneus from the semen. Edmondson and co-workers (46) have furthered the work of the above workers. They isolated the following organisms, listed in order of their predominance, from the semen of 36 dairy bulls used in artificial breeding work: bacilli, micrococci, coliform, hemolytic, streptococci, non-hemolytic streptococci, pseudomonas, actinomyces, proteus and yeasts. Five sires from which Pseudomonas pyocyaneus organisms were isolated were low in fertility, and eventually, all five became sterile. There was no apparent relationship between the fertility of the sires, livability of spermatozoa, and the total plate count of the semen. These workers observed that the addition of micrococci, yeast and non-hemolytic streptococci to fresh diluted semen caused a substantial increase in the storage time.

The control of certain bacteria by use of the bacteriostatic compounds is advocated and recommended by many investigators in the artificial insemination of dairy cattle. Shettles (131) observed that the survival and activity of human spermatozoa was not reduced by the addition of sulfapyridine or sulfanilamide in concentrations up to and including 160 mg. per 100 ml. of diluent.

Knodt and Salisbury (81), working at the Cornell station, were the first investigators to study the feasibility of using certain bacteriostatic compounds to control bacterial growth in bull semen. They found that the addition of 200 mg. or more sulfanilamide per 100 ml. of yolk-citrate diluter controlled the growth of bacteria in stored diluted semen for 20 days at 5° C. Later, these workers (122) observed that the use of 300 mg. of sulfanilamide per 100 ml. of diluter increased the fertility of bull semen used routinely in the breeding of dairy cattle.

The effects of penicillin added to bull semen were first reported by Almquist and co-workers (6). They found no significant decrease in the motility of diluted stored semen when concentrations of penicillin ranging from 0 to 1,000 units per ml. were added. However, levels of penicillin ranging from 1,000 to 2,000 units per ml. brought about a significant decrease in the motility during storage. Phillips and Spitzer (108) recommended that .03 percent sulfathalidine, sulfasuxidine or streptomycin be added to their L.G.B. (lipid, glucose, buffergum) diluter for the control of bacterial contamination.

They also mentioned that penicillin, used in proper amounts, was not toxic to the sperm. Later work by Almquist and associates (5) demonstrated that streptomycin will inhibit the growth of bacteria in diluted bull semen. The additions of 100, 250, 500, 750 and 1,000 gamma per ml. of diluted semen did not significantly affect the livability of bull spermatozoa during a 20-day storage period, but levels of 1,250, 1,500 and 2,000 gamma per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a storage period of 20 days. Easterbrooks and co-workers (44) studied the effect of streptomycin upon the fertility of 16 dairy bulls. They found that the addition of streptomycin to diluted semen increased the percent non-returns of 14 of the 16 bulls. This increase in percent of non-returns was 14.4 units over the untreated semen.

Foote and Salisbury (54) studied the effects of several sulfonamides upon the livability of spermatozoa and upon the control of bacteria in diluted bull semen. Twelve sulfonamides were added to bull semen diluted with citrate-phosphate and stored at 20'C. When the optimum level for each sulfa drug was determined for spermatozoan survival, nine of the 12 compounds increased the livability of the spermatozoa over that observed when no sulfonamide was added to the diluent. Of these nine drugs that increased livability of the spermatozoa, only two sulfonamides, sodium sulfamethazine and carboxysulfathiazole were significantly superior to sulfanilamide in maintaining motility of the sperm cells; they were inferior in controlling bacterial growth. Sulfanilamide slightly decreased the rate of motility, and N-benzoylsulfanilamide exerted a similar but more pronounced effect.

Foote and Salisbury (53), in studying the effects of a number of compounds, chemically unrelated to the sulfonamides, found that these effectively controlled bacterial growth. Furacin and phenoxethal proved to be highly bactericidal and at the same time were spermicidal at most of the levels tested. Pyridium was limited to its usefulness by its low solubility. Two commercial penicillins were equally effective in controlling bacterial growth and were slightly superior to sulfanilamide in this respect.

Various workers, Almquist and associates (4) and Mixner (98), have studied the effect of a combination of penicillin and streptomycin upon the livability and bacterial content of bovine semen. Almquist et al. found that a combination of penicillin and streptomycin in levels ranging from 100 to 1,000 units of each per ml. of diluted semen did not significantly decrease the livability of bull spermatozoa during a 20-day storage period. The latter authors conducted two experiments. The first experiment was run on a split sample involving a total of 13 bulls of two breeds, 51 semen samples, and 2,389 first and second services. The second experiment was a two treatment reversal trial with forced collection periods involving a total of 12 bulls, 48 semen samples and 801 first and second service breedings. For experiment one, the control fertility level was 64.8 percent while that of the

treated was 64.7 percent based on 60-90 day non-returns. For experiment two, the corresponding fertility levels were 68.4 percent and 70.4 percent. An analysis of variance indicated that there were no significant differences in these contrasted mean fertility levels.

Almquist (2) compared the effects of penicillin, streptomycin,and sulfanilamide upon the fertility of dairy bulls. He found that treatment of yolk-citrate diluted semen with 1,000 units penicillin per ml., 1,000 units of streptomycin, 1,000 units of each penicillin and streptomycin, and 300 mg. percent of sulfanilamide increased the fertility 21.7 percentage units by penicillin, 25.9 percentage units by streptomycin, and 21.3 percentage units by streptomycin plus penicillin. Sulfanilamide gave a slight decrease of 2.9 percentage units. Further studies by Almquist (3) showed that the addition of penicillin to semen of certain bulls of lowered fertility had a more beneficial effect while failing to be of significant value when added to the semen of other bulls. Mixner (99) substantiated the work of Almquist's by showing there were no significant differences in fertility levels of relatively high fertile bulls when 1,000 units of penicillin, 3 mg. of sulfanilamide, and 1,000 units of penicillin plus 3 mg. of sulfanilamide were added per ml. of diluter.

Myers and associates (100) and Sykes and Mixner (139) studied the influence of aureomycin upon the livability and bacterial content of bull semen. These authors found that the anti-bacterial activity of 1,000 ug. of aureomycin per ml.

compared favorably with the combination of 1,000 units each of penicillin and streptomycin per ml. of diluted semen. However, the addition of aureomycin ranging from 50 to 1,000 ug. per ml. of diluted semen caused toxic effects on motility when compared with portions of the same semen samples containing no aureomycin or 1,000 units each per ml. of penicillin and streptomycin. The latter workers also studied the toxicity of chloromycetin upon bull semen. It was noted that chloromycetin had a relatively low level of toxicity as the initial toxic levels seemed to be between one and two mg. per ml. of diluted semen.

Factors Affecting Quality of Semen

Season and Temperature

The well established fact that there is a seasonal effect on reproduction of domesticated animals suggests that climatic factors such as light and temperature may be responsible.

A number of workers have studied seasonal variation in semen quality and fertility of dairy bulls. Miller and Graves (97) observed that the Beltsville herd required more services per conception in July, August and September than during the rest of the year. Dawson (42) studying the fertility of aged bulls, used at various locations in the United States, found that the relative fertility of sires used at Southern stations was 36 percent as compared to 49 percent for sires used at Northern and Western stations. He concluded that the lesser variation in length of daylight may account for the relatively good winter results reported at low latitudes.

A study by Erb and associates (51) of the breeding efficiency in the Purdue University dairy herd for a 20 year period revealed considerable seasonal variations. The month of May with 74.3 percent had the highest average efficiency, and the month of August with 58.2 percent the lowest average efficiency for the year. Seath and Staples (130) studied an 11 year history of the North Louisiana State Experimental Herd and the Louisiana State University Herd. Seasonal differences in rate of conception were found in both herds. In each case the summer months required more services per conception. The best rate of conception in the Experimental herd was during the winter months followed by the fall period. The University herd had a reverse in this order with the fall months first and the winter months second.

Semen quality examinations by Erb and associates (48) showed it to be of superior quality in the spring and low in quality during the summer. The average semen volume and initial motility was least in July, August and September. The length of sperm survival was least in August and lower in July, September and November than during the other months. July, August and September were also months of high percentage of abnormal spermatozoa. Spermatozoa concentration and total spermatozoa per ejaculate were at high levels during

April, May and June.

Observations were made by Phillips and co-workers (103) on volume, motility, number of spermatozoa per c. c., total abnormal spermatozoa, and proportions of abnormal heads, necks, middle pieces and tails of semen collected from beef and milking shorthorn bulls. Significant or highly significant seasonal differences were found in volume, concentration, total sperm, and proportions of abnormal heads, necks, middle pieces and tails. Of 1,135 matings, 59.6 percent resulted in infertile matings in April and 40.8 percent in August.

Swanson and Herman (136) studied monthly variations in motility, volume, concentration, livability in storage, pH and morphology of semen from bulls used in the University of Missouri dairy herd. The pH of the semen was significantly lower in the summer than in the fall. Initial motility and viability in storage were lowest in the winter.

An analysis of 51,587 breeding months in the Cornell University herd by Clapp (38) showed an average of 2.11 services per conception. There appeared to be a trend toward greater efficiency from March through July. Breeding efficiency dropped sharply in August and continued at low level through February; July required 1.90 services per conception and February 2.29 services per conception.

Mercier and Salisbury (96) found that the lowest percent of successful services was obtained during the winter and spring and the highest during the summer and fall. The difference in fertility level between herds was not significant statistically, but those between seasons were significant at the five percent level of probability. The average monthly conception rate of the three herds studied in Eastern Canada was significantly correlated with the monthly average length of daylight. Temperature changes had no measurable effect on fertility level.

Lewis (88) found that monthly variation in semen quality was not significant in the dairy bull, but the quality was higher in the spring and summer than during the fall and winter. Light and temperature were both positively correlated with spermatogenesis. Light appeared to exert the greater effect.

A study of the seasonal effects showed a highly significant decrease in percentage of motile spermatozoa during the early spring months and a highly significant difference between months in spermatozoa counts, Salisbury (119). The lowest average counts was found in August, but there was no significant difference in fertility of the bulls from month to month.

An analysis of the breeding records at the Bureau of Dairy Industry, Hilder and associates (71) showed that a relatively large number of services were required for conception during midsummer followed by a sharp decrease in the fall.

Mercier and Salisbury (95) investigated on the seasonal variations in fertility of about 25,000 cows and 71 bulls of various ages bred artificially in New York. Winter was found to be the poorest breeding season of the year. The fertility level of the bulls was found to be significantly correlated with the length of daylight, there being a lag of one or two months before the effect of daylight reached its maximum. They concluded that young bulls probably are more subject to changes in light and temperature than are those bulls from six to ten years of age.

Table II shows the seasonal variation found by the authors reviewed in the foregoing as well as data concerning other species. Table III presents a summary of the seasonal effects upon the characteristics of semen.

Age and Frequency of Ejaculation

A difference in breeding efficiency between young and old bulls was found by Miller and Graves (97) in a study of the Beltsville herd. Young bulls appeared to be more fertile than mature bulls when bred to fertile cows. Tanabe and Salisbury (140) found that bulls between the ages of one to three years inclusive, had the highest breeding efficiency of all age groups.

An analysis of 2,636 dairy bulls by Winters (157) indicated their average useful age to be 10.63 years. Sixty-one percent of the 2,636 bulls were in active service when they reached 10 years old, and 4.3 percent of the bulls were still fertile at 15 years of age. Baker and Queensberry (18) were unable to find any fertility differences between old and young range beef bulls. Since the older bulls had been selected for

TABLE II

SEASONAL VARIATION IN FERTILITY

Author	Species	High Fertility	Low Fertility
Parker (101)	Fowl	Spring	Fall
Asdell (17)	Goat	Fall	Late Spring Early Summer
Turner (145)	Goat	Fall	Spring-Summer
Bissonnette (24)	Goat	Fall	Spring-Summer
Green (59)	Ram	Late Fall	Summer
McKenzie (92)	Ram	Fall	Summer
Yeates (159)	Ram	Late Fall	Summer-Early Fall
Miller (97)	Bull		Summer
Dawson (42)	Bull	Late Winter Early Spring	
Erb (51)	Bull	May	August
Seath (132)	Bull	Fall-Winter	Spring-Summer
Phillips (103)	Bull	April	August
Clapp (38)	Bull	Spring	Late Summer-Fall
Mercier (95)	Bull	Summer-Fall	Winter-Spring
Lewis (88)	Bull	Spring-Fall	Fall-Winter
Hilder (71)	Bull		Late Winter Summer

TABLE III

SEMEN CHARACTERISTICS OF THE DAIRY BULL
AFFECTED BY SEASON

	Phillips (104)	Erb (51)	Phillips (103)	Swanson (136)	Mercier (96)	Anderson (12)
Volume	+	+				
Concentration	+	+	+			+
Total Sperm	+	+	+		+	
Motility		+		+	+	+
Percent Abnormals		+	+			
Survival Time		+		+		
pH				+		+

+Affected by Season

high fertility, it is likely that any differences were obscured. Werner and associates (152) and Dawson (42) showed that young bulls showed the highest level of conceptions in natural breeding.

Bowling and associates (28), in studying breeding records over a period of 18 years found that bulls 13 years of age or over were significantly less sure breeders than bulls of six years of age. The breeding efficiency of bulls as they increased in age did not become significant until the bulls reached an average age of six years. Hilder and co-workers (71) using data from 8,919 natural services reported that the number of services per conception for bulls of all ages was 2.67. Bulls seven to 10 years of age required 3.14 services per conception, while bulls under five years of age 1.52 services per conception.

An age difference in response to seasonal variation in fertility was observed by Mercier and Salisbury (95). The correlation between fertility level and average length of daylight for the same period was not significant for bulls under six years of age. These young bulls were relatively high in fertility in winter, gradually decreasing to a low in the hot temperatures of summer. The relationship was high for bulls of six to eight and eight to 10 years of age, low for bulls over 10 years of age, and high for all bulls.

Weatherby et al. (149) showed a marked decrease in spermatozoan concentration of the semen of the bull used once each day for a 57 day period. Lasley and Bogart (86) have reported that semen volume, total number of spermatozoa per ejaculate, percentage of morphologically abnormal spermatozoa, and fertility increased in range bulls as the interval between ejaculations increased from one up to 10 days. While statistically significant, the correlation coefficients which they obtained were quite small. Mercier and associates (93), working at the Cornell Station, concluded that a six day interval between ejaculations is not detrimental to spermatogenic activity. Three groups of four bulls each were ejaculated according to the following schedule during a period of 216 consecutive days; one ejaculate every six days; two ejaculates taken within a few minutes of each other at 12 day intervals; and three ejaculates taken within a few minutes of each other at 18 day intervals. The volume of semen per ejaculate average 7.9, 6.6, and 5.3 ml. for the six, 12 and 18 day intervals respectively.

Libido

The problem of improving the libido of bulls is rarely mentioned in the studies of sterility in the male bovine.

McKenzie and Berliner (92) reported that libido is relatively independent of spermatogenesis in the ram. They found that sterile rams might exhibit normal sex drive and fecund rams might show very little. Hart and associates (67) noted that semen quality for artificial insemination from three bulls was reduced due to a lack of sexual drive. Hellstrom (68) found that in five cases when bulls were allowed to serve immediately, no semen was ejaculated, and in 12 others only 1-3 ml. (average 1.7) was produced. When the bulls were restrained for 1-6 minutes, the semen volume was increased to 4.9 ml. and after a longer wait the volume increased to 5.3 ml.

The Role of the Endocrine System in Fertility

Endocrine Interactions

Knowledge of the interactions of the hormones affecting sexual activity is meager. The internal rhythm of reproduction in our higher animals is brought into relation with seasonal changes and other external environmental phenomena. In particular, there is now general agreement as to the dominating role played by the anterior pituitary gland, which is recognized as being the organ directly stimulated, while activation of the gonads is brought about indirectly through the pituitary. The thyroid gland also exerts an effect upon the gonads through its effect on cell metabolism. The anterior pituitary gland secretes a thyrotrophic hormone which regulates the amount of thyroxine secreted by the thyroid gland. Thyroxine, in turn, depresses the rate of secretion of the thyrotrophic hormone by the anterior pituitary. Gonadotrophic hormones from the anterior pituitary affects the activity of the gonads. In turn, the gonads secrete hormones which depress the rate of secretion of gonadotrophic hormones from the anterior pituitary gland. It is logical to expect, therefore, that these changes may condition the metabolic processes, but in part, at any rate, act exteroceptively through the nervous system and probably through the hypothalamus upon the anterior pituitary and then upon the testes and ovaries, Turner (144).

Effect of the Anterior Pituitary

It appears that the most widely supported and likely mechanism of pituitary stimulation by light is that involving the eye as a receptor. The transmission of impulses or stimuli to the pituitary is affected through a neural pathway as well as possible by humeral means.

A good deal of work has been done on the use of various hormones upon sterility and sexual inactivity in dairy bulls, but very little data on this condition has been reported in the literature. The interest of research workers in Dairy Husbandry is doubtless centered on the possible use of the gonad-stimulating hormones as having influence in correcting breeding troubles in the dairy bull. Those breeding difficulties in which infection is not the primary cause and in which abnormal testicular function impairs fertility would appear to offer some possibility of responding to treatment with spermatogenic stimulating hormones.

Although the chorionic gonadotrophin is assumed generally to activate chiefly the interstitial cells of the testes, favorable reports concerning its therapeutic employment in diverse grades of seminal failure have been shown. Pighine (109) treated eight sterile bulls with anterior pituitary tissue and six became active again. The treatment of three bulls which produced semen with low sperm motility with chorionic gonadotrophin improved the quality of the sperm as reported by Bottomley and associates (27). Though the existence of seminal failure due to inadequate gonadotrophic activation from the pituitary has been presumed from both the experimental and clinical observations, factual proof that pituitary deficiency may induce seminal failure without androgenic failure is lacking.

The androgen content of the testes increases at a uniform rate during the first two years of life, from two to five years it increases sharply, and thence forward it decreases. This may be one of the many reasons why young sires require fewer breeding services per conception. There are no striking changes in Leydig cells or in the androgen content of the testes at puberty as reported by Asdell (15). Bottomley et al. (27) reported injection of 2 cc. of human pregnancy urine extract. The results of these experiments indicate that chorionic gonadotrophin may be of great value in treating sterility in the bull when endocrine deficiencies are suspected.

Erb and Andrews (49) noted that the injection of 2,250 R. U. of gonadotrophin into a dairy bull caused a decrease in venous blood plasma ascorbic acid of 42 to 67 percent during the first 24 hours. The recovery of ascorbic acid to approximate per injection levels required longer than eight to 10 days. Durrell (43) treated four bulls, with low ability to mount,

with 300 mg. of testosterone propionate. All of the bulls responded favorably, but in one, the increased response was only temporary. Hart (66) reported that a valuable Hereford sire injured his penis in service and would not make any effort to breed. One year after the injury, 1,500 I.U. of gonadotrophin in pregnant mares' serum were injected. This stimulation brought about by the injection of the pregnant mares' serum overcame the inhibition, and in two days he bred a cow normally.

The injection of large quantities of sheep pituitary extract and of pregnant mares' serum into bull calves one to four months of age, by Casida (35), did not produce any mature sperm. The size of the testicles was increased, but it was not definitely determined whether this was due to increased interstitial tissue or size of seminiferous tubules. The seminal vesicles of these calves were definitely stimulated which indicated an increased activity of the interstitial cells. In no case was there definite evidence of development of sexual desire even with extreme development of the gonads. Asdell and associates (16), however, were unable to note any results from administering gonadotrophic hormones to sterile cows. The latter workers caution that the high rate of spontaneous recoverings often encountered render it difficult to give correct interpretation to findings.

Effect of the Thyroid Gland

A good deal of work has been done on the use of various hormones upon sterility and sexual inactivity in males, but judging from the literature on this work with various species most of the work with our domestic animals has been with thyroxine. This hormone, secreted by the thyroid gland, controls the basal metabolism and this is essential for the efficient function of every cell of the body. There is evidence available that hypothyroidism often is associated with infertility, so this hormone must be considered in any account of hormonal therapy for spermatogenesis.

When there are lowered basal metabolic levels, thyroid substances often prove to be effective in therapy of seminal failure. An extensive review of the physiology of thyro-active substances has been made by Reineke (115). Out of a total of 14 bulls that received thyro-protein treatment by Reineke (114), 10 were observed to show definite improvement in vigor and libido. There was no change in the other four bulls receiving the thyro-protein treatment. The time noted for an observable effect to occur averaged 16 days. Definite evidence of improvement in the conception rate was inadequate, but the limited records were suggestive of an improvement in spermatogenesis. Definite improvement of libido of bulls with some indications of improved fertility were observed on a dosage of approximately 0.5 to 1.0 gram of thyro-protein daily per 100 lbs. of body weight.

Schultze and Davis (129) fed1.0 to 1.24 gms. of thyroprotein per hundred pounds body weight daily to seven bulls for 30 days. Initial motility of the group showed definite improvement. The percent of abnormal spermatozoa changed little except in one bull where it decreased markedly. Conception rates of five of the bulls increased from an average of 51.7 percent to 55.0 percent within the first 10 days. After 10 days, the conception rate increased to 58.6 percent, and during the 10 days after feeding stopped, the conception rate further increased to 60.4 percent. Seven other bulls, used as controls, exhibited no changes in breeding efficiency during this time.

Spielman and associates (133) reported that a thyroidectomized bull produced visible spermatozoa, but libido was completely absent. The administration of thyroid, dinitrophenol, and testosterone propionate temporarily restored normal breeding behavior.

Eaton and associates (45) observed the effects of feeding thyroprotein on semen characteristics of rams. Comparison was made between groups of rams fed 2 gm., 1 gm. and no thyroprotein. These results indicated that the feeding of thyroprotein to rams was not beneficial since neither fed group was as good as the unfed group in spermatozoa concentration, percentage of normal sperm, and motility score. However, the volume of the ejaculate was somewhat higher in the lot fed 2 gms. of thyroprotein in the daily ration for a period of 15 weeks. Bogart and Mayer (25) showed that thyroxine or thyroid active

protein, administered orally by injection, alleviated symptoms of summer sterility in rams resulting from impaired spermatogenic activity.

Warwick and co-workers (148) substantiated the work of Eaton and associates (45) by showing that treatment of rams with thyro-protein in the months of April and May had an apparent deleterious effect on semen quality as measured by initial fertility, livability, density, and ability to reduce a solution of methylene blue. These workers also furthered the work of Bogart and Mayer by presenting data to show that treatment of rams with thyroprotein during the summer months produced semen which was either equal or slightly above the semen produced by the controlled group.

Martinez (89) reported that thyroprotein fed as 0.04 percent of the ration caused a definite stimulation of spermatogenesis in the domestic fowl. Jaap (74) noted that doses of 0.25 to 1.0 gms. of desiccated thyroid gave an increase in the size of the testes of drakes during late winter and early spring months.

Nutritional Factors

Vitamin A

The importance of vitamin A in developing and maintaining the normal germinal epithelium, and the breeding ability of young bulls has been amply demonstrated by Sutton et al.(134)

Hodgson et al. (72) and Erb et al. (50).

Phillips (150) reported that cellular sloughing is noted in vitamin A deficiency, but spermatogenesis continues. The condition could also be repaired after 60 to 90 days of vitamin A. administration.

Sutton et al. (134) noted the following changes in the young male bovine from a ration low in vitamin A: degeneration of the germinal epithelium of the testes, absence of spermatozoa in the epididymis, and an accumulation of fluid in the cleft between the anterior and posterior lobes of the pituitary gland. Regeneration of the germinal epithelium following vitamin A and carotene therapy has been observed by Hodgson and associates (72) and Erb et al. (50). These latter workers reported that a bull fed a low vitamin A ration for four months became blind, had staggers, and gastric intestinal disturbances. By using testicular biopsy, they found severe degeneration of the seminiferous tubules and nearly complete disappearance of sperm from the lumen of the tubules. Feeding of 60,000 to 150,000 units of vitamin A per day resulted in recovery within two to five months.

Six dairy bulls were fed dry roughages low in carotene and a concentrate deficient in carotene and vitamin A for a period of 16 months by Bratton and associates (34) without inducing clinical manifestations of the deficiency. When the roughage component of the ration was changed from hay and/or straw to dried beet pulp, development of incoordination, edema of the extremities and papillary hemorrhage resulted. They also noticed a decrease in the percent of motile spermatozoa and a gradual increase in the percent of abnormal spermatozoa.

Vitamin C.

Phillips and associates (105) reported that bulls having a low ascorbic acid value for semen (two mg. or less) were brought back to normalcy in a large majority of cases within five weeks by the subcutaneous injection of Vitamin C. Phillips also pointed out that semen ascorbic acid values above eight mg. percent were toxic and the quality of the semen may be as poor as when ascorbic acid values are low.

The mechanism of how vitamin C improves spermatogenesis is not definitely known. Berg and co-workers (21) found that the ascorbic acid is concentrated in the seminal vesicle. These authors assume that the spermatozoa are kept viable after spermatogenesis is completed by the ascorbic acid in the seminal vesicles until they are ejaculated.

Phillips and co-workers (105) showed that blood plasma ascorbic acid declines with the development of vitamin A deficiency. Twenty cc. of cod liver oil per day was required to maintain normal ascorbic acid values in Guernsey bulls. That there is a difference between breeds, with the Holsteins lower than the Guernseys, has been reported by Phillips and associates (104).

Bortree et al. (26) showed that the feeding of chlorobutanol to cattle resulted in a marked increase in plasma ascorbic acid. Recent work has shown that the feeding of chlorobutanol to the bovine results in a marked increase in the level of ascorbic acid in the blood plasma. Fifteen slow breeding bulls were fed chlorobutanol orally by Scheidenhelm (126). The libido of the bulls improved in 76.6 percent of the cases. The average time required to serve a cow was reduced from 45 minuted to 3.7 minutes. Only two sires were able to withstand doses of 20 grams of chlorobutanol per day for more than a three week period without developing muscular incoordination.

Vitamin E

Somewhat contradictory claims are presented in the literature in regard to the value of vitamin E for normal reproduction of dairy cattle.

That vitamin E has no value in improving spermatogenesis in the dairy bull, has been shown by Gullickson and co-workers (61). These workers fed seven bulls on a ration devoid of vitamin E and found that growth, physical, and sexual development were normal in every respect. The feeding of one ounce daily of solvent, process wheat germ oil, in addition to the normal ration, by Salisbury (119), did not increase the volume, concentration, motility, or shorten the time required for service.

On the contrary, Timin and Perelurian (141) reported decidedly favorable results from the feeding of vitamin E in the form of wheat germ concentrate. A comparison between the bulls fed the concentrate and those used as controls showed a 31.5 percent increase in volume, 63.0 percent increase in sperm viability, and a 32 percent increase in sperm concentration in favor of the wheat germ concentrate. These beneficial effects were noted 25 to 30 days after feeding started. They recommend 800 to 1,000 gms. per bull per day.

Kafka (79), studying the case history of 126 non-breeding cows, found that treatment with wheat germ oil gave 65 percent positive conceptions, seven percent negative conceptions, and 28 percent non-conclusive results. Jones and Ewalt (78) injected wheat germ oil in 49 females at the Oregon State College Dairy Herd. The dosage varied from 10 cc. to 20 cc. per injection. Forty of the cows injected, calved, three were disposed of as sterile, and no records were available for the remainder of the cows. They concluded that vitamin E had a favorable influence in the case of 15, indefinite results in the case of 23, and negative results with five females.

Amino Acids

The optimum amount of dietary protein that is needed for producing high quality semen in the dairy bull has been under investigation for some time.

Holt and co-workers (73) working with humans found that a diet deficient in arginine over a ten day period caused a reduction in the number of spermatozoa to one-tenth of the normal values. They concluded that atrophy of the spermatogenic tissue took place. Guterman (64) studied five men patients before, during and after amino-acid therapy. One month prior to the amino acid therapy the average sperm count was 11.2 million per cc.; after eight months of treatment, the average concentration had increased to 47.0 million per cc. The amino-acids were then discontinued, and eight months later the sperm averaged 21.8 million per cc. This worker also treated 18 infertile men with oligospermia in which they received 1.8 - 2.7 grams of arginine and minimal amounts of lysine, tryptophane, and pyridoxine in tablet form for eight months. The tendency for sperm concentration to increase was as great in the untreated group as in the treated groups.. It appears that amino-acid therapy for oligospermia should be reserved for those patients who exhibit or give the history of inadequate protein in-take. Berg and Rohse (22) found that a period of uncomplicated trytophane deficiency in young male rats, lasting as long as 20 days, does not induce subsequent sterility.

Jones and associates (77) using two groups of bull calves, starting at five to seven months of age and continuing to 32 to 41 months of age, compared the effect of two supplemented alfalfa hay rations upon fertility. The basal ration consisted of alfalfa hay, disodium phosphate, salt, and potassium iodide and the supplemented basal ration consisted of the basal ration plus one pound each of skim milk powder and oat groats. Bulls fed the basal ration produced good quality semen after 18 months of age and were fertile on service between two and 3.5 years of age. Bulls fed the supplemented basal ration produced good quality semen after 12 months of age, and were fertile on service. The average initial motility was rated 52 percent of the samples on the basal ration as compared with 72 percent of the samples on the supplemented basal ration. The authors believed that the earlier maturity and better condition of the bulls fed the supplement was due to the greater energy intake.

Branton and co-workers (30) studied the measurable semen characteristics and relative fertility of 18 bulls when they were fed the protein supplement and timothy hay as the only roughage. Based on 60 to 90-day non-returns to first service cows, the average fertility levels of the semen produced when the bulls were fed corn gluten feed, skim milk powder, and soybean oil meal were 63.5, 61.6 and 65.7 percent respectively. The results showed that corn gluten feed, skim milk and soybean oil meal were approximately equal in value as protein supplements in the concentrate mixture. It is doubtful whether any real advantage lies with the soybean oil meal because the difference is relatively small.

Reid and associates (112) indicated that the ingestion of a simple and/or complex concentrate gave similar characteristics of good quality semen. Branton and co-workers (31) found comparable results in semen characteristics, relative fertility and body weight changes when the T. D. N. intake levels varied from 100, 120 and 140 percent of recommended maintenance requirements for dry dairy cows of equivalent weights fed simultaneously with concentrate mixtures containing 12, 16 and 20 percent total protein.

Minerals

Lardy and associates (85) studied the effect of a low manganese ration of corn, corn gluter, timothy hay, and minerals upon semen production. They found that ejaculates from the three bulls reared on the low manganese diet produced a small volume, low concentration, poor spermatozoa motility, and a short storing period. In contrast, the ejaculates from three bulls receiving the same ration plus a supplement of manganese sulfate produced a normal volume, high concentration, excellent motility, and the spermatozoa maintained motility for a long storage period. These authors indicated that an adequate amount of manganese is essential for sperm production in the bull. Marked improvements in semen quality was observed by Knoap (80) in seven of the 11 problem sires who daily received 50 mg. potassium iodide plus .8 pound of skim milk powder for a period of two to six months. Improved spermatogenesis was indicated by one or all of the following factors: libido, quantity of semen, output of sperm cells, percentage of living sperm cells, and the motility activity rating of the cells.

Boyer and associates (29) found that a manganese deficiency in male rats did not result in lowered ascorbic acid content of the various tissues. However, the rats on the deficient diet showed complete lack of spermatozoa.

SUMMARY OF THE REVIEW OF LITERATURE

The factor of exercise in management of dairy bulls has not received adequate study. Various investigators (19, 65, 87, 151 and 160) have indicated that exercise may give favorable effects on fertility; however, the data presented is somewhat contradictory. Exercise may increase semen quality and libido in the bull. If exercise affects spermatogenesis in the dairy bull, the physiology involved is not well defined.

Semen characteristics have been shown to be influenced by various factors. That the seasonal variation affects semen quality and fertility of bulls is evident (38, 51, 96 103 and 130). Several investigators (42, 51, 88, 95, 96, 103 and 130) have demonstrated that fertility is affected by season. These authors concluded that light and temperature were two of the factors involved. The quality of the semen appears to be lower during the summer and fall months.

The age of the bull appears to have an affect upon breeding efficiency (28, 42, 71, 97, 140 and 152). It appears that bulls six years of age and younger produce higher quality of semen than older bulls. That the frequency of ejaculation may affect the breeding abilities and fertility of the bull has been shown by (86, 93 and 149). From these data, it is reasonable to conclude that a six-day interval between ejaculations is not detrimental to spermatogenic activity.

The factors affecting libido in bulls is not definitely known. Two workers (67 and 68) indicate that libido is relatively dependent of spermatogenesis in the bull. The data showing the effect of the ration on libido is contradictory. Several workers (26, 105 and 126) showed that a ration which improves vitamin C synthesis or the injection of ascorbic acid will aid in maintaining sex drive. Improvements in semen quality was obtained by manganese sulfate and potassium iodide therapy as indicated by (80 and 85). The feeding of manganese deficient diets to male rats by (29) resulted in sterility. The experiment work conducted in the United States (61, 78 and 119) indicates that the feeding of wheat germ oil as a source of vitamin E is of no value in maintaining semen quality. The importance of vitamin A in developing and maintaining breeding efficiency of young bulls has been demonstrated (34, 50, 72 and 134).

There is evidence in the data (114, 129 and 133) that thyroid substances often prove to be effective in therapy of seminal failure. Hypothyroidism is associated with impaired sex interest. Gonad-stimulating hormones (27, 43, 66 and 109) may have an influence in correcting breeding troubles.

Data on the relationship of semen quality to breeding efficiency have varied (20, 46, 94, 135 and 137). Investigations (9, 41, 69 and 70) to date, indicate that initial motility of the spermatozoa is the best laboratory measure of semen fertility.

Values (13, 51, 69 and 70) for good quality of semen varied somewhat, but it appears that semen to be inseminated should have the following characteristics: (1) normal volume -- 4 ml., (2) concentration -- 800,000 permatozoa per cu. mm., (3) motility rating -- 70 percent progressively motile spermatozoa, (4) abnormal spermatozoa -- less than 30 percent, (5) hydrogen-ion concentration -- 6.8.

Various authors (75, 76, 124 and 142) have studied the relationship of hyaluronidase titre to fertility levels. No conclusive evidence has been presented.

Many investigators have studied the storage of semen (7, 13, 52, 70, 102, 106, 107, 128 and 154). Most workers are of the opinion that a storage temperature of 5° C is optimum, and that semen, if used for insemination, should not be stored over four days. Various diluents (20, 33, 106, 107, 120, 121 and 151) have been used in the United States to preserve semen. Best results in maintaining the fertility of semen have been obtained with the buffered egg-yolk-citrate and the buffered egg-yolk-phosphate diluents. Many workers (5, 6, 54, 81, 98, 100, 108 and 139) have studied bacteriological control of semen. A combination of penicillin and streptomycin added to the above accepted diluents has more desirable anti-biotic properties than any other bacteriostatic compound.

OBJECT

The object of this experiment was to observe the effects of forced exercise of dairy bulls upon their semen characteristics (volume, concentration, motility and percent abnormals), and the fertilizing capacity of their semen by computing the percentage non-returns on first and second service cows based on 60-90 days. These cows were owned by patrons of the Michigan Artificial Breeders Cooperative Incorporated.

EXPERIMENTAL PROCEDURE

Selection of Animals

Thirty-two bulls of the Michigan Artificial Breeders Cooperative Incorporated varying from four to 15 years of age were divided into eight groups according to breed and to high or low fertility as shown by Table IV.

Feeding and Management

Approximately one-half of the bulls in this experiment were housed in 9' by 11' box stalls and the other one-half in 5' by 8' tie stalls. All bulls received a 15 percent protien concentrate, (Table V), and an average quality alfalfa-brome-grass hay. In addition, grass silage was fed for the first 122 days, followed by corn silage for the last 59 days. The analysis of the grass silage and corn silage is shown in Table VI.

TABLE IV

SUMMARY OF BREEDING EFFICIENCY FOR THE INDIVIDUAL BULLS
FROM OCTOBER, 1948 THROUGH SEPTEMBER 30, 1949

EXERCISE								NON-EXERCISE							
High Fertility				Low Fertility				High Fertility				Low Fertility			
Holstein		Guernsey		Holstein		Guernsey		Holstein		Guernsey		Holstein		Guernsey	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	69	9	65	5	58	13	46	17	75	25	61	21	65	29	62
2	68	10	67	6	55	14	50	18	68	26	72	22	57	30	66
3	70	11	60	7	66	15	52	19	72	27	65	23	62	31	59
4	74	12	74	8	50	16	54	20	74	28	65	24	64	32	64
Tot.	281		266		229		202		289		263		248		251
Av.	70.3		66.5		57.3		50.5		72.3		65.8		62.0		62.8

% Non-returns: 60-90 days

TABLE V

CONSTITUENTS OF GRAIN RATION

Feed	Pounds	
Oats	1,200	*D. P. = 15.8% D. M. = 23.01bs. T.D. N. = 81.71bs.
Corn	300	
Wheat bran	600	
Wheat middlings	300	
Meat scraps	200	
Linseed oil meal	100	
Soybean oil meal	100	
M. V. P. poultry concentrate	100	
Salt	1.5%	
Cobalt	3.0 oz.	
M_n S O_4	1.0 lb.	

Legend = *According to the Morrison Standard

D. P. = Digestible protein
D. M. = Dry Matter
T. D. N. = Total Digestible nutrients

TABLE VI

ANALYSIS OF THE
CORN SILAGE AND GRASS SILAGE

	Moisture %	Protein Wet Basis %	Protein Dry Basis %
Corn	70.36	2.41	8.10
Grass	50.70	7.70	15.50

*Association of Official Agricultural Chemists

The average daily level of feeding the grain mixture, silage, and hay was 3.5 lbs., 12 lbs., and 15 lbs. respectively. This ration contained sufficient digestible nutrients to meet the Morrison Standard.

The original plan called for weekly semen collection. However, because of the fluctuations in demand of semen, condition of the bull, and the management practices, this time schedule could not be strictly followed. In general, the interval between collections was longer depending on the individual bull.

An artificial vagina was used for collection. In most cases the bulls were ejaculated twice at each collection time with a short interval between the first and second ejaculates. In no case was a third collection made. For each ejaculation the bulls were restrained depending upon their habits and libido.

Exercising Procedure

During the first 35 days, one-half the bulls were exercised for 15 minutes in the afternoon on a mechanical exerciser with a speed of 1.24 mi. per hr. The amount of daily exercise was increased to 23 minutes at the same rate of speed for the following 14 days. For the remaining 119 days of the experiment, these bulls were exercised daily for 30 minutes at the rate mentioned above on the mechanical exerciser. No bulls were exercised on Sunday.

Method of Semen Handling

Semen collections were made on Monday, Wednesday and Friday from 3 a.m. to 7 a.m. All bulls were ejaculated in or near the breeding stable adjacent to the processing laboratory. Immediately after each collection the vial containing the semen was taken into the laboratory. The following procedures were used:

1. The semen sample was observed for any gross abnormalities.

2. The semen volume was measured and recorded.

3. The spermatozoa concentration and motility was determined by the following technique:

A. A 1 ml. T. D. pipette was inserted into the semen receptacle and .2ml. of semen was pipetted.

(a) .1 ml. of the pipetted semen was transferred to a 3 ml. (10 x 75 mm.) test tube containing 1 ml. of sodium citrate dihydrate. This tube, with the diluted semen, was inserted into the photoelectric colorimeter and the reading recorded as thousands of spermatozoa per cu. mm.

(b) .1 ml. of the pipetted semen was placed on a sterile microscopic slide (body temperature) and the spermatozoa concentration was estimated objectively under the microscope.

4. Semen which showed a concentration of 800 spermatozoa per cu. mm. or above and also had motility rating of 70 percent or above was further processed. The semen was diluted with buffered egg-yolk citrate prepared by the method of Salisbury and associates (138). The range of dilution for the processed semen varied from 1:30 to 1:40. Sixty-days after the start of the experiment, a combination of 1,000 international units each of penicillin and streptomycin was added per ml. of diluted semen.

5. Semen was then gradually cooled to 5^{o} C. at the rate of 15^{o} per hour.

6. The semen was then placed in 8 c. c. vials, one-fourth inch from the top. Paraffined corks were used as stoppers.

7. The tubes were packaged in special containers containing ice for parcel post, special delivery and air shipment to the inseminators that morning or afternoon. Thus, the semen used for insemination was never over three days old when the cow was bred.

<u>Procedure of Making Semen Smears for Morphological Studies</u>

Smears were made of the fresh semen from each bull to determine the percentage of abnormal spermatozoa. The semen smears were made immediately after recording the volume by the following technique: (1) A small, sterile stiring rod was cautiously introduced into the calibrated semen receptacle to

transfer a small drop of the semen to a small, two-inch test tube, 2 ml. capacity, containing .5 ml. of physiological saline. (2) The contents of the test tube were then poured on the entire flat surface of a sterile microscopic slide and allowed to air-dry at laboratory temperature. The semen smears were then taken to the nutritional laboratory for the staining technique. The following staining method was used to illustrate the head, midsection, and tail of the sperm, Gradwohl (60).

A. Fixation in Schaudinn's solution:

1. Immerse for one minute in 7 percent solution of corrosive mercuric chloride, 2 parts, and absolute alcohol, 1 part.
2. Immerse for a half-minute in 50 percent alcohol.
3. Immerse for a half-minute in 3 ounces of distilled water, and 2 drops of tincture of iodine.
4. Wash in tap water.

B. Staining Process:

1. Immerse for a half-minute in aqueous solution of 5 percent eosin.
2. Immerse for one minute in 3 ounces of 50 percent alcohol and two drops of concentrated hydrochloric acid.
3. Wash in distilled water.
4. Immerse for two and one-half minutes in

hematoxylin.

5. Immerse for one minute in 3 ounces of distilled water, and 2 drops of glacial acetic acid.
6. Wash in distilled water.
7. Dry at laboratory temperature.

Method of Counting Spermatozoa

Each stained slide was placed on a movable microscope stage and following a standard technique of moving the slide about, 250 individual spermatozoa were examined and classified as to morphological type.

Spermatozoa were classified into the following groups suggested by Salisbury and associates (39):

1. Morphologically normal.
2. True abnormals, which included such types as those possessing any abnormality with respect to the shape or size of the head, spermatozoa with enlarged, abaxial, beaded or filiform middle-pieces, and those with thickened or double tails.
3. Tailless spermatozoa.
4. Spermatozoa with broken necks.
5. Spermatozoa with coil tails.

The number and types of normal and abnormal spermatozoa found were recorded and these figures multiplied by four to give the number of abnormal spermatozoa per 1,000. The examinations were made at 430 X magnification with a high-dry objective. In addition to noting the abnormal spermatozoa, observations were made as to the presence of debris, epithelial, and primordial sperm cells in the semen. In recording the abnormal spermatozoa, the following system was followed: using a bacterial counter, 62 spermatozoa were counted in each corner of the microscope slide, in some cases, this procedure could not be followed due to the absence of cells on some slides. Each sperm cell was recorded in its respective classification as it was counted. No spermatozoon was included in more than one group. Hence, a pyriform head that was tailless or coil-tailed. For this reason the percentage of each abnormality is not absolute but represents only the abnormal types that fall in their group after other forms have been separated out. Since many of the coil-tailed and tailless spermatozoa possessed other defects, it can be seen that the values for these two forms will be somewhat high in comparison to the other two types of abnormals.

Weights of the Bulls

Weights of the individual bulls were taken approximately at six-week intervals during the course of the experiment. All bulls were weighed at the same time each day. The regular weighing time was 10:00 a.m. so as to eliminate the possible error of feed consumption, upon body weight.

Evaluating the Degree of Libido

The procedure used was to note the length of time in minutes or fractions of a minute that elapsed from the time the bull was two to three feet from the teaser and the completion of service to the artificial vagina. In a few cases some bulls were retarded in their sex drive by the leader forestalling the bull's mount. This time that a bull was hindered in libido was not recorded.

Observations on Appetite and General Activity

During the experimental period, close observations were made upon each bull to detect any noticeable changes in appetite and activity.

EXPERIMENTAL RESULTS

Breeding Efficiency

A summary of the numbers of cows bred monthly and the breeding results for treatments and breeds are found in Tables VII and VIII.

The Holstein bulls had a considerably higher breeding efficiency than the Guernseys. The percent of non-returns for the Holstein breed was much higher on both exercise and non-exercise than those for the Guernseys. The Holsteins not exercised exceeded the exercised group by .8 percent in total 60-90 day non-returns; whereas, the Guernseys that were exercised showed 1.6 percent more total non-returns than the non-exercised group. Figure 1 shows graphically the monthly percent of 60-90 day non-returns for treatments and breeds.

The data for breeding efficiency were analyzed on fertility levels by analysis of variance and the results are presented in Tables IX and X. A highly significant difference of the average of non-returns was found between breeds and between months in the high fertile bulls. No significant differences were shown by the low fertile bulls. The treatment interaction, exercise X breeds, was significant in the fertile group, but not in the bulls of low fertility, meaning that in the high fertile bulls, the Holstein did not respond the same as Guernsey bulls for exercise and non-exercise. As shown by the analysis of variance, there were not

TABLE VII

SUMMARY OF MONTHLY BREEDING DATA
FOR THE EXERCISED AND NON-EXERCISED BULLS BY BREEDS
REGARDLESS OF HIGH AND LOW FERTILITY

EXERCISE

Month	Number of Cows Bred		Number of 30-60 day Returns		Percent of Non-returns	
	Holstein	Guernsey	Holstein	Guernsey	Holstein	Guernsey
Oct.	1,326	775	453	254	65.8	67.2
Nov.	1,649	690	600	274	63.6	60.2
Dec.	2,457	1,272	828	475	66.3	62.6
Jan.	2,204	1,369	777	464	64.7	66.1
Feb.	2,190	1,043	686	400	68.6	61.6
Mar.	2,057	840	624	307	69.6	63.4
Total	11,883	5,989	3,968	2,174	----	----
Average	1,980	998	661	362	65.6	64.1

TABLE VII(continued)

SUMMARY OF MONTHLY BREEDING DATA
FOR THE EXERCISED AND NON-EXERCISED BULLS BY BREEDS
REGARDLESS OF HIGH AND LOW FERTILITY

N O N - E X E R C I S E

Month	Number of Cows Bred		Number of 30-60 day Returns		Percent of Non-returns	
	Holstein	Guernsey	Holstein	Guernsey	Holstein	Guernsey
Oct.	1,344	691	456	231	66.1	66.5
Nov.	1,587	939	512	362	67.7	61.4
Dec.	2,286	876	732	327	61.6	62.6
Jan.	1,785	685	549	264	69.2	61.4
Feb.	1,851	727	556	282	69.9	61.2
Mar.	1,654	995	478	372	70.0	62.6
Total	10,507	4,913	3,283	1,838	----	----
Average	1,751	818	547	306	67.4	62.5

TABLE VIII

MONTHLY BREEDING DATA OF HOLSTEIN BULLS
COMPARED TO GUERNSEY BULLS

Month	Number of Cows Bred		Number of 30-60 day Returns		Percent of Non-returns	
	Holstein	Guernsey	Holstein	Guernsey	Holstein	Guernsey
October	2,670	1,466	909	485	66.0	65.6
November	3,236	1,629	1,112	636	65.6	60.1
December	4,743	2,148	1,560	802	67.1	62.6
January	3,989	2,054	1,326	728	66.7	64.5
February	4,041	1,770	1,246	682	69.1	61.4
March	3,711	1,835	1,122	679	69.7	62.9
Total	22,390	10,902	7,275	4,012	----	----
Average	3,731	1,817	1,212	668	67.5	63.1

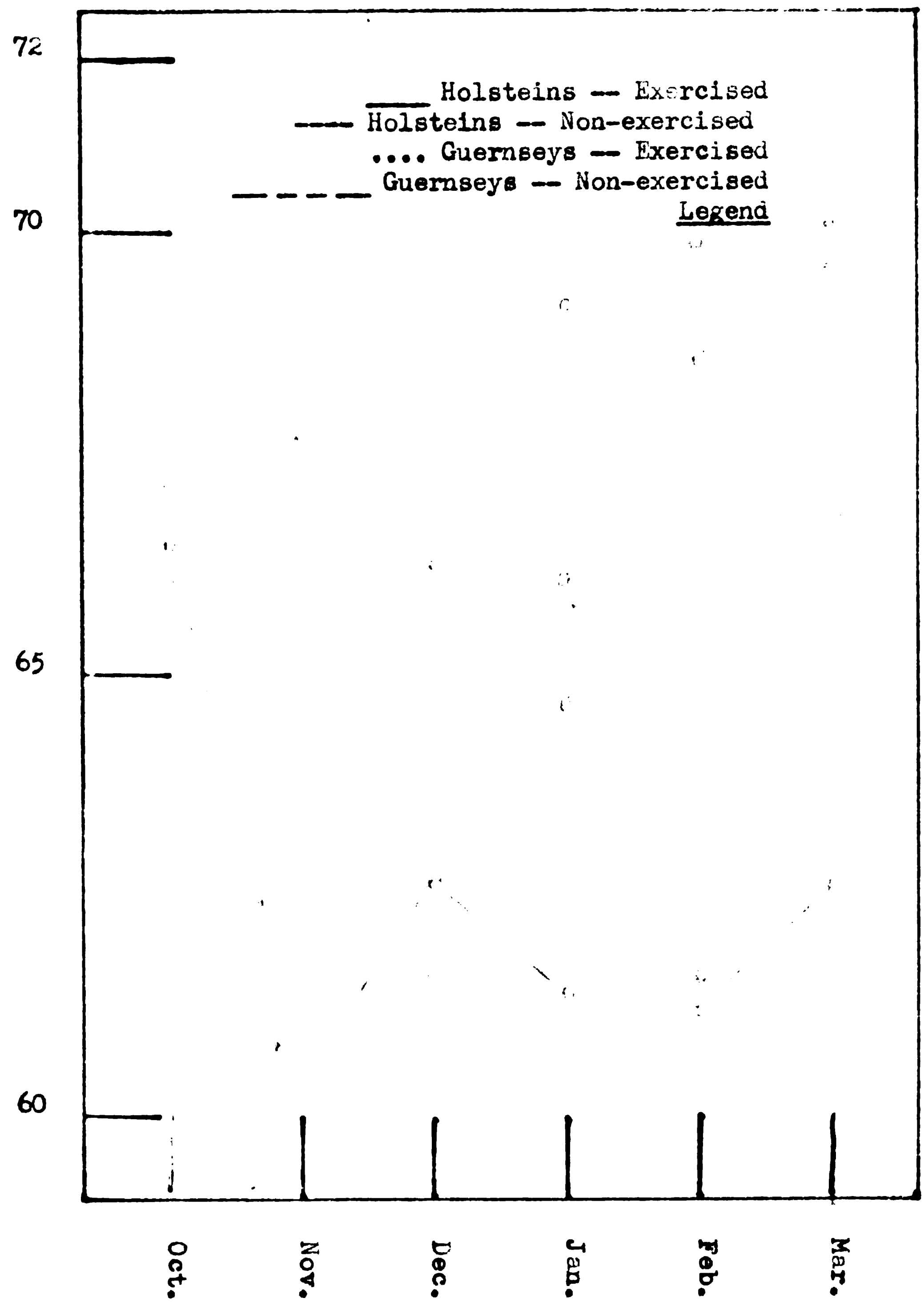


Fig. 1. Percent of 60-90 Day Non-Returns

TABLE IX

ANALYSIS OF VARIANCE OF PERCENT NON-RETURNS FOR HIGH FERTILE BULLS

Source of Variation	Degrees of Freedom	Mean Square	F
Exercise	1	15.0	.39
Breeds	1	1,073.0	28.01**
Months	5	160.5	4.19**
Exercise x Months	5	48.6	1.27
Exercise x Breeds	1	251.0	6.55*
Breeds x Months	5	46.2	1.21
Exercise x Breeds x Months	5	17.9	.47
Error	72	38.3	---

*Significant at the five percent level of probability
**Significant at the one percent level of probability

TABLE X

ANALYSIS OF VARIANCE OF PERCENT NON-RETURNS FOR LOW FERTILE BULLS

Source of Variation	Degrees of Freedom	Mean Square	F
Exercise	1	141.1	3.8
Breeds	1	1.2	.03
Months	5	14.4	.39
Exercise x Months	5	3.5	.09
Exercise x Breeds	1	.5	.01
Breeds x Months	5	11.6	.31
Exercise x Breeds x Months	5	12.3	.33
Error	55	37.0	----

significant differences in percent non-returns, in breeds and treatments between months regardless of fertility levels.

Tables XI and XII summarize the breeding efficiency of the exercised and non-exercised, high and low fertility Holstein and Guernsey bulls. This data includes the average percent non-returns during the course of the experiment, six months prior to the experiment, and the corresponding six months, one year before the experiment. An analysis of variance of these data showed no significance between the percent non-returns for the three periods. However, this analysis showed that there were highly significant differences between the bulls for the three periods. This was more pronounced with the Guernseys than the Holsteins.

Statistical analysis showed significant differences between the Holstein bulls in percent non-returns. The variation between the Guernsey bulls was highly significant.

Correlation coefficients between percent non-returns and various factors were calculated in order to ascertain any real relations between them. A highly significant correlation (0.536) was obtained between percent non-returns and abnormal spermatozoa. The higher the percentage of non-returns the lower the percentage of abnormal spermatozoa. Significant correlations were obtained between months in percentage of non-returns for all bulls which means that non-returns for one month are closely associated with those of other months. A correlation of-0.539, which is highly significant, was obtained between percent non-returns and age of the bulls. The younger,

TABLE XI

SUMMARY OF THE BREEDING EFFICIENCY OF THE EXERCISED BULLS BY BREEDS*

HIGH FERTILITY								LOW FERTILITY							
	Holstein				Guernsey				Holstein				Guernsey		
Bull	x	xx	xxx	Bull	x	xx	xxx	Bull	x	xx	xxx	Bull	x	xx	xxx
1	63.8	69.0	71.8	9	64.5	63.5	67.2	5	57.5	58.3	58.0	13	58.6	37.6	46.4
2	71.5	69.0	68.5	10	69.5	67.1	66.6	6	62.0	52.3	54.2	14	52.3	51.5	50.2
3	63.1	70.3	75.2	11	60.0	61.3	63.8	7	66.4	66.0	61.4	15	53.8	56.8	68.0
4	74.6	72.6	74.0	12	74.1	73.6	74.7	8	----	----	----	16	57.5	55.5	62.8
AV.	68.2	70.2	72.8		67.0	66.4	68.1		61.9	58.8	57.9		55.5	50.3	56.9

*% non-returns
x -- average for the 6 months one year before experiment
xx-- average for the 6 months before experiment
xxx- average for the 6 months experiment

TABLE XII

SUMMARY OF THE BREEDING EFFICIENCY OF THE NON-EXERCISED BULLS BY BREEDS*

HIGH FERTILITY								LOW FERTILITY							
	Holstein				Guernsey				Holstein				Guernsey		
Bull	x	xx	xxx	Bull	x	xx	xxx	Bull	x	xx	xxx	Bull	x	xx	xxx
17	72.8	78.4	75.7	25	62.0	55.1	64.1	21	69.6	63.8	73.4	29	60.3	60.5	66.6
18	67.6	70.5	71.1	26	---	67.5	68.1	22	57.0	56.2	50.0	30	100.0	73.1	66.1
19	67.6	75.0	75.9	27	67.1	67.8	60.0	23	62.3	58.8	65.2	31	58.5	59.2	60.6
20	72.8	71.8	70.0	28	65.8	63.8	61.0	24	62.8	62.1	67.8	32	78.0	63.0	60.1
AV.	70.2	73.9	73.1		64.9	63.5	63.3		62.9	60.2	64.1		74.2	63.4	63.4

*% non-returns
x -- average for the 6 months one year before experiment
xx-- average for the 6 months before experiment
xxx- average for the 6 months experiment

the bull (age 4 - 15 years), the higher was the percentage of non-returns.

The correlation was less between the monthly responses of the exercised and non-exercised groups of bulls, than when all bulls were thrown together. Hence, it was thought justifiable to do an analysis of variance for the exercised and non-exercised groups separately. In some cases there was a slight correlation between monthly responses and in others there was no evidence of correlations.

Semen Characteristics

Volume

A monthly summary of the average semen volume for exercised and non-exercised, high and low fertile, Holstein and Guernsey bulls is presented in Table XIII. This table shows that the non-exercised bulls produced .3 ml. more semen per ejaculate than the exercised group.

Figure 2 shows the monthly differences in volume between high and low fertile bulls exercised compared to those not exercised. The high fertile bulls on exercise slightly excelled the high fertile group not on exercise; whereas, the non-exercised low fertile group was slightly superior to the low fertile bulls receiving exercise.

Exercised, high and low fertile Holsteins showed a smaller average ejaculate than the corresponding groups not

TABLE XIII

MONTHLY SUMMARY OF AVERAGE SEMEN VOLUME FOR EXERCISED AND NON-EXERCISED HIGH AND LOW FERTILE GUERNSEY AND HOLSTEIN BULLS*

	EXERCISED				NON-EXERCISED			
Month	High Fertility		Low Fertility		High Fertility		Low Fertility	
	H	G	H	G	H	G	H	G
Oct.	6.3	6.3	7.5	6.6	6.4	6.2	8.7	7.9
Nov.	5.3	6.0	8.4	6.5	5.5	5.7	8.9	8.7
Dec.	6.5	6.4	7.9	5.8	6.4	5.5	7.6	8.6
Jan.	5.5	6.1	6.9	5.7	6.1	5.1	7.9	7.3
Feb.	5.8	6.3	7.0	8.1	6.5	5.7	7.4	8.5
Mar.	6.0	6.1	8.6	7.2	5.3	5.1	7.3	7.8
Av.	5.9	6.2	7.7	6.7	6.0	5.6	8.0	8.1

*ml.

TABLE XIII (continued)

MONTHLY SUMMARY OF AVERAGE SEMEN VOLUME FOR EXERCISED AND NON-EXERCISED HIGH AND LOW FERTILE GUERNSEY AND HOLSTEIN BULLS*

Average for all months:

1. Exercise vs. non-exercise (6.6-6.9) = -.3.
2. High fertile exercised vs. non-exercised high fertile (6.0-5.8)= + .2.
3. Low fertile exercised vs. non-exercised low fertile (7.2-8.0) = -.8
4. Exercised high fertile Holsteins vs. non-exercised high fertile Holsteins (5.6-6.0) = -.4.
5. Exercised low fertile Holsteins vs. non-exercised low fertile Holsteins (7.7-8.0) = -.3.
6. Exercised high fertile Guernsey vs. non-exercised high fertile Guernseys (6.2-5.6) = +.6.
7. Exercised low fertile Guernseys vs. non-exercised low fertile Guernseys (6.7-8.1) = -1.4.
8. Exercised and non-exercised high and low fertile Holsteins vs. exercised and non-exercised high and low fertile Guernseys (6.9-6.6) = +.3.

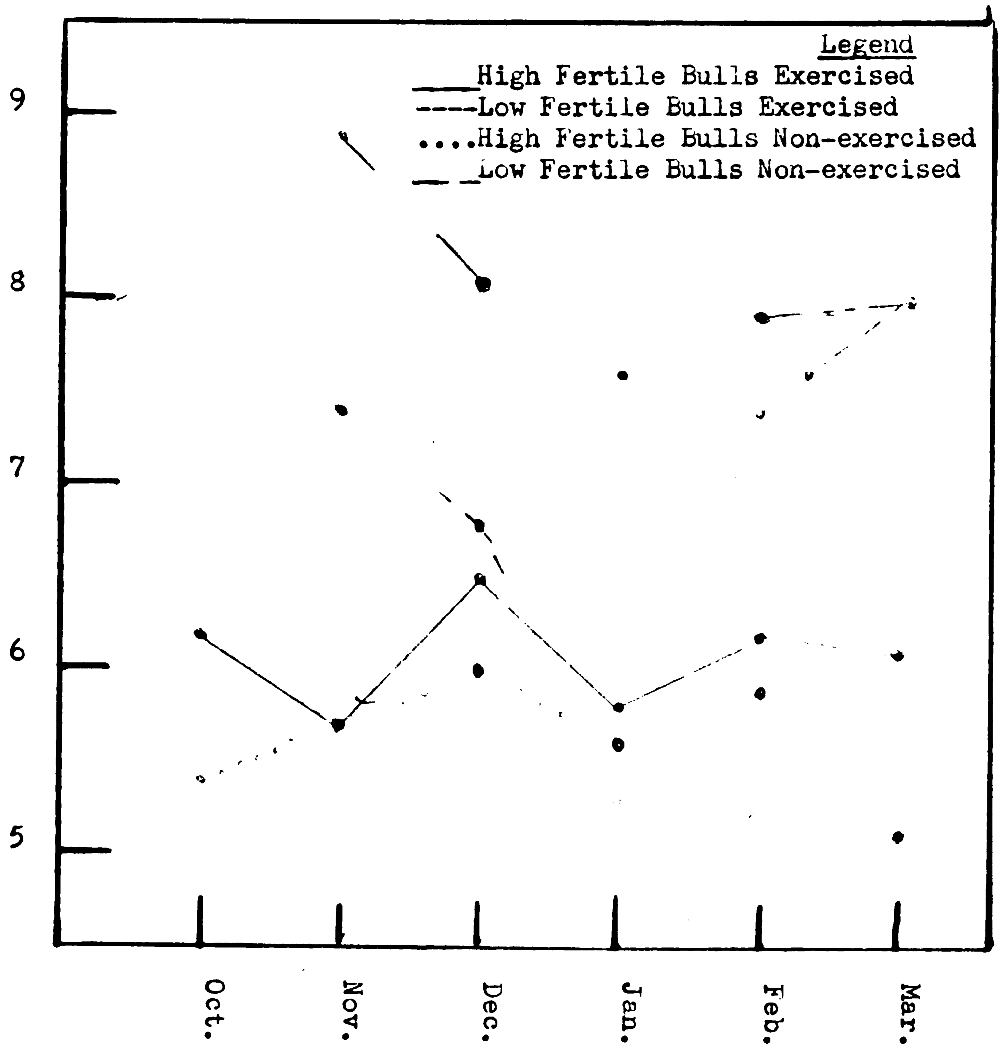


Fig. 2. Ml. Semen Per Ejaculate.

exercised. High fertile Guernsey bulls exercised showed 1.6 ml. more semen per ejaculate as compared to the non-exercised Guernseys of high fertility. This was not true with the low fertile groups as the non-exercised, low fertile Guernsey bulls exceeded the group of low fertility on exercise.

Figure 3 is a graphical summary of the monthly volume differences between exercised Holstein and Guernsey bulls to that of the non-exercised. An examination of the monthly averages shows that the Holstein bulls on exercise were, in general, slightly superior to the Guernsey bulls not exercised; whereas the non-exercised Guernseys were superior to the exercised Holsteins. The Holstein bulls were slightly higher in semen volume.

As shown in Table XIV and Figure 4, the volume of the second ejaculate was larger than that of the first for both the exercised and non-exercised bulls. The volume of the first and second ejaculate of the bulls on exercise was somewhat lower than those not receiving exercise.

Bulls of both breeds gave a greater volume of semen in the second ejaculate than the first ejaculate. The Guernsey bulls produced a greater volume of semen in the first (.5 ml. per ejaculate) and second (.7 ml. per ejaculate) ejaculates than the Holstein bulls. This was the only semen characteristic in which the Guernseys were superior to the Holsteins. These data are summarized in Table XIV and presented graphically in Figure 5.

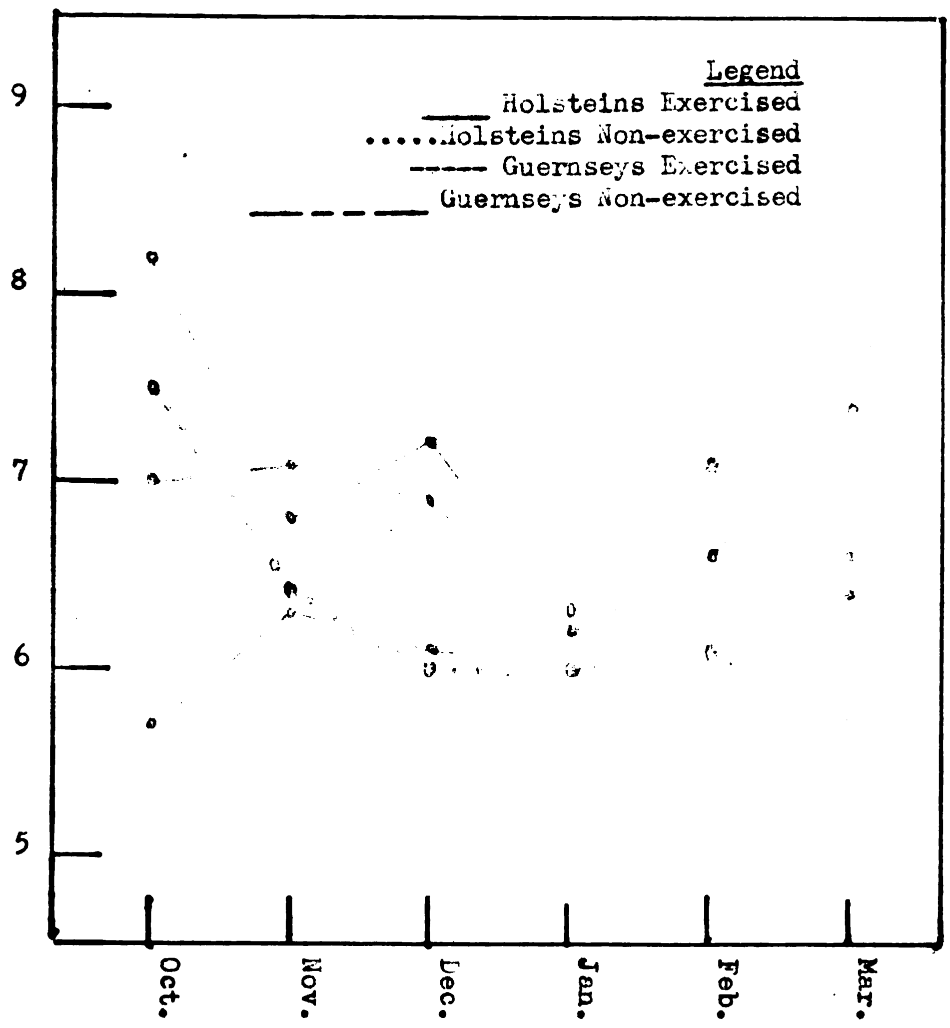


Fig. 3. Ml. Semen Per Ejaculate.

TABLE XIV

THE VOLUME OF SEMEN OF FIRST AND SECOND EJACULATIONS REGARDLESS OF FERTILITY LEVELS*

Month	Exercise		Non-exercise		Holstein		Guernsey	
	1	2	1	2	1	2	1	2
October	6.6	7.0	7.4	6.8	6.9	6.9	6.6	6.8
November	6.6	7.3	6.7	7.0	6.4	7.5	6.9	6.6
December	6.5	6.5	5.0	7.4	6.1	5.9	5.3	6.2
January	5.7	6.0	5.6	5.5	5.7	5.6	5.6	6.2
February	6.2	7.1	6.7	7.4	5.8	6.4	7.5	8.6
March	5.9	6.5	7.5	8.0	5.6	6.1	7.5	8.0
Average	6.3	6.7	6.5	7.0	6.1	6.4	6.6	7.1

*ml.

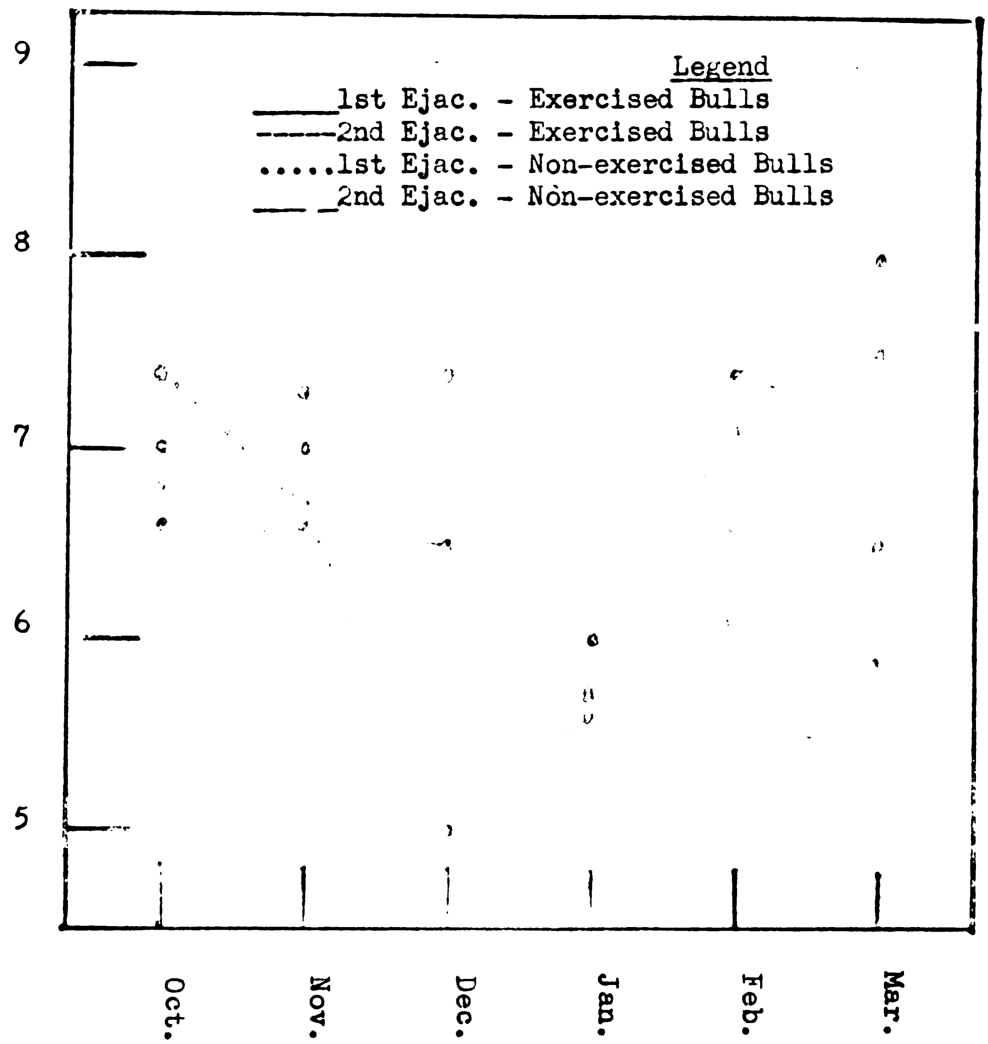


Fig. 4. Ml. Semen Per First and Second Ejaculates.

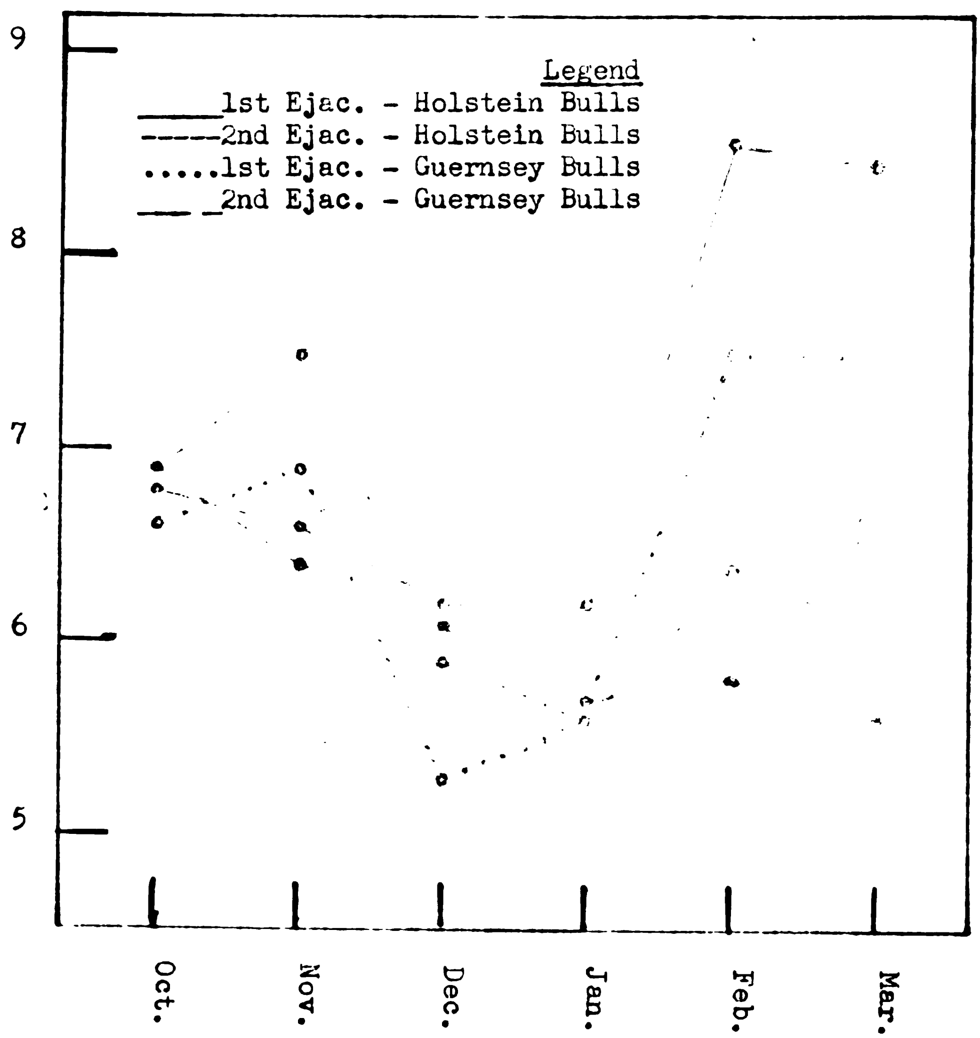


Fig. 5. Ml. Semen Per First and Second Ejaculates.

Since the data indicated a variation in volume of semen between exercised and non-exercised, high and low fertile Holsteins and Guernseys, an analysis of variance according to the method of Snedecor (132) was made. No significant differences in volume were obtained between exercised and non-exercised, high and low fertile Holstein and Guernsey bulls.

Total Spermatozoa

Table XV presents a monthly summary of the average, total spermatozoa per ejaculate for exercised and non-exercised, high and low fertile, Holstein and Guernsey bulls. This table shows that the exercised bulls were lower in total number of spermatozoa per ejaculate than the non-exercised group.

Considerable monthly variation in total spermatozoa per ejaculate was found between treatments and fertility levels as indicated in Figure 6. The high fertile bulls that were exercised produced .15 billions more spermatozoa per ejaculate than low fertile exercised bulls.

Non-exercised, high and low fertile, Holsteins were superior to the corresponding groups exercised. High fertile, exercised Guernseys were slightly superior to the non-exercised low fertile group.

A monthly summary of total spermatozoa per ejaculate for exercised and non-exercised Holstein and Guernsey bulls is presented in Table XV and Figure 7.

TABLE XV

MONTHLY SUMMARY OF AVERAGE TOTAL SPERMATOZOA FOR EXERCISED AND NON-EXERCISED HIGH AND LOW FERTILE HOLSTEIN AND GUERNSEY BULLS*

	EXERCISED				NON-EXERCISED			
Month	High Fertility		Low Fertility		High Fertility		Low Fertility	
	H	G	H	G	H	G	H	G
Oct.	8.02	7.11	8.52	6.49	8.29	7.80	8.19	9.74
Nov.	6.10	7.78	7.55	6.34	6.54	7.16	11.25	11.75
Dec.	9.11	8.28	7.93	6.07	9.21	7.38	8.54	11.88
Jan.	7.70	7.38	7.72	6.45	8.57	6.46	7.94	8.37
Feb.	7.76	8.27	7.19	9.28	9.04	7.02	7.98	10.80
Mar.	8.74	7.20	10.12	6.94	7.45	6.75	6.70	8.31
Av.	7.91	7.67	8.17	6.93	8.18	7.10	8.43	10.12

*Billions per ejaculate

TABLE XV (continued)

MONTHLY SUMMARY OF AVERAGE TOTAL SPERMATOZOA FOR EXERCISED AND NON-EXERCISED HIGH AND LOW FERTILE HOLSTEIN AND GUERNSEY BULLS*

Average for all months:

1. Exercise vs. non-exercise (7.67-8.46) = -.79
2. High fertile exercised vs. non-exercised high fertile (7.79-7.64) = + .15
3. Low fertile exercised vs. non-exercised low fertile (7.55-9.28) = -1.73
4. Exercised high fertile Holsteins vs. non-exercised high fertile Holsteins (7.91-8.18) = -2.7
5. Exercised low fertile Holsteins vs. non-exercised low fertile Holsteins (8.17-8.43) = .25
6. Exercised high fertile Guernsey vs. non-exercised high fertile Guernsey (7.67-7.10) = +.57
7. Exercised low fertile Guernsey vs. non-exercised low Guernsey (6.93-10.12) =-3.19
8. Exercised, and non-exercised high and low fertile Holsteins vs. exercised and non-exercised, high and low fertile Guernseys (8.17-7.93) = + .24

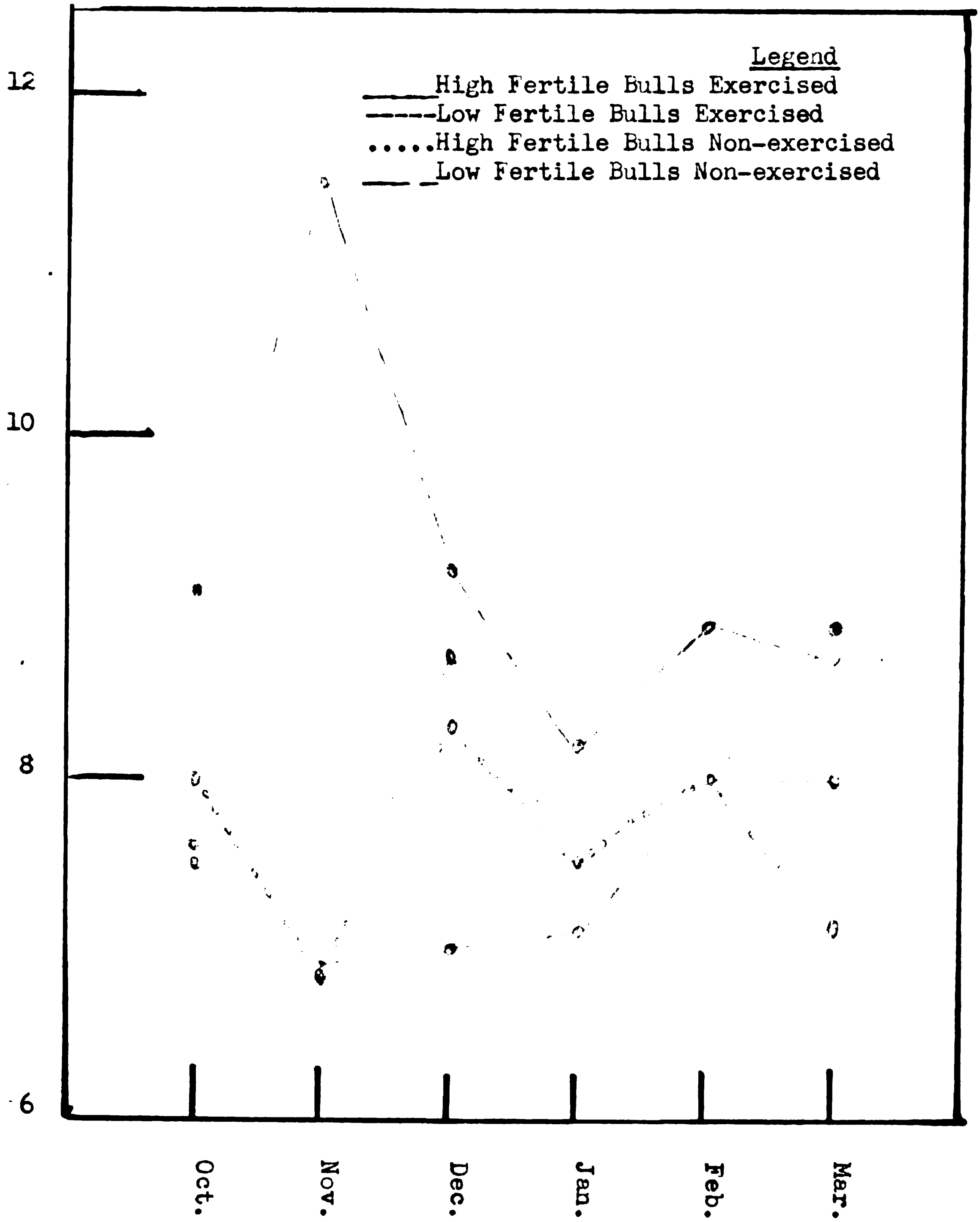


Fig. 6. Billions of Spermatozoa Per Ejaculate.

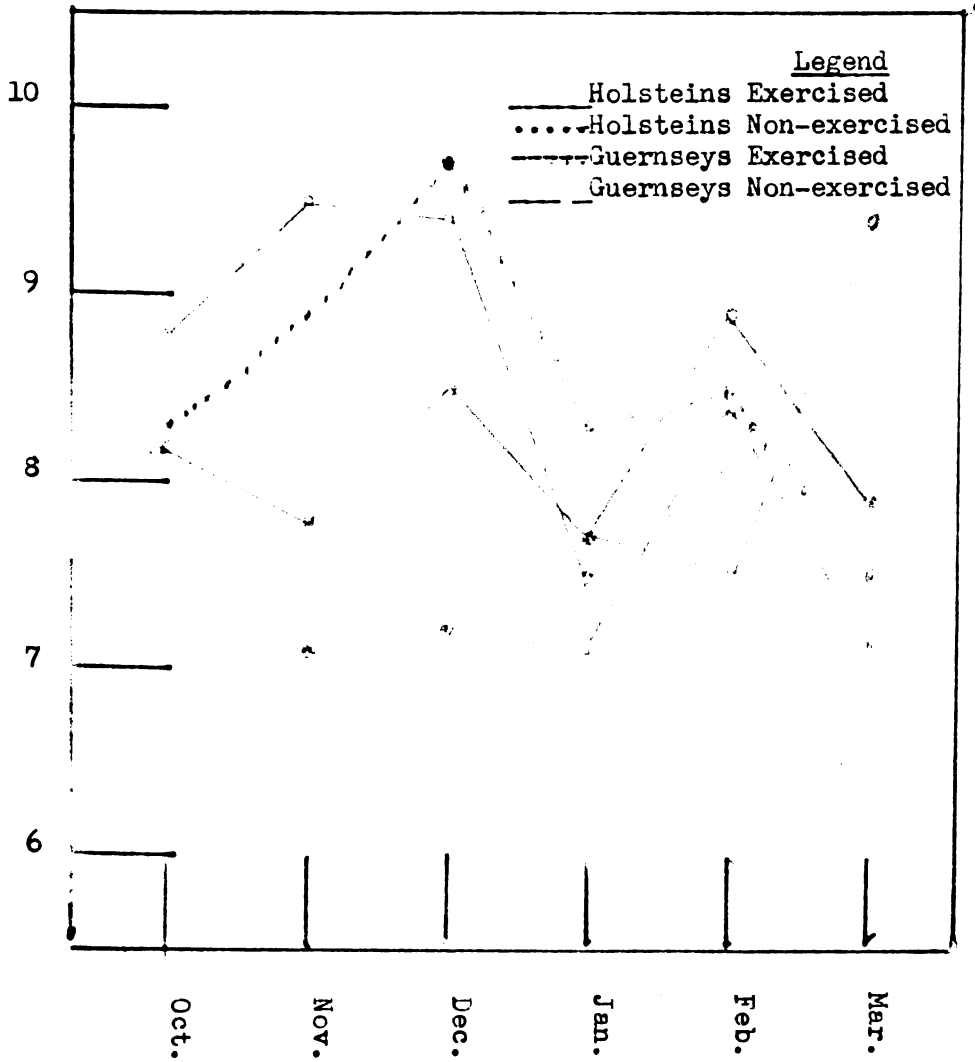


Fig. 7. Billions of Spermatozoa Per Ejaculate.

In non-exercised groups, the Guernseys were superior to the Holsteins in numbers of spermatozoa per ejaculate. Except for the month of February, the semen from the exercised Holsteins was consistently of higher spermatozoa concentration than that from the exercised Guernseys. The Holsteins slightly excelled the Guernsey bulls in the total number of spermatozoa per ejaculate.

The semen from the first ejaculates showed a higher total number of spermatozoa than that from the second ejaculates. These data are presented in Table XVI and Figures 8 and 9. These data show that the first ejaculates of the exercised and non-exercised bulls contained a higher number of spermatozoa than did the second ejaculates. The first ejaculates from the bulls not exercised had a larger number of spermatozoa per ejaculate as compared with the ones from the exercised group. The total number of spermatozoa per second ejaculate was higher for the bulls on exercise than those receiving no exercise.

Both breeds gave a greater number of spermatozoa in the first ejaculates than in the second.

In total spermatozoa, the first ejaculates of the Holsteins slightly excelled the first ejaculates of the Guernseys; whereas, the second ejaculates of the Guernseys had .2 billions more spermatozoa per ejaculate than the second ejaculate from the Holsteins.

An analysis of variance of the variation in total spermatozoa

TABLE XVI

TOTAL SPERMATOZOA OF FIRST AND SECOND EJACULATIONS REGARDLESS OF FERTILITY LEVELS*

Month	Exercise		Non-exercise		Holstein		Guernsey	
	1	2	1	2	1	2	1	2
October	7.7	7.5	9.0	7.7	8.8	7.7	6.6	6.8
November	8.1	7.7	9.5	7.5	8.2	7.9	6.9	6.6
December	8.7	8.0	6.7	5.5	8.8	7.0	5.3	6.2
January	7.8	7.1	7.8	6.6	8.1	6.4	5.6	6.2
February	8.1	7.7	8.7	7.4	8.3	6.9	7.5	8.6
March	7.9	8.7	10.3	9.0	7.9	8.8	7.5	8.0
Average	8.1	7.9	8.7	7.3	8.4	7.5	8.2	7.7

* In billions

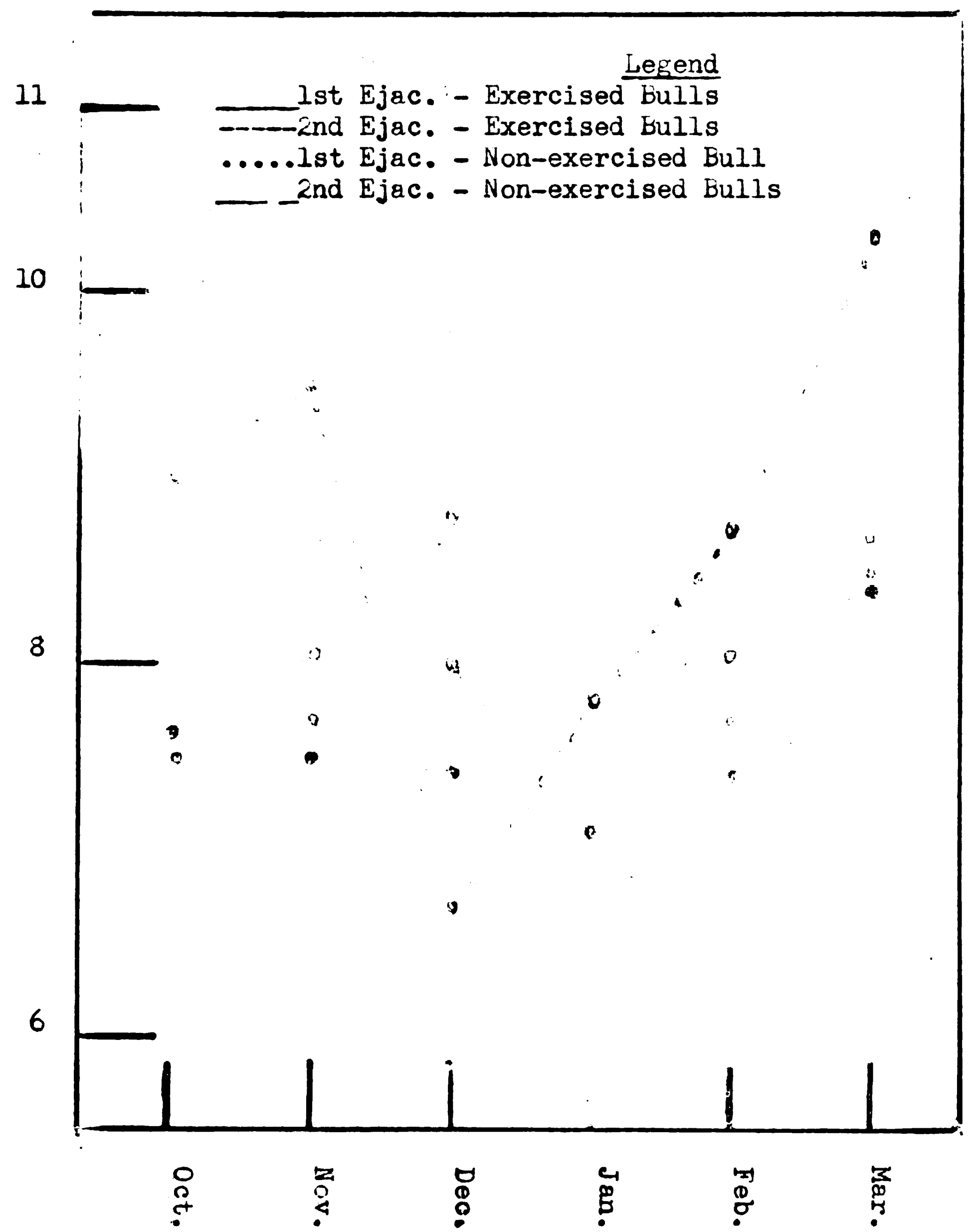


Fig. 8. Billions of Spermatozoa Per First and Second Ejaculations.

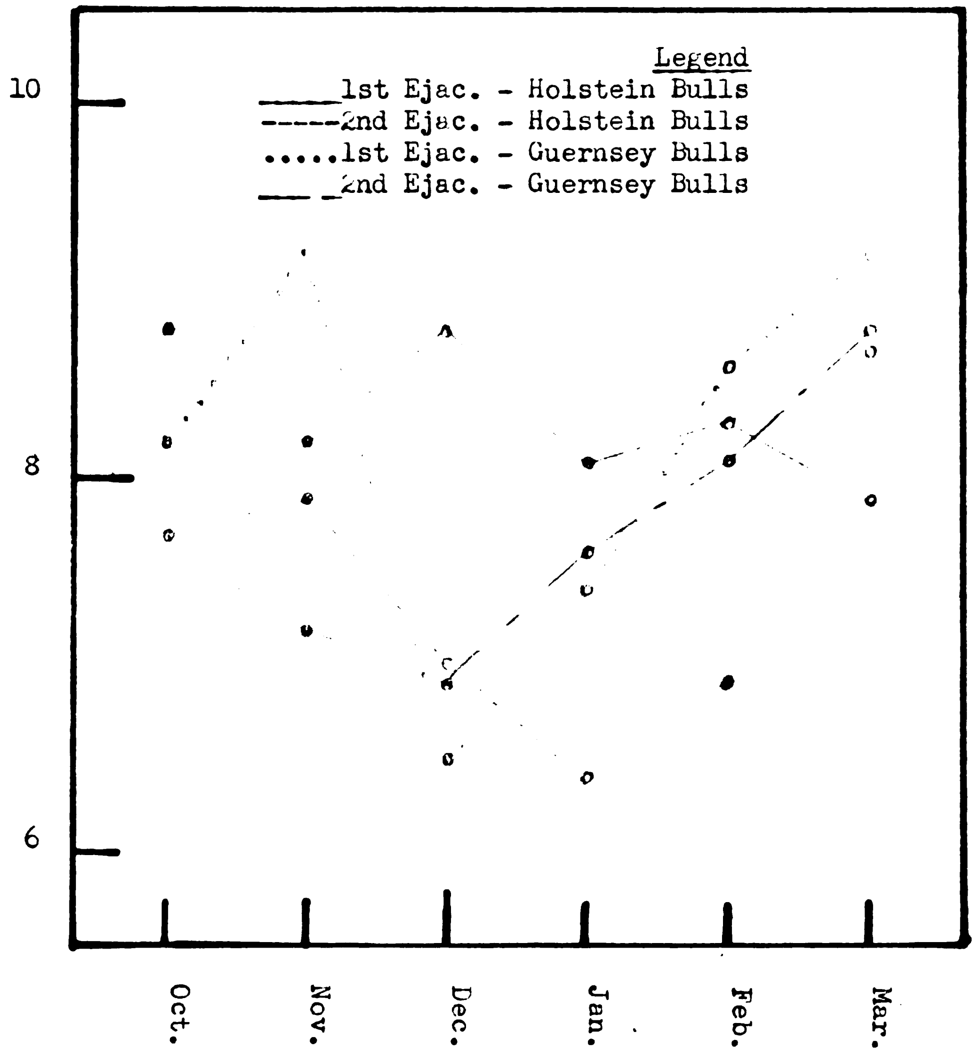


Fig. 9. Billions of Spermatozoa Per First and Second Ejaculates.

per ejaculate, for the exercised and non-exercised, high and low fertile, Holstein and Guernsey bulls, showed no significance between treatments, fertility levels and breeds.

Initial Motility

The percentage of initial motility showed less variation than any other semen characteristic. A monthly summary of initial motility for exercised and non-exercised, high and low fertile Holstein and Guernsey bulls, is presented in Table XVII. This table shows that the non-exercised bulls exceeded the exercised group by 1.7 percentage units in initial motility of the spermatozoa.

Figure 10 presents graphically a summary of the monthly variations in initial motility for high and low fertile, exercised, and for the same group, not exercised. The high fertile group, exercised, was superior to the high fertile group not exercised; whereas, the low fertile group not exercised was higher in initial motility than the exercised low fertile bulls.

Both groups, the low fertile Holsteins and Guernseys non-exercised, were superior in initial motility as compared to the high fertile Holsteins and Guernseys exercised; whereas, the exercised, high and low fertile Holsteins and Guernseys show a higher percentage of progressive motile spermatozoa than the corresponding groups not exercised.

A summary of the monthly differences in initial motility

TABLE XVII

MONTHLY SUMMARY OF INITIAL MOTILITY FOR EXERCISED AND NON-EXERCISED HIGH AND LOW FERTILE HOLSTEIN AND GUERNSEY BULLS*

	EXERCISED				NON-EXERCISED			
Month	High Fertility		Low Fertility		High Fertility		Low Fertility	
	H	G	H	G	H	G	H	G
Oct.	75.1	77.1	59.4	58.1	75.6	73.8	75.3	66.8
Nov.	75.8	77.5	52.8	69.6	76.4	70.0	73.9	70.3
Dec.	74.6	76.8	58.9	60.5	74.2	74.9	73.9	69.1
Jan.	74.9	76.4	72.8	74.9	74.5	74.7	74.5	65.0
Feb.	77.1	76.4	75.4	74.8	76.0	73.3	74.2	73.8
Mar.	75.6	77.6	68.8	75.0	75.5	72.1	74.2	74.0
Av.	75.5	77.0	64.7	68.8	75.4	73.1	74.3	69.8

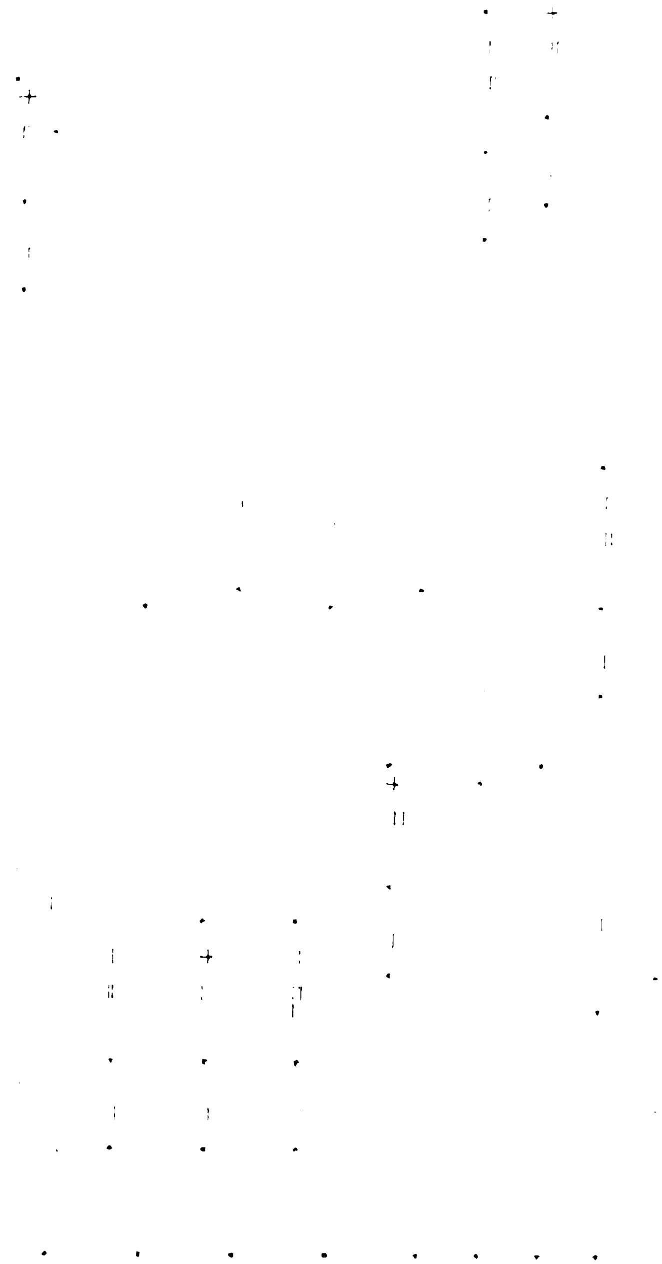
*Expressed in percentage

TABLE XVII (continued)

MONTHLY SUMMARY OF INITIAL MOTILITY FOR EXERCISED AND NON-EXERCISED HIGH AND LOW FERTILE HOLSTEIN AND GUERNSEY BULLS*

Average for all months:

1. Exercise vs. non-exercise (71.5-73.2) = -1.7
2. High fertile exercised vs. non-exercised high fertile (76.3-74.3) = +2
3. Low fertile exercised vs. non-exercised low fertile (66.8-72.1) = -5.3
4. Exercised high fertile Holsteins vs. non-exercised high fertile Holsteins (75.5-75.4) = +.1
5. Exercised low fertile Holsteins vs. non-exercised low fertile Holsteins (64.7-74.3) = -9.5
6. Exercised high fertile Guernseys vs. non-exercised high fertile Guernseys (77.0-73.1) = +3.9
7. Exercised low fertile Guernseys vs. non-exercised low fertile Guernseys (68.8-69.8) = -1
8. Exercised and non-exercised high and low fertile Holsteins vs. exercised and non-exercised high and low fertile Guernseys (72.2-72.1) = +.1



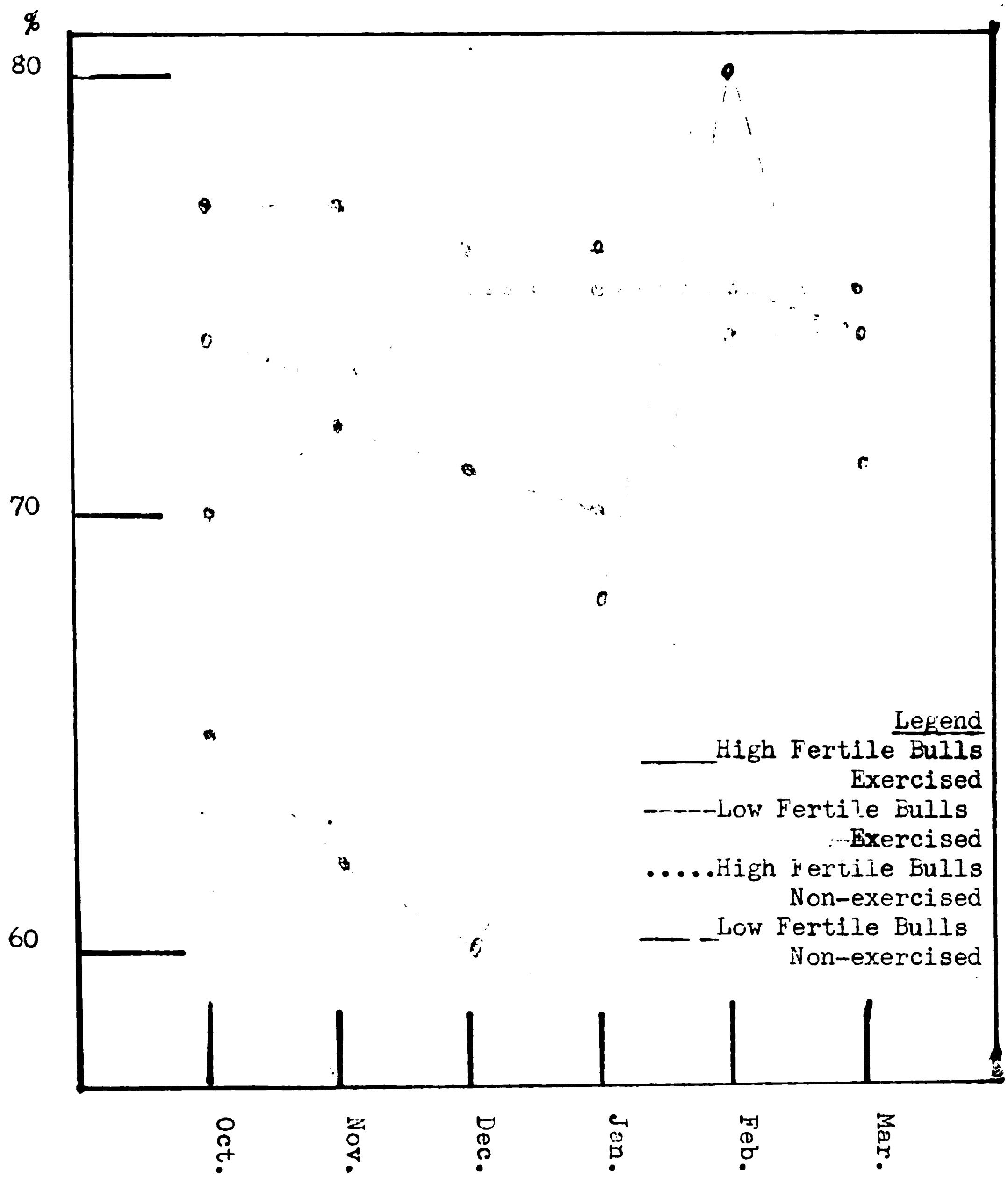


Fig. 10. Initial Motility Per Ejaculate.

between exercised Holsteins and Guernseys may be found in Table XVII. Table XVII and Figure 11 indicate that the initial motility of exercised Holsteins was lower than that of the Guernseys receiving exercise. In the non-exercised group, the Holsteins were higher than the Guernseys. The Holstein bulls slightly exceeded the Guernseys in percent of progressively motile spermatozoa.

Table XVIII shows that spermatozoa motility in the first ejaculation is poorer than in the second ejaculate except for bulls on exercise. The average motility ratings of the first and second ejaculates for the treatments are graphically presented in Figure 12. It can be seen that the motility from the second ejaculates of the non-exercised bulls was 1.3 percentage units higher than the first ejaculates. However, a comparison of the exercised bulls shows that the first ejaculations contained .8 percent more motile spermatozoa than the second ejaculates. In the two treatments, the motility of the first and second ejaculates of the exercised bulls were superior to those of the non-exercised bulls.

Figure 13 presents the monthly and breed variations in percent of motile spermatozoa per first and second ejaculates. As shown by this Figure and Table XVIII, the motility of the first and second ejaculates did not show a striking difference between the Holsteins and Guernseys.

As with the volume and total number of spermatozoa, an analysis of variance of average motility ratings for the exercised and non-exercised, high and low fertile Holstein and

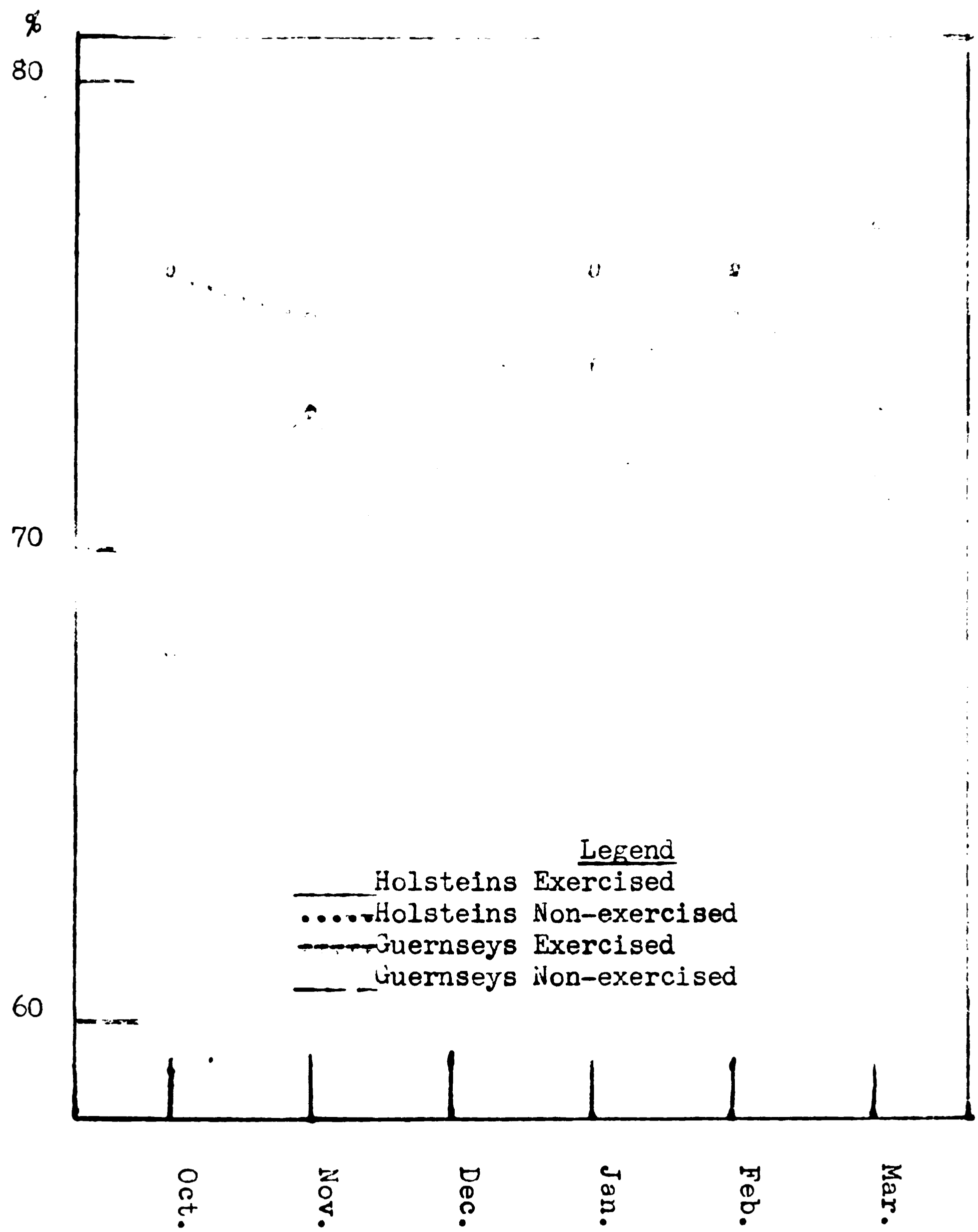


Fig. 11. Initial Motility Per Ejaculate.

TABLE XVIII

PERCENTAGE OF INITIAL MOTILITY OF FIRST AND SECOND EJACULATIONS REGARDLESS OF FERTILITY LEVELS

Month	Exercise		Non-exercise		Holstein		Guernsey	
	1	2	1	2	1	2	1	2
October	70	75	72	74	74.0	75.5	71.5	73.6
November	73	73	71	73	72.0	73.3	71.3	72.7
December	76	72	76	72	72.8	75.4	75.4	75.7
January	76	75	72	75	72.3	75.8	74.8	76.4
February	76	75	73	75	74.6	76.9	74.9	75.0
March	77	73	70	73	73.6	76.9	72.2	76.7
Average	74.6	73.8	72.3	73.6	73.2	75.6	73.3	75.0

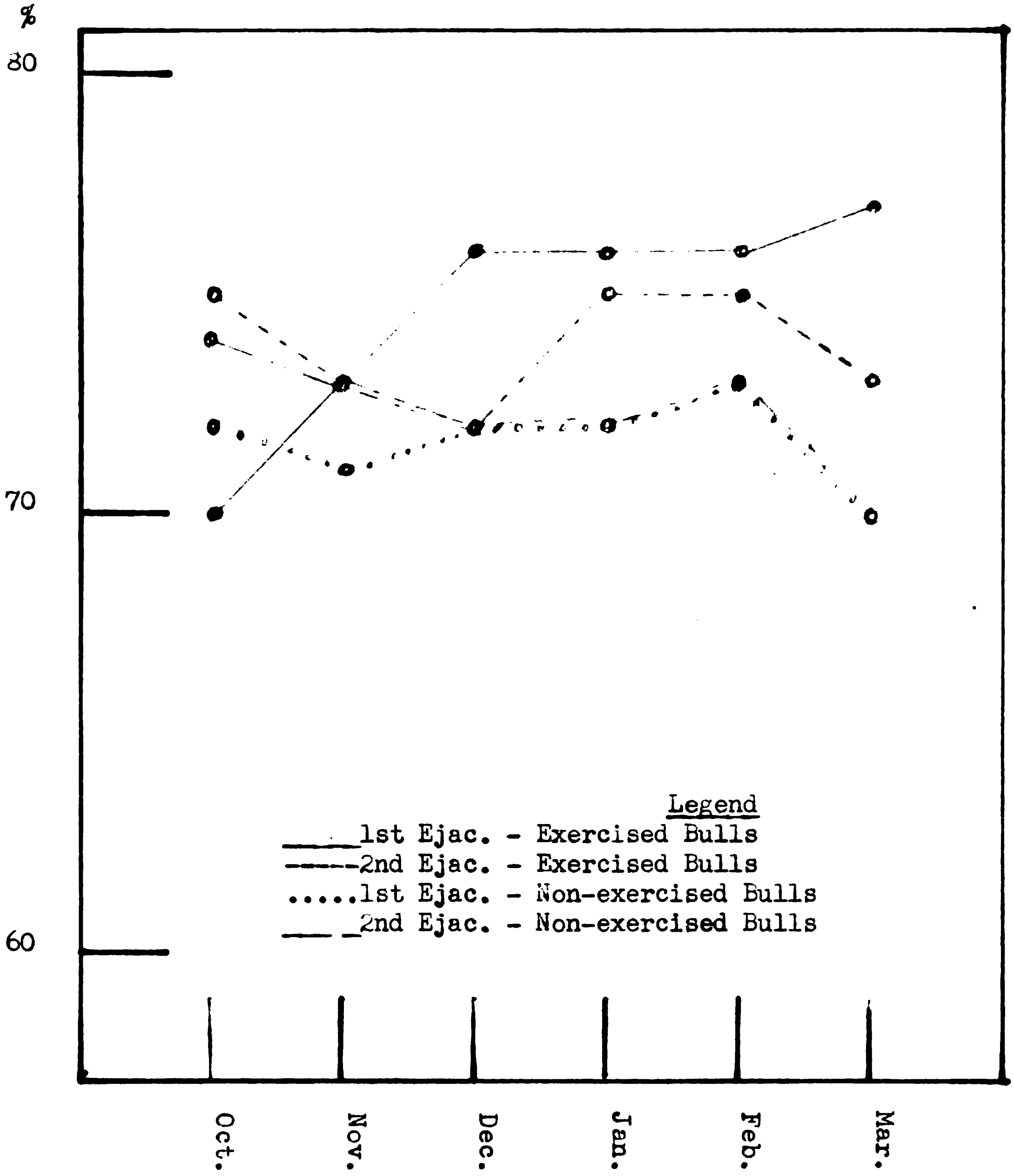


Fig. 12. Initial Motility Per First and Second Ejaculates.

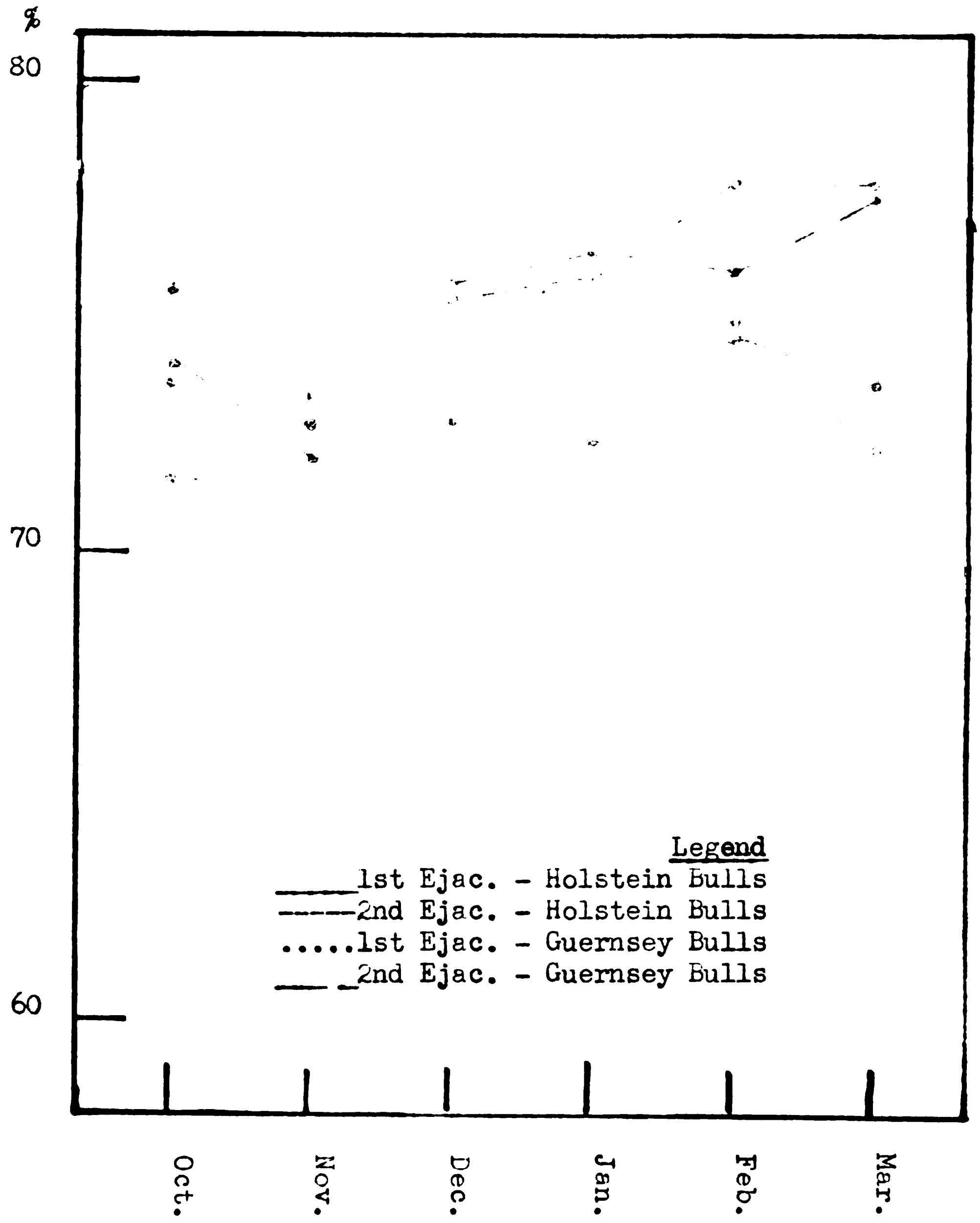


Fig. 13. Initial Motility Per First and Second Ejaculates.

Guernsey bulls, showed no significance in treatments, fertility levels and breeds.

Abnormal Spermatozoa

A monthly summary of abnormal spermatozoa for exercised and non-exercised, high and low fertile Holsteins and Guernsey bulls is given in Table XIX. This table indicates that the non-exercised bulls produced 1.4 percent more abnormal spermatozoa per ejaculate than the exercised bulls.

The exercised high fertile bulls, showed 3.9 percent less spermatozoa abnormalities as compared to the high fertility group not exercised; whereas, the low fertile exercised bulls reproduced 1.0 percent more than the low fertile non-exercised group.

The monthly variations of the treatments by breeds according to the percentage of abnormal spermatozoa in their semen are shown in Figure 14. These variations are markedly pronounced. The Guernsey bulls exceeded the Holsteins in percent of abnormal spermatozoa. The semen from exercised Holsteins showed an average of 5.9 percent less abnormalities than those not exercised; whereas, the semen from the exercised Guernseys had an average of .2 percent more abnormal spermatozoa than those not receiving exercise.

A comparison of the non-exercised groups indicated that bulls of high fertility produced .1 percent more abnormal spermatozoa than the low fertile bulls, and the exercised bulls of

TABLE XIX

MONTHLY SUMMARY OF ABNORMAL SPERMATOZOA FOR EXERCISED AND NON-EXERCISED HIGH AND LOW FERTILE HOLSTEIN AND GUERNSEY BULLS*

	EXERCISED				NON-EXERCISED			
Month	High Fertility		Low Fertility		High Fertility		Low Fertility	
	H	G	H	G	H	G	H	G
Oct.	10.3	12.6	21.7	17.5	16.6	17.4	15.6	15.9
Nov.	11.3	11.3	13.1	16.2	13.5	14.3	24.3	19.4
Dec.	13.0	12.0	10.1	25.9	11.3	16.5	10.9	15.8
Jan.	13.8	12.0	11.2	21.6	14.8	12.8	12.6	11.5
Feb.	12.5	15.5	13.8	17.0	16.9	16.7	14.1	11.4
Mar.	7.0	6.5	9.5	13.4	11.8	15.9	13.6	12.9
Av.	10.3	11.7	13.2	18.6	14.2	15.6	15.2	14.5

*Expressed in percentage

TABLE XIX(continued)

MONTHLY SUMMARY OF ABNORMAL SPERMATOZOA FOR EXERCISED AND NON-EXERCISED HIGH AND LOW FERTILE HOLSTEIN AND GUERNSEY BULLS*

Average for all months:

1. Exercise vs. non-exercise (13.5 - 14.9) = -1.4

2. High fertile exercise vs. non-exercised high fertile (11.0-14.9) = -3.9

3. Low fertile exercised vs. non-exercised low fertile (15.9-14.9) = -1.0

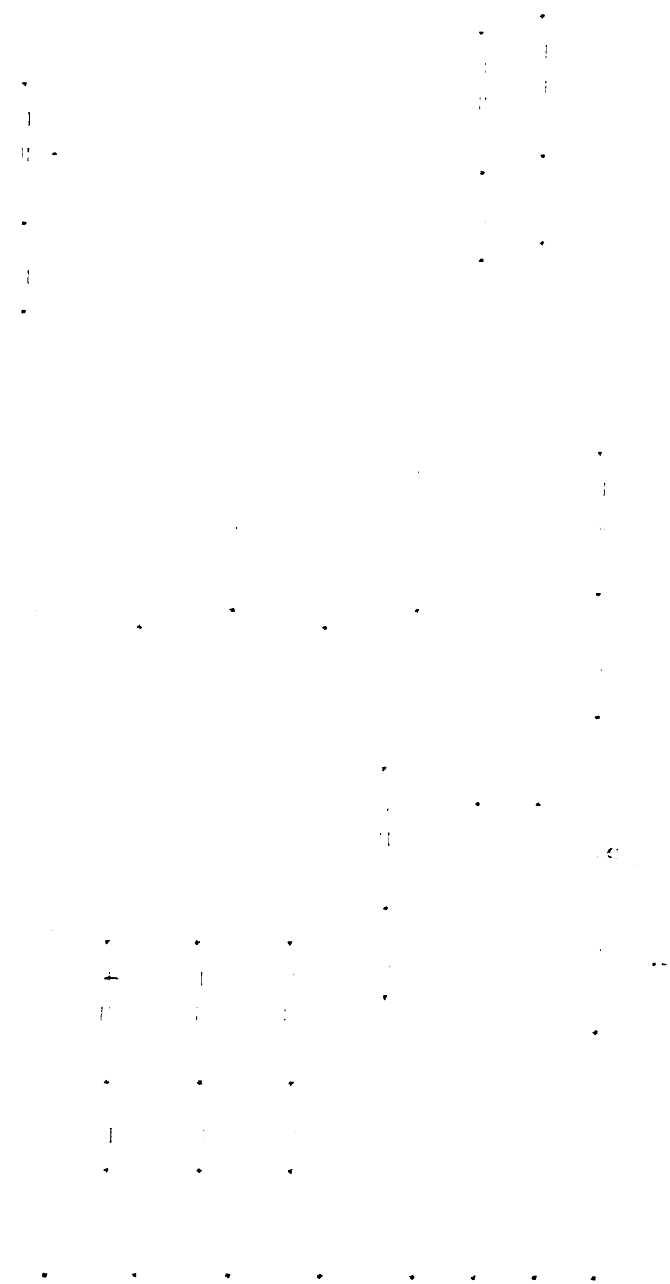
4. Exercised high fertile Holsteins vs. non-exercised high fertile Holsteins (10.3-14.2) = -3.9

5. Exercised low fertile Holsteins vs. non-exercised low fertile Holsteins (13.2-15.2) = -2.0

6. Exercised high fertile Guernseys vs. non-exercised high fertile Guernseys (11.7-15.6) = -3.9

7. Exercised low fertile Guernseys vs. non-exercised low fertile Guernseys (18.6-14.5) = +4.1

8. Exercised and non-exercised high and low fertile Holsteins vs. exercised and non-exercised high and low fertile Guernseys (13.2-15.1) = -1.9



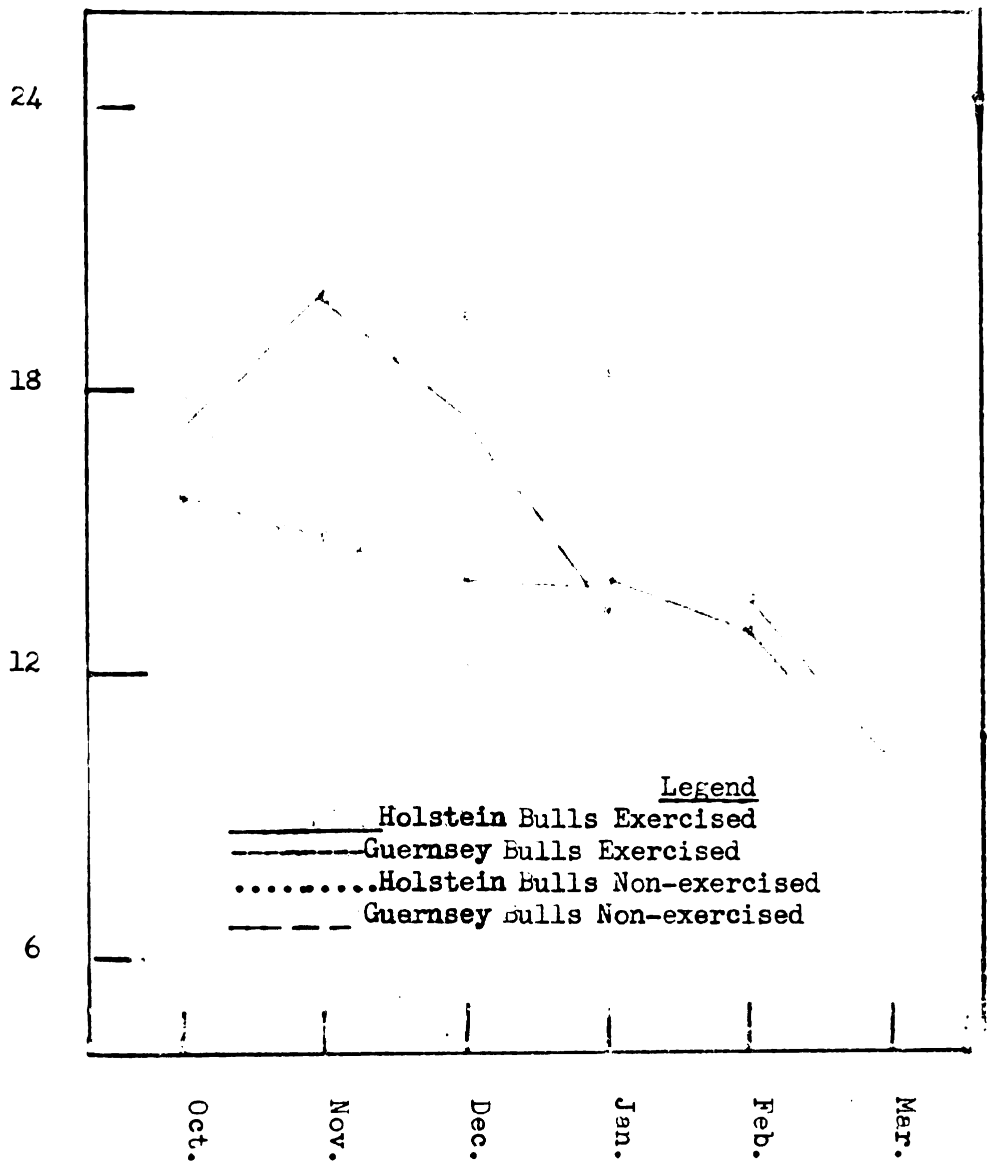


Fig. 14. Percent Abnormal Spermatozoa.

high fertility contained 9.8 percent less abnormalities than the low fertile group. These data are presented monthly in Figure 15.

Exercised high fertile Holsteins contained 3.9 perdent less spermatozoa abnormalities per ejaculate than the non-exercised group. This was also true with the low fertile exercised Holstein bulls which had 2.0 percent less abnormal spermatozoa per ejaculate as compared with the non-exercised group.

The data shown in Table XX indicates that the first ejaculation contained more abnormal spermatozoa than the second ejaculates. It is evident that the first and second ejaculates of the exercised group were decidedly lower in abnormalities as compared to the group not receiving exercise, Figure 16.

A summary of the monthly variation in percent abnormal spermatozoa for first and second ejaculates of the Holstein and Guernsey breeds is graphically presented in Figure XVII. These data show that the first and second ejaculates of the Guernsey bulls were higher in percent abnormal spermatozoa than those of the Holsteins.

According to an analysis of variance, there were no significant differences in the percent abnormal spermatozoa between treatments, fertility levels, and breeds.

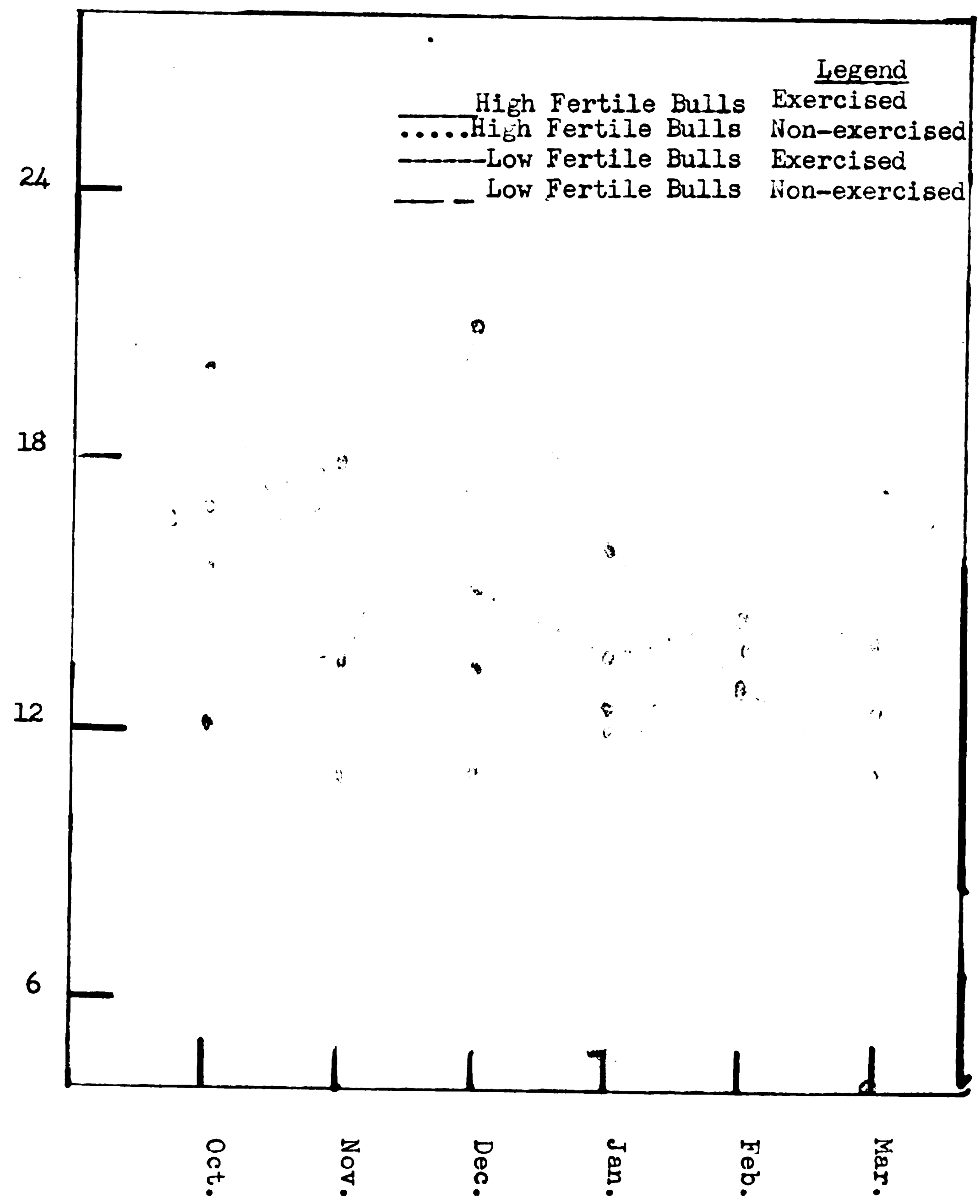


Fig. 15. Percent Abnormal Spermatozoa.

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TABLE XX

PERCENTAGE OF ABNORMAL SPERMATOZOA OF FIRST AND SECOND EJACULATIONS REGARDLESS OF FERTILITY LEVELS

Month	Exercise		Non-exercise		Holstein		Guernsey	
	1	2	1	2	1	2	1	2
October	8.5	6.7	11.8	10.1	8.0	6.2	6.8	6.0
November	12.0	10.7	20.2	18.0	13.4	11.4	18.2	17.6
December	15.0	12.0	16.0	15.5	12.4	13.0	20.8	14.0
January	14.0	8.5	12.0	11.9	14.6	10.8	12.6	10.2
February	12.0	12.3	18.1	14.0	15.2	14.2	10.8	8.8
March	8.0	6.1	10.2	12.0	9.0	7.8	10.8	8.0
Average	11.6	9.4	14.7	13.5	6.1	5.3	6.7	5.4

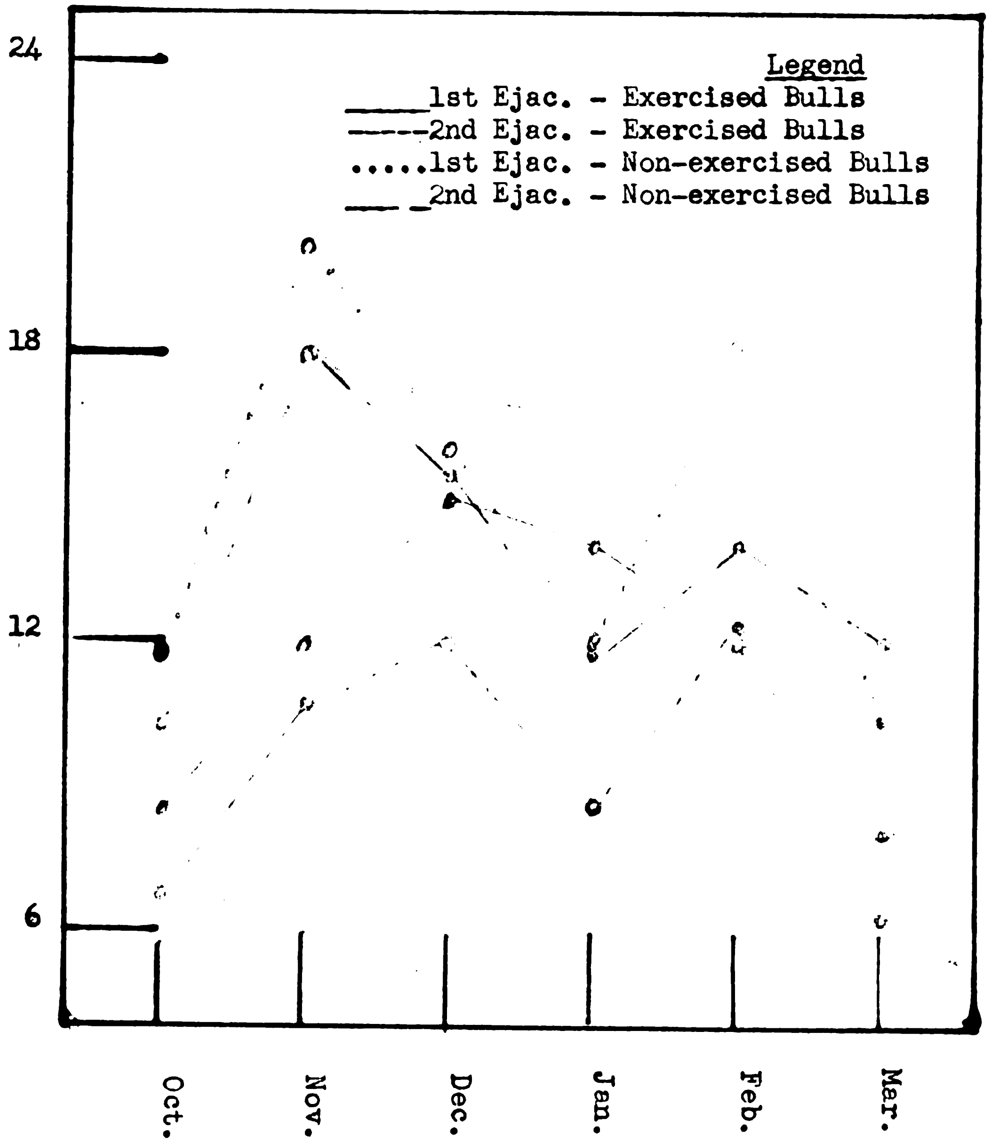

Fig. 16. Percent Abnormal Spermatozoa Per First and Second Ejaculates.

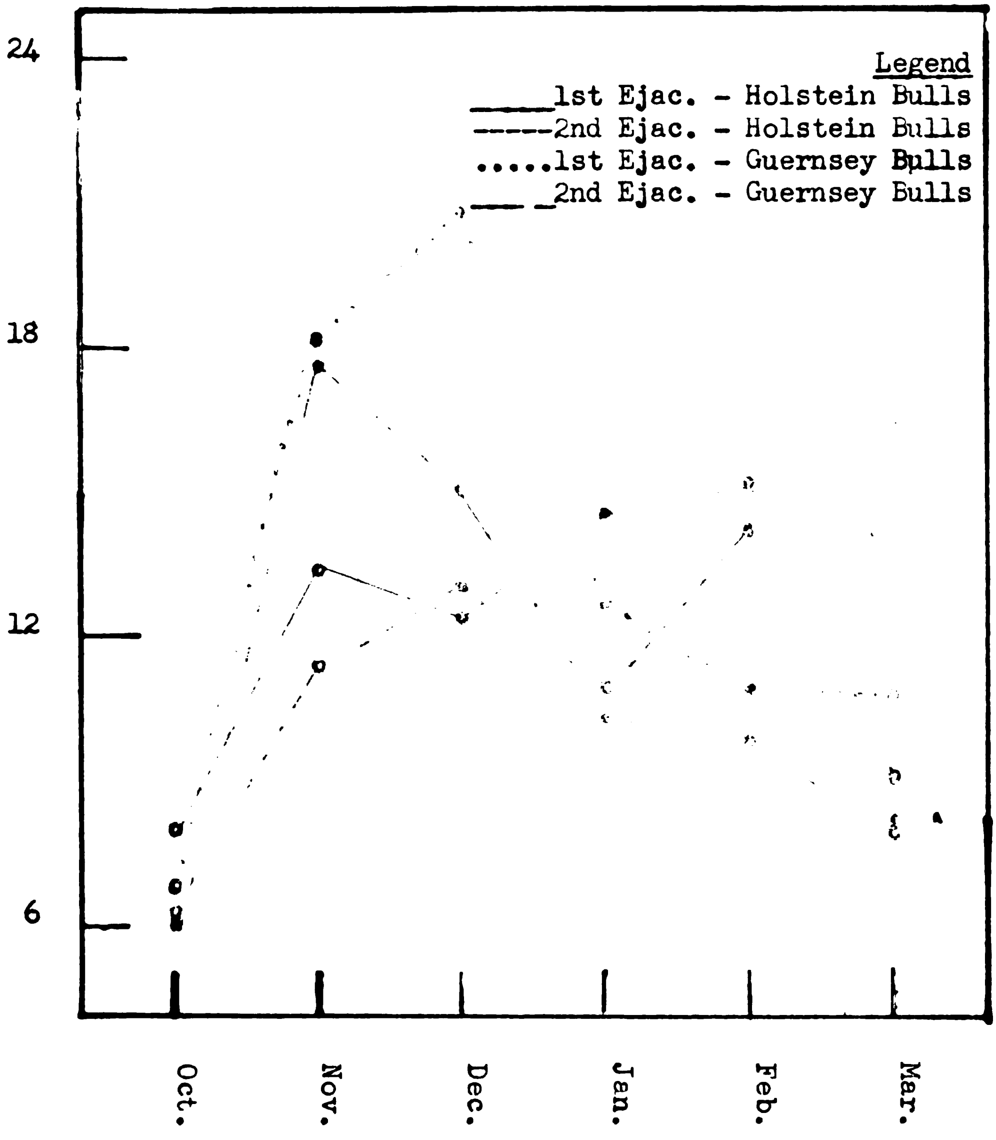

Fig. 17. Percent Abnormal Spermatozoa Per First and Second Ejaculates.

Types of Abnormal Spermatozoa

A summary of the abnormal types of spermatozoa for the treatments and fertility levels, regardless of breeds, is presented in Table XXI. There seemed to be no one particular type of abnormality associated with exercised or non-exercised bulls. This was also true with fertility levels. The main distinguishing characteristic between spermatozoa of bulls of high fertility and those of low fertility were the percentage of total abnormal spermatozoa. There were exceptions to the above statement in that some bulls with semen of abnormal morphology were high in percentage of non-returns, and that other bulls of low fertility had semen with few morphological defects.

The abnormal types of spermatozoa for exercised and non-exercised Holstein and Guernsey bulls, regardless of fertility level, is summarized in Table XXII. This table shows that the non-exercised Holsteins and Guernseys exceeded the exercised group in percentage of coiled tailed, tailless, and true abnormal spermatozoa; whereas, the broken neck spermatozoa were slightly higher in the exercised Holstein and Guernsey bulls than in the non-exercised groups.

The most common abnormal form encountered was coiled tailed spermatozoa followed closely by the tailless. The next most common abnormal type was true abnormal including such forms as those possessing any abnormality with respect to the shape or size of the head, spermatozoa with enlarged, abaxial,

TABLE XXI

TYPES OF ABNORMAL SPERMATOZOA
REGARDLESS OF BREEDS*

EXERCISE

Month	High Fertility					Low Fertility				
	C. T.	T.	B. N.	T. A.	TOT.	C. T.	T.	B. N.	T. A.	TOT.
Oct.	3.5	6.1	.9	1.6	12.1	6.0	10.0	1.0	3.0	20.0
Nov.	4.9	3.3	.7	2.1	11.0	5.3	4.1	.9	3.1	13.5
Dec.	3.4	3.1	3.0	1.8	11.1	9.7	6.4	3.6	2.1	21.0
Jan.	4.0	3.1	2.5	2.4	12.0	6.9	4.2	2.5	2.4	16.0
Feb.	4.0	3.7	3.7	2.5	13.9	4.6	4.1	2.2	2.2	13.1
March	1.9	2.9	1.2	1.0	7.0	5.2	2.9	1.0	2.0	11.1
Average	3.6	3.7	2.0	1.9	11.0	6.3	5.3	1.9	2.5	15.9

Legend = *Expressed in percentage

C. T. = Coiled tailed
T. = Tailless
B. N. = Broken necks
T. A. = True abnormals

TABLE XXI(continued)

TYPES OF ABNORMAL SPERMATOZOA
REGARDLESS OF BREEDS*

NON-EXERCISE

Month	High Fertility					Low Fertility				
	C. T.	T.	B. N.	T. A.	TOT.	C. T.	T.	B. N.	T. A.	TOT.
Oct.	8.0	3.2	1.8	4.0	17.9	6.3	4.6	.9	3.5	15.3
Nov.	6.7	4.1	1.2	6.0	18.0	6.6	6.1	1.2	3.5	17.4
Dec.	5.9	3.3	3.1	2.8	15.1	5.0	3.1	3.0	2.2	13.3
Jan.	5.1	4.0	2.3	2.3	13.7	5.6	2.9	1.7	2.5	12.7
Feb.	6.8	3.6	2.1	2.5	14.5	6.2	3.3	.7	2.8	13.0
Mar.	6.5	2.8	1.9	2.8	14.0	4.6	3.7	2.1	2.6	13.0
Average	6.5	3.5	2.1	3.4	15.5	5.7	3.9	1.6	2.8	14.1

Legend = *Expressed in percentage

C. T. = Coiled tailed
T. = Tailless
B. N. = Broken necks
T. A. = True abnormals

TABLE XXII

TYPES OF ABNORMAL SPERMATOZOA FOR BREEDS REGARDLESS OF FERTILITY LEVELS *

EXERCISE								
Month	Coiled Tails		Tailless		Broken Necks		True Abnormals	
	H	G	H	G	H	G	H	G
Oct.	2.1	3.2	3.2	3.0	1.0	.8	1.6	1.0
Nov.	5.0	5.2	3.7	3.5	.7	.9	2.9	2.6
Dec.	4.1	8.0	3.3	5.7	2.6	3.9	2.2	1.8
Jan.	4.3	4.0	3.2	5.9	2.3	2.8	2.2	2.7
Feb.	3.1	3.0	2.7	2.4	2.7	1.8	1.8	1.3
Mar.	3.0	3.1	3.1	2.1	.7	2.1	1.2	1.4
Average	3.6	4.4	3.2	3.8	1.7	1.9	2.0	1.8

*Expressed in percentage

TABLE XXII(continued)

TYPES OF ABNORMAL SPERMATOZOA FOR BREEDS
REGARDLESS OF FERTILITY LEVELS*

N O N - E X E R C I S E

Month	Coiled Tails		Tailless		Broken Necks		True Abnormals	
	H	G	H	G	H	G	H	G
Oct.	6.4	8.6	4.1	4.1	1.8	.9	4.2	3.4
Nov.	5.8	7.5	4.0	7.6	.8	1.7	3.8	5.8
Dec.	4.3	6.7	2.6	3.8	2.3	3.8	2.4	2.6
Jan.	5.3	5.6	3.8	3.2	2.6	1.5	2.4	2.2
Feb.	4.7	7.1	3.5	2.7	1.7	2.3	2.5	2.2
Mar.	5.0	7.3	2.7	4.0	1.8	.9	2.8	2.5
Average	5.3	7.1	3.5	4.2	1.8	1.9	3.0	3.1

*Expressed in percentage

beaded or filiform middle pieces, and those with thickened or double tails. Spermatozoa with broken necks were relatively rare as compared to the coiled tailed and tailless.

Coiled Tails

Figure 18 presents graphically the monthly variations in coiled tailed spermatozoa for treatments and fertility levels. A study of this graph indicates that the semen of exercised high fertile bulls contained decidedly less coiled tails than any of the other experimental groups. In the non-exercised groups, the high fertile bulls produced .8 percent more coiled tails per ejaculate than the low fertile bulls. A comparison of the bulls of low fertility shows that the group on exercise had .6 percent more coiled tailed spermatozoa per ejaculate as the bulls not exercised.

Table XXIII shows the percentage of the types of abnormal spermatozoa for treatments and ejaculates one and two. A study of this table shows that first and second ejaculations of the bulls on exercise contained a lower percentage of coiled tailed spermatozoa than those not receiving exercise. Of the non-exercised group, the second ejaculation contained .5 percent more coiled tailed spermatozoa per ejaculate than the first ejaculates; whereas, the first ejaculates of the bulls on exercise showed .1 percent more per ejaculate of this abnormality than the second ejaculation.

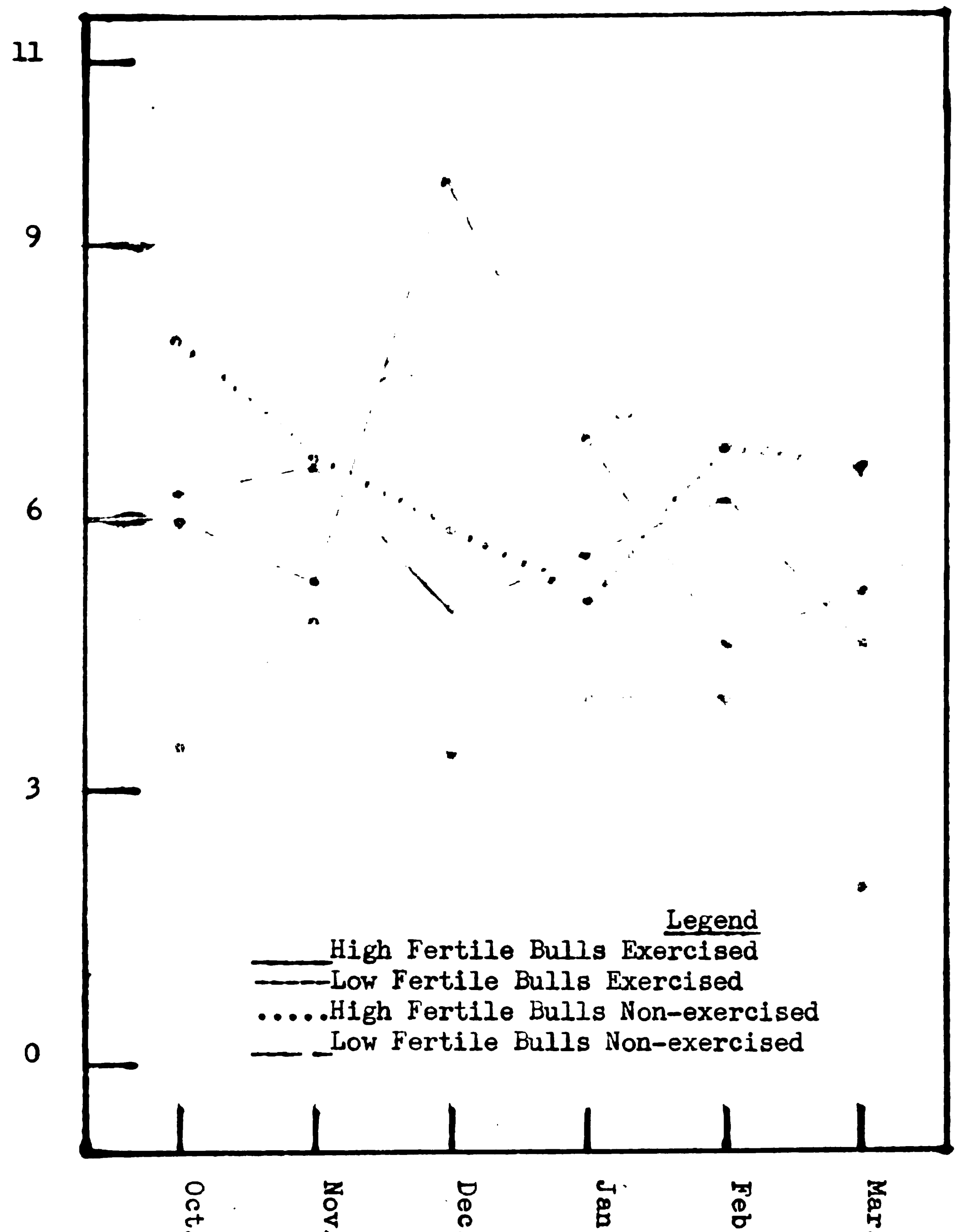

Fig. 18. Percent of Coiled Tails.

TABLE XXIII

PERCENT ABNORMAL SPERMATOZOA OF THE FIRST AND SECOND EJACULATES

EXERCISE								
Month	Coiled Tails		Tailless		Broken Necks		True Abnormals	
	1	2	1	2	1	2	1	2
Oct.	1.4	2.0	1.1	.6	.2	.1	1.2	.4
Nov.	2.3	2.1	1.9	1.7	.4	.5	.2	.9
Dec.	2.2	1.8	2.5	1.8	2.2	1.4	.7	1.0
Jan.	2.9	1.8	1.8	1.1	1.6	.5	1.4	.8
Feb.	1.5	1.9	1.8	1.9	1.5	1.4	1.2	1.1
Mar.	1.3	1.1	1.2	1.0	.7	.1	.8	.6
Average	1.9	1.8	1.7	1.4	1.1	0.7	0.9	0.8

TABLE XXIII (continued)

PERCENT ABNORMAL SPERMATOZOA OF THE FIRST AND SECOND EJACULATES

NON - EXERCISE

Month	Coiled Tails		Tailless		Broken Necks		True Abnormals	
	1	2	1	2	1	2	1	2
Oct.	1.2	1.9	.8	1.0	1.1	.1	.8	.3
Nov.	3.2	3.9	3.1	3.1	.5	.5	2.9	2.0
Dec.	2.3	3.5	2.7	1.2	.6	1.8	1.7	1.0
Jan.	2.0	2.5	1.8	2.2	1.0	.8	1.0	1.1
Feb.	2.4	2.6	1.6	1.3	.9	1.0	1.1	1.4
Mar.	2.2	2.0	1.4	1.6	.9	.6	1.2	1.0
Average	2.2	2.7	1.9	1.7	0.8	0.8	1.5	1.1

Tailless Spermatozoa

Figure 19 shows graphically the wide variation of tailless spermatozoa for the treatments and fertility levels. The number of spermatozoa without tails was slightly higher in the non-exercised bulls, (Table XXII). Both the low and high fertile bulls receiving exercise were higher in tailless spermatozoa as compared to the corresponding groups not exercised. In both treatments, bulls of low fertility were higher in percentage of tailless spermatozoa than the high fertile groups.

As was true with coiled tails, this type of spermatozoa abnormality was slightly higher in the first and second ejaculation of the non-exercised bulls as compared to the exercised group, (Table XXIII). In both groups, the first ejaculate contained a higher percentage of this abnormality.

Broken Necks

This abnormality was not noticed in all ejaculates. Bulls that received exercise showed .1 percent fewer broken necks per ejaculate than those bulls not on exercise, (Table XXII). Exercised bulls of high fertility had slightly more broken necks than those of low fertility. This was also true in the case of the corresponding groups not on exercise. These data are presented graphically in Figure 20.

A comparison of the first and second ejaculates in Table XXIII indicates that the first ejaculates generally contained more of this type of abnormal spermatozoa than the second

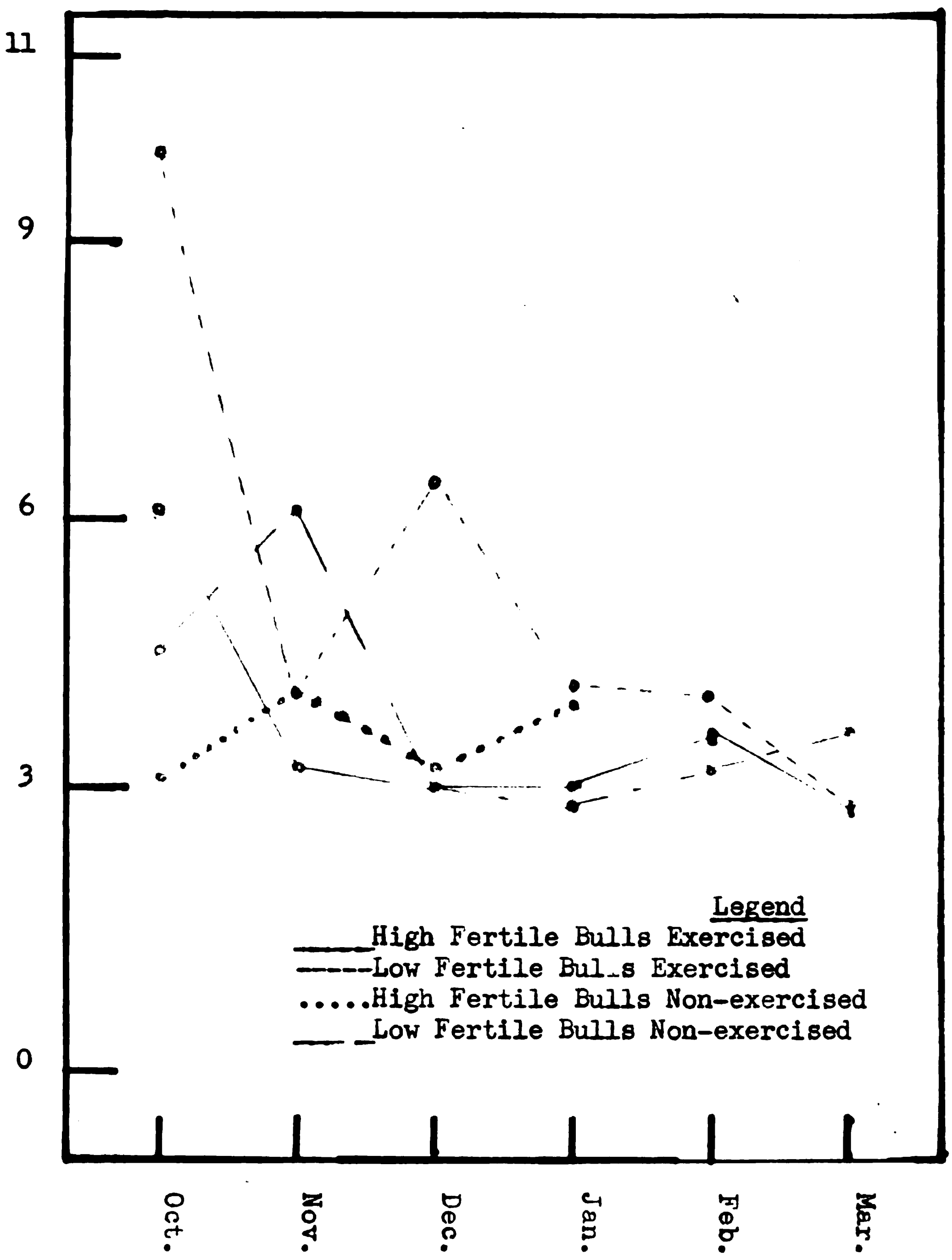

Fig. 19. Percent Tailless Spermatozoa.

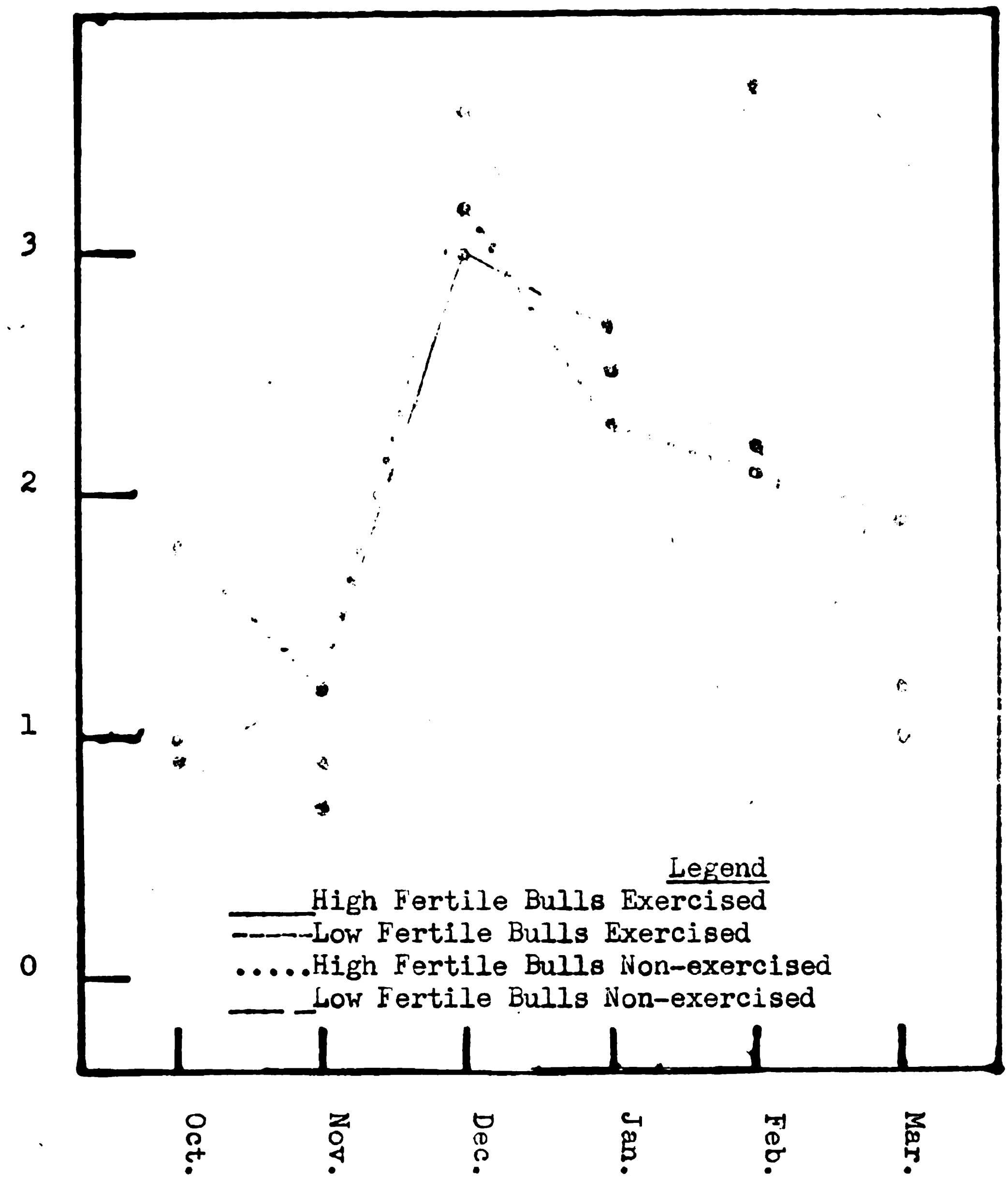

Fig. 20. Percent Broken Necks Spermatozoa.

ejaculations. This fact was especially true of the exercised bulls.

True Abnormal Spermatozoa

This type of abnormality consists of forms of spermatozoa. Figure 21 shows the wide variation between months, treatments, and fertility levels. The non-exercised groups had a larger number of true abnormal spermatozoa than the exercised bulls, (Table XXI). In the non-exercised group, the bulls of high fertility showed .6 percent more true abnormals per ejaculate than those of low fertility; whereas in the exercised groups, the low fertile bulls produced .6 percent more of the abnormality per ejaculate than the high fertile bulls.

The Holstein bulls produced more true abnormals than the Guernsey bulls (Table XXII). The non-exercised Holstein and Guernseys had a larger number of this abnormality than those breeds on exercise.

The first ejaculates were higher in true abnormals than the second ejaculates for both exercised and non-exercised bulls, (Table XXIII).

Body Weight of Bulls

Individual body weights of the bulls were taken at approximately six week intervals. The initial and final weights of the exercised and non-exercised groups are shown in Table XXIV. Both groups of bulls gained in body weight. The 16 bulls not

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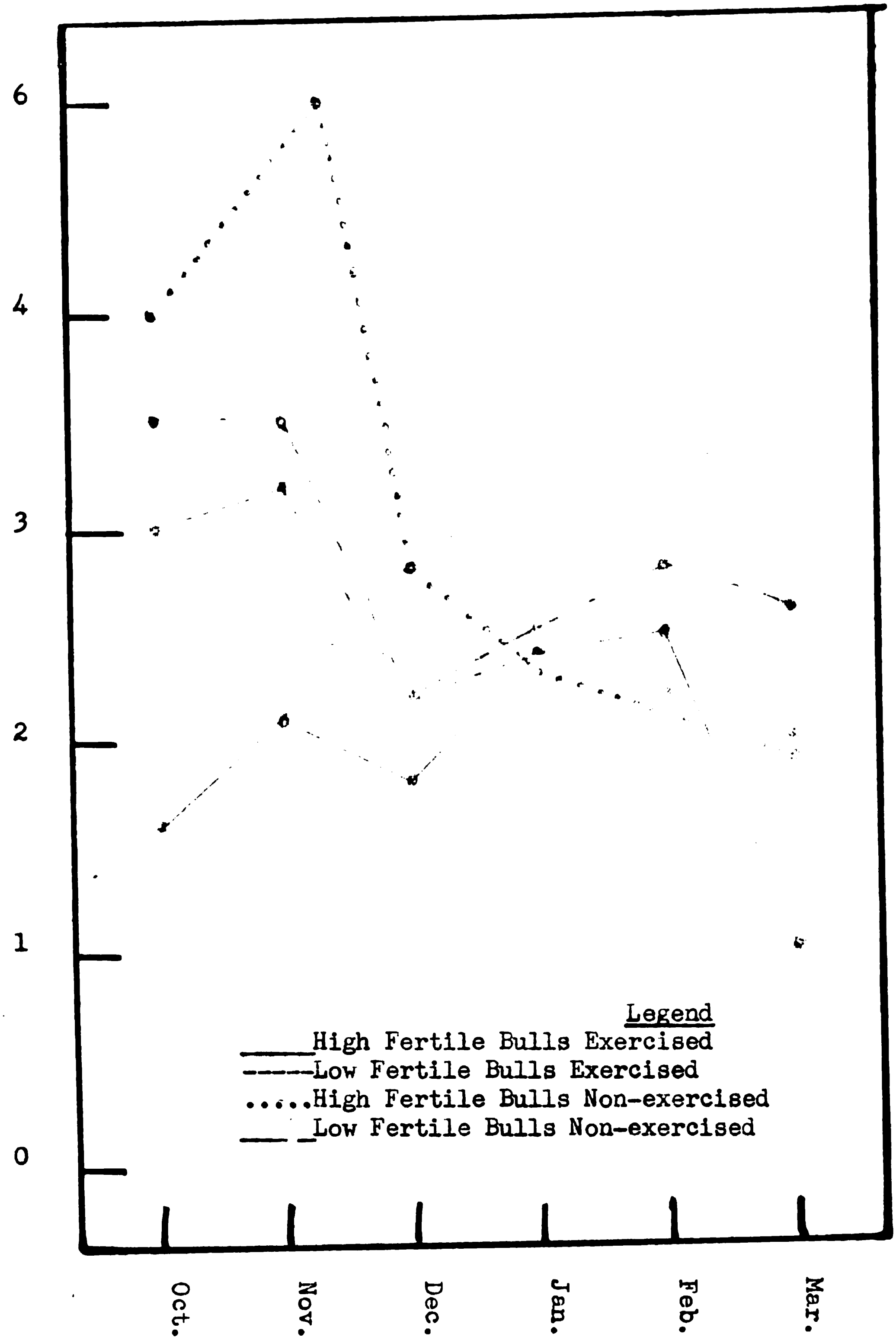

Fig. 21. Percent True Abnormal Spermatozoa.

TABLE XXIV

BODY WEIGHTS OF THE EXERCISED AND NON-EXERCISED BULLS*

EXERCISE				NON-EXERCISE			
Bull	Initial Weight	Final Weight	Difference	Bull	Initial Weight	Final Weight	Difference
High Fertile Holsteins							
1	2,060	2,130	70	17	2,000	2,271	271
2	2,237	2,350	113	18	2,480	2,515	35
3	2,326	2,445	119	19	2,125	2,080	-45
4	2,275	2,404	129	20	2,317	2,423	106
Low Fertile Holsteins							
5	1,990	2,032	42	21	2,254	2,200	-54
6	2,103	2,195	92	22	2,185	2,268	83
7	2,145	2,222	77	23	2,107	2,195	88
8	2,300	2,330	30	24	2,165	2,250	85

TABLE XXIV (continued)

BODY WEIGHTS OF THE EXERCISED AND NON-EXERCISED BULLS*

E X E R C I S E				N O N - E X E R C I S E			
Bull	Initial Weight	Final Weight	Difference	Bull	Initial Weight	Final Weight	Difference
			High Fertile Guernseys				
9	1,620	1,680	60	25	1,852	1,830	-22
10	1,507	1,630	123	26	1,660	1,770	110
11	1,564	1,645	81	27	1,695	1,712	52
12	1,960	2,075	115	28	1,366	1,450	84
			Low Fertile Guernseys				
13	1,790	1,735	-55	29	1,560	1,643	83
14	1,910	2,008	98	30	1,635	1,731	96
15	1,507	1,490	-17	31	1,794	1,903	109
16	1,736	1,800	64	32	1,720	1,910	190
Total gain in body weight:			1,141				

*In pounds

on exercise only gained 95 pounds more than the exercised group. Only three bulls not exercised lost body weight; whereas, only two bulls exercised lost body weight.

Degree of Libido

The time required for each bull to mount the teaser for each collection was recorded. Ejaculation frequencies and the mean mounting times for the bulls are given in Table XXV. It is evident from these data, that no real differences existed.

TABLE XXV

EJACULATION FREQUENCIES AND MEAN MOUNTING TIMES FOR THE INDIVIDUAL BULLS

EXERCISE			NON-EXERCISE		
No.	Ejaculations Number	Time Min.	No.	Ejaculations Number	Time Min.
		High Fertile Holsteins			
1	50	.6	17	23	2.0
2	25	.1	18	22	.2
3	51	1.3	19	25	1.8
4	27	1.0	20	52	.2
Total	153	2.1		122	4.2
		Low Fertile Holsteins			
5	29	1.7	21	11	1.6
6	29	.9	22	16	3.8
7	25	1.4	23	43	1.4
8	5	2.0	24	19	.5
Total	88	6.0		89	7.3

TABLE XXV (continued)

EJACULATION FREQUENCIES AND MEAN MOUNTING TIMES FOR THE INDIVIDUAL BULLS

EXERCISE			NON-EXERCISE		
No.	Ejaculations Number	Time Min.	No.	Ejaculations Number	Time Min.
High Fertile Guernseys					
9	48	.0	25	25	.8
10	24	.1	26	29	1.5
11	29	1.2	27	19	.2
12	17	1.9	28	23	.0
Total	118	3.2		96	2.5
Low Fertile Guernseys					
13	5	.0	29	29	.6
14	18	.3	30	16	.1
15	10	4.1	31	18	1.5
16	24	1.3	32	11	.6
Total	57	5.7		74	2.8
Grand Tot.	416	17.0		381	16.8
Av. Sec.		2.4			2.6

DISCUSSION

From the days of our forefathers, dairymen have been particularly concerned about the role of exercise in management of bulls, and its relationship to maintainance and/or its improvement of reproductive efficiency.

A few previous workers have reported varying results. Bartlett and Perry(19) found that regular systematic exercise of three bulls for one month gave a 51 percent increase in volume. Woodward (160) reported that the exercising of bulls for 30 minutes a day increased the motility (with exercise 60 to 100 percent of the spermatozoa were motile, while with no exercise there was only 5 to 45 percent motility) and longevity of the spermatozoa and greatly diminished the time of service with a slow bull. Hamilton and Symington (65) observed that daily exercise increased the volume, sperm concentration and motility of the ejaculate. Lepard and associates (87) noted no significant effect of exercise on the morphology, concentration and viability of the spermatozoa. The volume of the semen showed a slight but insignificant increase in bulls receiving exercise. They reported that the conception rate of the exercised and non-exercised bulls followed the same general tend. These results only show the value of exercise on the amount and quality of semen for a sixteen weeks' trial.

It is not unlikely that some of this variability of previous results stems from a multiplicity of factors affecting the potential fertility and characteristics of semen. No doubt, the method of selecting the animals and the period of time the bulls were exercised was a major problem. Bowling (28) and Dawson (42) have indicated that age and health of the bulls may have an effect upon breeding efficiency. That the frequency of ejaculation may effect the breeding abilities and fertility of the bull has been shown by Weatherby and associates (149) and Lasley and Bogart (86). The season of the year effects semen quality and fertility of bulls as shown by Lewis (88) Mercier and Salisbury (96). The data showing the effect of nutrition upon reproduction is limited and somewhat contradictory, but several investigators, Erb and co-workers (50) and Hodgson and associates (72) have suggested that certain nutrients are concerned in normal breeding efficiency.

It is commonly thought by many dairymen that exercise is an important factor in maintaining the production of high quality semen in bulls over a long period. They believe that exercise stimulates the appetite, influences the degree of fleshing, and may be responsible for normal blood circulation.

It is appreciated that the percentage of 60-90 day non-returns is not as accurate in measuring bull fertility as 90-120 day non-returns, however, the trends observed would have probably remained the same.

As shown by the analysis of variance there were no significant differences in the percentage of non-returns between the exercised and non-exercised bulls during the course of this experiment. These results are in accord with those reported by Lepard and associates (87).

Highly significant differences between breeds and between months were found in the bulls of high fertility, whereas, the low fertile bulls showed no significant differences between breeds and between months. The interaction, breeds X exercise, was significant in the high fertile bulls which indicates that the high fertile Holsteins did not respond to exercise the same way as the high fertile Guernsey bulls.

Highly significant differences in fertility of individual bulls were found during the experimental period. This was more pronounced in the Guernseys than the Holsteins. These data are similar to those reported by Lewis (88) and Salisbury (119). The monthly variation in the percentage of non-returns was significant between some months but not for all months. The Holstein bulls showed a consistently higher fertility than the Guernseys.

Statistical analysis showed that there was no significance between the percentage of non-returns for the six months that the bulls were exercised, the six months prior to the experimental period and the corresponding six months, one year before the exercised period.

Bowling and associates (80) and Dawson (42) have indicated that the age of the bull may have an effect upon the breeding efficiency. The results of this study indicate that young bulls have a higher percentage of non-returns than older bulls.

The findings of this study confirm the earlier reports of Erb and associates (51) and Anderson (13) that the higher the percentage of non-returns the lower the percentage of abnormal spermatozoa. In view of the difference in fertility level observed between the Holsteins and Guernseys, it was interesting that the semen of the Guernsey bulls contained more abnormal spermatozoa than the Holsteins. This may be one of the factors responsible for the lower fertility observed in the Guernsey bulls.

The bulls on exercise produced semen with less volume, with fewer total spermatozoa, with lower initial motility, and less abnormal spermatozoa than the non-exercised bulls, but the differences were not significant. These data confirm the work of Lepard and associates (87). The findings of this study disagree with those of Woodward (160) who found that exercise greatly diminished the time of service with a slow bull. The results obtained here showed that libido was not affected by exercise.

These data indicate that the management practice, exercise, did not have an effect upon the body weights, appetites and general activities of dairy bulls for a six months period. If the aged theory, that exercise has an effect upon the breeding efficiency is to be clarified, an experiment involving a large number of young and old bulls will have to be planned for a long period.

SUMMARY

1. Thirty-two Holstein and Guernsey bulls, owned by the Michigan Artificial Breeders' Association, varying from four to 15 years of age, were divided into eight groups according to their breed and to high or low fertility. All animals were fed and managed similarly except for exercise. During the first 35 days one-half of the bulls were exercised for 15 minutes a day on a mechanical exerciser with a speed of 1.24 mi. per hr. The amount of daily exercise was increased to 23 minutes at the same rate of speed for the following 14 days. For the remaining 119 days of the experiment these same bulls were exercised daily for 30 minutes at the rate mentioned above on the mechanical exerciser. The other 16 bulls were left in their stalls during the six months period. 452 semen collections and 33,292 breeding records were tabulated in this study.
2. No significant differences in the percentage of non-returns were found between the exercised and non-exercised bulls.
3. Highly significant differences in percentage of non-returns were found for individual bulls during the experimental period.
4. Highly significant differences in the percentage of non-returns between breeds and between months were observed in the high fertile bulls.
5. No significant differences were found in the percentage

of non-returns between breeds and between months in the low fertile bulls.

6. A breed difference in response to exercise was significant in the high fertile bulls.
7. The monthly variation in the percentage of non-returns was significant between some months, but not for all months.
8. The Holstein bulls showed a consistently higher fertility than the Guernseys.
9. No significant differences were found in the percentage of non-returns for the six months that the bulls were exercised, the six months prior to the experimental period, and the corresponding six months, one year before the exercised period. This was also true with the non-exercised bulls.
10. A highly significant correlation (-0.539) was obtained between the age and the percentage of non-returns.
11. A correlation of -0.536, which is highly significant, was obtained between the percentage of non-returns and the percentage of abnormal spermatozoa.
12. No significant differences were noted between the exercised and non-exercised bulls in the semen characteristics (volume, total spermatozoa, initial motility and abnormal spermatozoa).
13. A breed difference in percentage of abnormal spermatozoa was evident, but not significant.
14. Libido was not affected by exercise.

15. The body weights between the exercised and non-exercised bulls show little variation.

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