FEBRUARY, 1941 RESEARCH BULLETIN 326

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION

M. F. MILLER, *Director*

Variations in Dairy Bull Semen With Respect to Its Use in Artificial Insemination

H. A. HERMAN AND ERIC W. SWANSON

(Publication Authorized February 11, 1941)

COLUMBIA, MISSOURI

TABLE OF CONTENTS

Variations in Dairy Bull Semen With Respect to Its Use in Artificial Insemination

H. A. Herman and Eric W. Swanson

INTRODUCTION

The widespread use of artificial insemination for the breeding of dairy cattle has greatly increased the breeding responsibility of many bulls. Failure of many bulls to consistently breed efficiently has long been recognized and has often been traced to the production of semen abnormal in characteristics and of low vitality. The question arises as to whether or not such infertile semen can be detected, predicted, or remedied. This point is especially important in artificial insemination associations where it is very necessary that every sample of semen be effective. Apparently fertile bulls, in natural service, sometimes fail to impregnate cows with good breeding histories. There are evidences of normal variation of semen quality with even the most fertile bulls. The knowledge of such variations and the ability to detect inferior samples of semen before their use is a matter of prime importance in artificial insemination.

This study was undertaken in order to determine the quality of semen produced by bulls whose breeding efficiency was known, to compare the actual efficiency of semen used in artificial insemination with the results of physical and chemical examinations of the sample, and to determine, if possible, the best measure for evaluation of semen to be used for artificial insemination of dairy cows under practical field conditions.

REVIEW OF LITERATURE

The earlier researches regarding the reproductive capacity of the dairy bull were largely concerned with the pathology of the reproductive organs and variable breeding efficiency exhibited in dairy cattle bred by natural mating. The recent emphasis on artificial breeding, however, is stimulating much research concerning the normal, chemical and physiological characteristics of dairy bull semen with the object of formulating suitable diluents and to perfect methods of storage of the semen.

Physical, Chemical and Physiological Properties of Bull Semen

Physical Characteristics of Semen.—The normal ejaculate of the bull was described by Lagerlof (1934) and Kuhne (1936) as an opaque, white, or yellow-white fluid with milky or creamy appearance. The stronger the spermatozoa concentration the whiter the fluid. Watery-like semen, sometimes of light yellow color, contained few spermatozoa.

Gilman (1932) stated that thin, watery semen was caused by hypersecretion of one or all of the accessory sex glands and that hyposecretion of these glands resulted in semen that was too viscid. Sterile semen, or semen with spermatozoa of lowered vitality, was characterized by rapid precipitation of "Boettcherchen" crystals and a smaller amount of sediment than was observed in fertile semen.

A small ejaculate of high viscosity was also ascribed by Williams (1920) to unhealthy seminal vesicles or those with the excretory ducts occluded.

Volume.—The volume of the ejaculate of the bulls seems to vary within wide limits. Thus, Lagerhof (1934) and McKenzie (1939) listed an average of 3 cc. with a range of 2 to 8 cc. Herman and Ragsdale (1939) reported average values of 4.2 cc., ranging from 2.1 to 6.1 cc., for 161 samples collected from 12 bulls. Williams (1939) suggested that mature bulls produced a much greater volume of semen per ejaculate than did young bulls. The method of collection may also influence the volume. Davis (1939), Miller (1934), and Kuhne (1936) reported much larger volumes, as high as 23 cc. for a single ejaculate, in collection by rectal massage.

Concentration of Spermatozoa.—The concentration of spermatozoa in the semen of bulls with good fertility was reported by Lagerlöf (1934) as usually between 300,000 and 1,200,000 per cu. mm., or an average of about 820,000. He considered bulls in which the concentration was below 250,000 per cu. mm. to be of poor fertility. McKenzie (1939) listed the most common concentration of spermatozoa in bull semen to be 800,000 per cu. mm., with a satisfactory range of 300,000 to 2,000,000. The semen of bulls studied by Herman and Ragsdale (1939) ranged from 421,000 to 1,950,000 spermatozoa per cu. mm.

Chemical Composition.—Gilman (1922) stated that bull semen was weakly alkaline in reaction and that it contained 80 to 90 per cent water. The solid matter was 40 per cent ash, three-fourths of which was calcium phosphate. He often observed the presence in semen of lecithin bodies and the precipitation of characteristic phosphoric acid salts when the semen was cold.

Nesmejanova (1938) analyzed ram and bull sperm serum and the secretions of the ampulla, epididymis and seminal vesicles for K, Na, and Ca content. The amounts found are listed in the table below.

Mg. per cent of K, Na, and Ca in Seminal Secretions of the Bull

	Epididymis	Ampulla	Seminal Vesicles	Sperm Serum
K	278.4	320.8	326.1	227.8
Na	115.0	165.4	135.8	277.8
Ca	81.7	26.7	32.6	33.9

It was observed that values for K and Ca were much higher and those for Na much lower in semen serum than in blood serum.

Hydrogen-ion Concentration.—The pH of semen was found by Shergin (1935) usually to be lower than the pH of the blood of a given species. Although the semen of the majority of animals is alkaline, that of the bull had an average pH of 6.74. Schneerson (1936) emphasized that the pH of semen for use in artificial insemination was an important factor and that the optimum was pH 5.8 to 6.6. He found that values for number of spermatozoa, survival of spermatozoa, at room temperature, and their resistance to NaCl solution all declined as the pH of the semen rose.

The optimum pH of bull semen to be used in artificial insemination was reported by Kuhne (1936) to be below 6.60. He suggested alkaline semen to be an indication of urine contamination, and observed that semen of very high concentration was below pH 6, while thin or discolored semen was above pH 6.7 and often above pH 7.

The method of collection of the semen affects its pH. Davis (1938) and Kuhne (1936) reported that bull semen collected by the massage method was ordinarily alkaline, while that collected with the artificial vagina was usually acid.

The change in pH of bull semen during storage has been attributed to the production of lactic acid from glycolysis. Thus, Bernstein and Slovochotov (1933) reported that fresh ejaculates of bull and human semen contained about 40 to 50 mg. per cent of lactic acid and that the content of lactic acid increased as the semen was kept outside the body. The motility of the spermatozoa also decreased but complete cessation of motility occurred at different levels; hence it was not thought to be due to lactic acid alone. The lactic acid content of fresh semen was given by Shergin (1937) as 63 mg. per cent.

Metabolism and Spermatozoan Survival.—In his study of the glucose metabolism of semen, Bernstein (1933a) found bull semen quite high in glucose content, 300 mg. per cent, as compared with 116 and 82 mg. per cent respectively in dog and stallion semen. The glucose was found in both the seminal fluid and the spermatozoa. It remained relatively constant in the spermatozoa, but varied in the seminal fluid. During storage the glucose content of the fluid decreased but that of the spermatozoa remained the same. Fluid from which the spermatozoa had been centrifuged, however, had a very small decrease in glucose; hence Bernstein concluded that the spermatozoa metabolize the glucose of the fluid. He also reported (1933b) that low temperatures (12°C.) caused a reduction of glycolysis which paralleled the loss of motility. Immotile spermatozoa were capable of glycolysis, however.

Comstock (1939) used the rate of glycolysis of semen as a measure of semen quality since it was closely correlated with survival of motility during storage and observed significant differences independent of motility and cell number between the glycolytic powers of fresh semen from different males. Glycolysis decreased as the sperm aged.

Many experiments have been conducted to find the source of the energy used by spermatozoa. Ivanov (1931) showed that oxygen was not necessary for the motility of spermatozoa but he believed that it was necessary, as in muscles, for the elimination of waste products. He concluded that although the energy of the spermatozoa does not form itself at the expense of the oxidating process, the latter is helpful in prolonging motility for a longer period than is possible anaerobically.

Because motility of bull and boar spermatozoa was maintained for several hours in the presence of monohaloacetic acids, Ivanov (1935 and 1936) concluded that motility did not depend on glycolysis. What the reserve intracellular resources of the spermatozoa are remains unknown. Ivanov (1936) reported that although spermatozoa remain motile for a long time in the absence of glucose with an R.Q. of 0.78 in the presence of glucose their R.Q. becomes 1.0.

Shergin (1939) found that the respiration of spermatozoa of farm animals was much less than that of other body tissues—only 29 cm. compared to 100 cm. for brain tissue. He believed that respiration was of greater value for maintaining life and activity of the spermatozoa than glycolysis because it liberated 10 to 30 times as much energy as the latter. MacLeod (1939), on the other hand, reported that the motility of human spermatozoa was maintained much better anaerobically than in air or pure oxygen.

Windstosser (1935), in measuring respiration of spermatozoa secured from the epididymis, noted no definite correlation between respiratory rate and motility or pH.

Chemical and Physical Factors Affecting Sperm Survival.—Dubincik (1934) reported that motility of spermatozoa in hypotonic solutions was reduced and often ceased. In hypertonic solutions motility ceased immediately.

Lewis (1911) stressed the fact that chemicals, water, and excessive exposure to rubber or sunlight are injurious to spermatozoa.

Strocenko (1934) placed spermatozoa under a bell jar with eighteen different drugs and determined the killing time of each. All of the drugs tried proved noxious, with NH_3 and HNO_3 causing instantaneous death.

Some drugs, however, may be used to reactivate spermatozoa which have lost their motility in storage. Steensma (1938) found that a strychnine solution mixed 1:1 with immotile bull and ram semen caused motility to be resumed for 24 to 36 hours. Bretschneider (1936) reported that tutocaine reactivated spermatozoa which had been removed from the testes or epididmes of the bull but not those in a normal ejaculate.

Weber (1936) found that when motility of bull semen had ceased in storage it could be restored with isotonic salt or sugar solution. Lactic acid was used by Milovanov and Khabibulin (1933) to activate semen in which anabiosis had been induced by the removal of oxygen and the prevention of glycolysis.

Steensma (1938) reported that when the pH of bull semen was artificially lowered before the semen was centrifuged, the time of survival was increased and Dubincik (1934) found that the motility of spermatozoa of various farm animals ceased at pH 4.2 but after a shift to alkalinity it was resumed again. He reported that an irreversible immotility occurred at pH 3.5 to 3.4.

Bernstein and Petropavlovsky (1937) found that mammalian and bird spermatozoa were highly resistant to alcohol. In low concentrations of various alcohols, spermatozoan vitality was maintained even longer than in seminal fluid or salt solution.

Bernstein and Lasarev (1933) reported that normal blood serum of cows and bulls agglutinate the spermatozoa of the same species. Spermatozoa of the bull were even agglutinated by serum of the same animal. The process, however, was reversible. Disagglutination occurred in about 24 hours and the spermatozoa regained motility. Serum inactivated by heat was favorable to the retention of activity of the spermatozoa. Williams (1920) stated that the bleeding of heifers at service might account for some temporary sterility.

Vigorous shaking of bull semen for three minutes destroyed motility, according to Bretschneider (1936). He also reported that spermatozoa from the normal ejaculate showed more resistance to shaking than did those secured from the testicles or epididymis.

Studies on Storage and Dilution of Semen for Artificial Insemination

As artificial insemination of dairy cattle became practical on a large scale the necessity for dilution and successful storage of semen in order to inseminate a larger number of females from a single ejaculate became apparent.

Dilution, it has been claimed, may also aid in prolonging the storage of semen by furnishing a favorable environment for the spermatozoa. Miller (1934) gives the purposes of diluting semen as follows: (1) to increase volume, (2) to improve the surrounding media by furnishing a supply of glucose, buffering against lactic acid, decreasing the electrolyte concentration to approximately the same as that in the epididymis, and strengthening the lipoid capsule by replacing the chlorides with sulfates and tartrates.

The Russian investigators have formulated many diluents for use with bull semen. In a comprehensive review of the diluents proposed, Walton (1933) listed formulae and directions for the use of seven diluents taken mainly from the report of Milovanov and Selivanova (1932). These diluents were of the glucose-type, two of which contained lecithin and one of which contained peptone. The SGC-2 diluent which was also developed by Milovanov and contains glucose, Na_2SO_4, and peptone has been reported to be in wide use in cooperative artificial breeding associations in this country and Denmark by Perry and Bartlett (1939), Herman and Ragsdale (1939), and Kingman (1936).

Some of the earliest diluents employed with semen were isotonic solutions of salts and sugars. Milovanov (1933) reported, after three years work with diluters for the sperm of livestock, that ordinary physiological media such as Ringer's, Locke's, Tyrode's, etc. were entirely unsuitable as diluters. Bederke (1933) found that physiological solutions greatly reduced the survival time of dog sperm. Other investigators (Kust, 1936, and Steensma, 1938) have reported, on the other hand, that physiological salt solution was as satisfactory a diluent as measured by motility as the more complicated Russian diluents. Bernstein (1933) states nutrient substances in solution do not cause an increase in time of survival of spermatozoa, and that spermatozoa have a very high resistance to pure solutions containing a single neutral of salt or sugar.

Phillips (1939) and Lardy and Phillips (1939) proposed the use of a diluent containing fresh egg yolk plus a phosphate buffer mixed 1:1 and adjusted to pH 6.7 to 6.8 for dairy bull semen preservation. Pregnancies were secured with semen stored up to 100 hours.

Although diluters have been used widely in attempting to increase the survival time of stored semen, their value in this respect is doubtful, according to many investigators. Hatziolos (1937) reported that Ringer's, Tyrode's Russian formulae, etc. failed to prolong survival of stored semen. Kufarev (1935), Weber (1936) and Komarov and Gladcinova (1937) also recommended the use of undiluted bull semen for storage and for insemination, also, where possible. Petrov and Schneerson (1933) recommended that for storage of bull semen it should be kept undiluted under vaseline oil for 24 hours to produce rapid anabiosis, then diluted not more than eight times to neutralize metabolic products. Vorobjev and Schneerson (1933) found that sperm kept up to 30 hours undiluted at 8° to 12°C. did not lessen the percentage of pregnancies. In a review of results of artificial insemination of sheep and cattle on Russian collective farms, Kersin (1937) stated that results definitely demonstrated that microdoses of undiluted semen were the most efficacious.

The Amount of Semen Necessary for Insemination.—Kufarev (1935) listed the optimum doses of undiluted bull semen to be 0.5 cc., containing an average of 300,000,000 to 400,000,000 spermatozoa. Over two years' time, 65 to 72 per cent of such insemination had resulted in pregnancy.

Kozlova (1937) likewise found no significant difference in the calving percentage of cows inseminated with 0.1, 0.2, or 0.5 cc. of undiluted semen high in spermatozoa and cows served naturally. Herman (1939) reporting the results of artificial breeding in Missouri dairy herds, stated that the services required per conception were practically the same for semen doses of 0.4 to 6.0 cc.

Effect of Fluids of Reproductive Tract on Spermatozoa.—Some natural secretions have been used in addition to the formulated solutions as diluents for bull semen with varying results.

Milovanov (1933) stated that the seminal fluid and the secretions of the accessory sex glands were entirely unsuitable as diluters and Donham and Simms (1931) reported that seminal vesicle fluid retarded or stopped motility of the spermatozoa.

Kingman (1936) concluded from the use of cattle mucous of estrum that the number of cows settled and the motility and longevity of the spermatozoa appeared the same as in the Russian diluters. Rumjancev and Flegmatov (1935) found motility of bull spermatozoa maintained for nearly four days in the uterine horns of cows at 10° to 15°C.

The desirable environment for spermatozoa storage which exists in the epididymis was demonstrated by Kirillov and Morozov (1936). After ligating the left epididymis of a two year old bull in the region of the deferent ducts, the right testis and epididymis were removed and the bull allowed to serve at intervals. Ejaculates obtained up to 32 days after the operation contained many spermatozoa of which about 70 per cent showed progressive motility.

Effect of Temperature on Spermatozoa Survival.—Although the natural temperature for the spermatozoa after ejaculation is body temperature of the cow (about 39°C.) it was found early that semen kept at this temperature rapidly loses vitality. Weber (1939), from a study of 81 ejaculates from 30 bulls, reported that low temperatures reduced motility of bull sperm but prolonged its duration, while room or body temperature had the reverse effect. He recommended 8°C. as the best storage temperature. Other investigators have observed the effect of body temperature in shortening the life of spermatozoa through stimulation to motility. Walton (1933), in a review, stated that the reduction of temperature is perhaps the most important factor for maintaining fertility of the spermatozoa outside the body.

Komarov and Gladcinova (1937) found that sperm at 0° to 5°C. deteriorated within 12 hours and that optimum survival was attained when semen was stored at 8° to 12°C., although fertilizing ability of semen kept at 15° to 25°C. for short periods was somewhat higher.

Birillo and Puhaljskii (1936) reported that bull and ram sperm were very sensitive to the rate of cooling. Cooling semen from 40° to 0°C. in 30 to 60 minutes resulted in a rapid decrease of resistance and loss of ability be reactivated. Gradual cooling for three to four hours (3° every 15 minutes) resulted in semen that was readily reactivated. Gladcinova (1937) likewise found that when cooling from body temperatures to 5° or 10°C. is rapid, activity was decreased and was not restored on reheating. He suggested that collection and insemination should be carried out at 20 to 25° C. and that cooling should be gradual.

Miller (1934) recommended that semen to be kept more than one hour should be gradually cooled to 45°F. For this he stated that a normally operating household refrigerator will cool gradually enough and normal allowance to return to room temperature will warm the semen gradually enough.

Davis (1938) recommended an hour's time to lower the freshly collected semen to a temperature of 50°F. After using storage temperatures of 35°, 42°, and 50°F., he considered 50°F the highest temperature that should be used for successful storage.

When inseminations are to be made 2 to 10 hours after collection of the semen, Herman and Ragsdale (1939) recommended that the semen be cooled gradually by insulating it with several thicknesses of paper around the storage tube and placing it in a refrigerator or thermos bottle at 45° to 50°F. They cautioned against frequent warming or cooling of the semen or rapid changes in temperature.

Steensma (1938) however was not able to confirm the observations of previous workers that bull semen must be cooled slowly. He found that cooling to 5°C. within 15 minutes gave better motility ratings than semen cooled slowly.

Some Additional Factors Concerned with Storage of Dairy Bull Spermatozoa.—Other factors may be important in the storage of bull semen. The use of paraffin oil placed atop the semen to exclude the air has been advocated by several investigators, including Vorobjev and Schneerson (1933), Petrov and Schneerson (1933), Winters and associates (1938). The desirability of using paraffin oil has not been proved, however. Steensma (1938) found that it gave no beneficial effect and Shergin (1939) found that spermatozoa survived better with free access to oxygen than with aeration restricted. Davis (1938) reported that the method of collection influenced the ability of sperm to withstand storage. He found that semen collected with the artificial vagina survived better than that collected by other methods.

Examinations of the Semen and Their Value in Forecasting Fertility

It is only within the last 20 or 30 years that the semen of the bull has been recognized as an important factor in bovine sterility. Previous to that time, according to Lagerlöf (1934), the leading workers in the field neglected the examination of the bull and his semen and laid all the fault to the cow. W. L. Williams, in his first book on the diseases of the genital organs of animals (1921), focused the attention more on the bull and his semen.

Previous to this time about the only method for evaluating the semen of the male had been motility. In his work with human spermatozoa, Reynolds (1916) described three types of motion: (1) progressive vibratile, (2) undulatory tactile, and (3) stationary bunting. He emphasized that just plain motion was not enough and that vigor of motion as seen in his first type was necessary for fertile semen. Gilman (1922) and 1923) supported Reynold's conclusions as to the value of the motility of the spermatozoa and stated that they applied to bull semen just as surely as to human semen.

Donham, Simms, and Shaw (1931) examined bull semen for motility after natural mating and concluded that there was a definite relation between the microscopic picture (motility) and the percentage of conceptions obtained.

W. W. Williams (1920a) (1920b) observed that varying degrees of sterility in the bull were associated with either an arrest in development of the spermatozoa or their disintegration after reaching maturity, and he believed that this destruction or devitalization was due to toxic substances derived from bacterial infection. He stated that disturbances of the testicular germinal epithelium and sperm vitality occur frequently in the absence of apparent orchitis. He found the most serious defect of the spermatozoa to be a lessened size or development of nuclear elements. Williams and Savage (1925) reported on semen examination of 237 bulls, 198 of which were accompanied by service records. The semen was collected and examined according to William's (1920a) method. Primordial cells were found in only

3 per cent of the cases examined and most of these were badly diseased bulls. Their presence to the extent of 1 per cent indicated a grave condition. The presence of epithelial cells, pus, bacteria, extruded cytoplasm, and blood was considered abnormal. They considered the morphology of the spermatozoa heads as the greatest single source of information as to the fitness of the cells for reproduction. None of the bulls examined that eliminated more than 16.6 per cent abnormal heads had a good breeding record and 27 bulls with poor breeding records averaged 50.1 per cent abnormal heads. The breeding inefficiency was not always detectable by spermatozoal abnormalities, however. Bulls of poor fertility had either a coefficient of variation above 4.0, or a significant skewness of the frequency curve, trailing off to more undersized heads.

These early observations of W. W. Williams and Savage have since been confirmed on observations of the semen of many different species. Thus Moench (1929) and Moench and Holt (1931) and Mason (1929) found that morphological variations of human spermatozoa were the best source of information as to the reproductive fitness of the individual that produced them. Biometrical methods were also found valuable for measuring the fertility of stallions by Savage, Williams, and Fowler (1930). McKenzie and Phillips (1934) concluded from an examination of fifteen rams in their breeding season that the morphology of the spermatozoa reveals within reasonable limits the degree of fertility of the ram. McKenzie and Berliner (1937) found that the percentage of abnormal spermatozoa rose in rams out of their breeding season. McKenzie and Phillips (1933) and Phillips (1935) stated that semen of highly fertile boars should not exceed 104 to 172 abnormal spermatozoa per 1000 spermatozoa.

Lagerlöf (1934 and 1935) concluded from an examination of 100 bulls with good fertility and 150 bulls with lessened or arrested fertility that one can judge the fertility of the bull by examination of the semen in most cases. The semen of bulls with good fertility did not have over 18 per cent abnormal spermatozoa (average was 10 to 12 per cent) while with the bulls of poor fertility the percentage of abnormal spermatozoa was 20 per cent or over.

Addis (1937) reported that three sterile bulls he examined were normal as far as volume, appearance, concentration, and motility of the semen were concerned but that 40 to 50 per cent of the spermatozoa were abnormal, and Generales (1938) stated that good fertility could be expected if the percentage of abnormal spermatozoa was not over 25. When it reached 60 per cent, the animal was sterile. Similar results are reported by Sciuchetti (1938).

In discussing methods of determining sexual fitness of the sire, W. L. Williams (1932) states that the most accurate one, next to the breeding record, is a microscopical examination of the semen. The most valuable examination was a morphological study of the fixed and stained spermatozoa. He considered motility of itself unreliable, but duration of motility was important in evaluating the sire.

Davis (1938) listed five methods of evaluating bovine semen as follows: (1) concentration, (2) morphology of spermatozoa, (3) activity of the spermatozoa, (4) pH of the semen, and (5) resistance of the spermatozoa to NaCl. He believed the third method to be one of the best single evidences of viability. Others have also stressed the importance of good motility. Donham, Simms and Shaw (1931) found that they could divide bulls into two groups according to the motility of their spermatozoa. Seven bulls with semen of normal motility settled 75 per cent of their cows served while ten with semen below normal motility settled only 57 per cent of their cows.

Kuhne (1936) observed that dense semen improved in motility whereas thin semen did not when phosphate diluent was added. He stated that semen which would not improve in motility when diluted was not well suited for artificial insemination.

Lagerlöf (1934) found that bulls with spermatozoa of poor motility were usually of poor fertility, though some bulls produced normal appearing semen yet were sterile. These bulls, however, had a high abnormality count, a large coefficient of variation of spermatozoan head length, or a large number of immature spermatozoa. Lagerlof considered the position of the protoplasmic drop on spermatozoa a factor in fertility. If the drop were around the neck, the spermatozoa were considered unripe, while if it were at the lower end of the middle piece, or absent, the spermatozoa were considered mature. He found that sexually healthy bulls had few unripe spermatozoa but bulls with lessened fertility produced many unripe spermatozoa, about 16% were found even in the first ejaculate. The unripe spermatozoa observed were motile and the non-motile spermatozoa did not have a protoplasmic drop.

Other methods of evaluating bull semen have also been proposed. Walton and Edwards (1938) found that the initial respiration rate of semen from 13 bulls was proportional to their breeding efficiency.

Comstock (1939) and Comstock and Green (1939) working with ram sperm, found a high correlation, r=0.8766, between the rate of glycolysis of fresh sperm and its persistence of motility during storage. At times the rate of glycolysis was higher with samples of high percentage of abnormal spermatozoa than in samples low in abnormals. Hence both determinations need to be made. They also found that the semen of highest quality contained the highest percentage of cells with a small hyaline vesicle at the anterior border of the cell. There was a negative correlation between the number of cells with vesicles and the number of abnormal heads and a positive correlation with the rate of glycolysis.

In applying any method or combination of methods of semen examination to determine the fertility of an animal, it must be kept in mind that variations occur in different ejaculates from the same bull. Thus, Sciuchetti (1938) recommends that animals should be tested by examining two samples taken at an interval of two to three hours before any conclusions concerning their breeding ability may be drawn, and Lagerlöf (1934) suggests that it is wise to follow the seminal picture of the bull for two or three months where this is possible.

Quality of Semen from Different Ejaculates and Rate of Service.—Especially after a prolonged sexual rest of the male are the properties of his semen apt to be distinctly abnormal according to Lagerlöf (1934), McKenzie and Phillips (1934), Polowzow (1928), Webster (1932), and Davis and Williams (1939).

The quality of the semen may also vary with the number of the ejaculate. Kingman (1936) and Lagerlöf stated that there was clinical evidence to indicate that the second and third ejaculates have more virile spermatozoa than have the first ones. Davis and Williams (1939) made an extensive study of the semen quality of the first, second and third ejaculates of eleven bulls. They found that the volume was relatively constant with a bit more being received at the second ejaculate. The concentration was found to decrease on the average with each subsequent ejaculate. The quality of the second ejaculate as measured by motility averaged slightly better than that of the first ejaculate and there was little difference observed between the second and third ejaculates. When bulls were being used frequently for service there appeared to be little difference between the first and second ejaculate.

Kirillov and Morozov (1933) in attempting to determine the maximum sexual activity of the bull mated one bull 24 times in 27 hours. The degree of concentration of spermatozoa was maintained at the same level up to the 15th mating when it was followed by a sharp fall. After a rest the concentration was again high and persisted so until the twenty-third mating. Nine other bulls allowed to mate 11 to 12 times daily gave similar results. They concluded that it was practically impossible to exhaust the bull. In another report Kirillov (1934) compared bulls mated once in two days, twice a day, and four times a day. The latter group seemed to show a decrease in sexual potency. He concluded that two matings per day was probably the optimum number for the bull.

Lagerlöf (1934) found that bulls differed in the number of ejaculates per day they could give without causing a decrease in concentration of spermatozoa. He stated that the sex strain which one bull can bear without harmful result may harm another bull. This fact was also stressed by Williams, W. L. (1939).

Effect of Management of Herd Sire on Semen Characteristics

Comparatively little attention has been paid to the effect that certain management practices may have on the semen of the bull and, consequently, his fertility.

The effect of exercise was shown by Bartlett and Perry (1939) with the bulls in a cooperative breeding project. Regular daily exercising of the bulls, one hour at 2¼ miles per hour, increased the semen output 51 per cent. Kelley (1940) reports that work at the Washington Agricultural Experiment Station showed that exercise at 2½ miles per hour for 30 minutes per day increased greatly the quality of the semen produced. Before exercise, motility of the spermatozoa was 5 to 45 per cent while after exercise it was 60 to 100 per cent. Without exercise, motion of the spermatozoa had ceased at 3 to 24 hours; but with exercise, motility was maintained for 22 to 39 hours.

The effect of different feeds on semen production and quality from six stud bulls was reported by Smirnov-Ugrjumov and Laptev (1938). They found that rations containing concentrates of higher protein content but less total food units were more favorable for sperm production than rations containing oats. Proteins, especially proteins of blood meal and skimmilk, caused a marked improvement in sperm resistance and concentration; but this effect was not manifested for 17 to 37 days. Fermented feeds, beets, and green grass caused an immediate favorable response, but green grass without concentrates resulted in an immediate decrease in sexual activity and amount of ejaculate. It is also recognized among experienced dairy cattle breeders that feeding large amounts of silage is detrimental to fertility in the bull.

A summary of the literature reveals many reports to the effect that bull semen may vary in its characteristics and fertility. The cause of these variations has not been specifically determined. Because of these variations investigators of bull fertility have not been able to ascribe to any one examination the power of evaluating the semen. Most workers on this subject have concluded that a consideration of all the properties of semen (motility, appearance, concentration, duration of motility, pathological spermatozoa, etc.) is necessary to evaluate the fertility of the semen or the bull that produces it. Certain of these examinations, such as the head length variation, per cent of abnormal spermatozoa, and duration of motility, seem to be of more significance than others, yet the use of any one alone has not been advocated. The investigation of energy requirements and storage of the spermatozoa has presented many conflicting ideas. Results of attempts to furnish nutrients for the spermatozoa have been inconclusive and seem to have failed. The latest method for attacking the problem has been mainly to conserve energy by preventing motility through refrigeration and to improve the surrounding media in some way—mainly by dilution with various diluents containing buffers. When these problems are better understood, or solved, artificial insemination and research in reproduction alike will have gained.

MATERIALS AND METHODS

Bulls Used

Semen from 55 dairy bulls totaling 342 ejaculates was examined in this study. All bulls included were in regular service in various dairy herds throughout the state of Missouri. They ranged in age from one and one-half years to thirteen years. All were purebred bulls, consisting of 14 Jerseys, 11 Guernseys, and 30 Holsteins, and they appeared to be in good physical condition at the time semen collections were made.

Breeding Records of the Bulls

Complete service records were available for 40 of the bulls. These records included matings to all cows known to be regular breeders along with the number of services resulting in conception. From these records the number of services required for conception was computed for each bull. The number of services for each cow and the classification of cows with poor breeding histories were made by the individual breeders from their herd records. Pregnancies were determined by examination in many instances and also by failure of the cows to come in heat following service. The period of time over which records were included varied with the different bulls ranging from 3 to 15 months. For many of the bulls records for the year ending April 1, 1940, were used. A few of the bulls, however, had been used only a few months. In these cases all of their records were used. For 15 of the bulls complete breeding records were not available, however, reliable information as to the general breeding efficiency of 11 was secured. These bulls were classified as to degree of fertility the same as were those for which full records were available.

Semen Collection

All semen collections were made by use of the artificial vagina, as decribed by Herman and Ragsdale (1939).

Immediately after collection of the ejaculate the collection tube was stoppered with a clean, freshly paraffined cork. If the ejaculate were two cc. or more in volume it was divided. One to two cc. were kept for initial examinations and the rest of the ejaculate was transferred to 4 to 6 cc. sterile glass vials. These vials were also stoppered with clean, freshly paraffined corks and stored as explained below.

Semen was collected from some of the bulls as regularly as twice a week for several months. From the others collections were made as the opportunity presented itself. Only one collection was made from several of the bulls, but two or more collections were made from a majority of them. All collections were made during the time interval from which the bulls' breeding efficiencies were determined.

Semen Storage

The semen from which the initial examinations were made was kept in the tube in which it was collected, insulated, and placed next to the body of the worker to prevent cooling until it could be examined in the laboratory. When collections from several bulls were made at the same time, all collecting was finished before any examination or dividing of semen was done. For this reason samples were not examined for a half-hour after collection. The vials of semen were wrapped with several thicknesses of paper toweling, labeled, covered with a rubber finger stall, and placed in a thermos bottle of water at 40° to 50°F. where they were allowed to cool gradually to the temperature of the water.

Paraffin oil was used atop the semen in the small vials for a few of the first samples stored. It seemed to give no beneficial effect, however, when compared with semen from the same sample stored without a covering of oil; and, since the oil interfered with some of the examinations of the semen, its use was early discontinued. A sample of each ejaculate was stored undiluted in all cases where possible.

In the laboratory the small vials of semen were removed from the thermos bottle, placed in a tray filled with water at 40°F., and put into a refrigerator. The refrigerator maintained a temperature of 40°F. with a range from 36° to 45°F. The semen was stored in this manner until its vitality was such that examinations of it were discontinued. Stored semen was examined daily for motility. After the motility rating had been 1 for three days, the pH of the sample was again taken, a smear was made for morphology examination, and the sample was discarded.

Semen Examination

The semen examinations made were those which were believed to be practical for the operator of any artificial breeding association where a laboratory would be accessible. The examination of the fresh semen included the following: (1) Observations of the appearance of the semen as to viscosity, color, opaqueness, or any abnormal qualities, (2) Concentration of spermatozoa. This was determined by use of a haemacytometer and was expressed as number of spermatozoa per cubic millimeter. The diluting fluid used for counting was physiological salt solution (0.875 per cent NaCl). (3) Initial motility of the spermatozoa. For motility examinations a large drop of semen was transferred from the tube to a clean slide by means of a sterile medicine dropper. The slide and drop of semen were then placed on the microscope stage in an incubating chamber which was thermostatically controlled to a constant temperature of 100°F. After allowing two or three minutes for the drop of semen to warm, it was spread out in a thin layer so that the motility could be observed. A magnification of 250x was used. The semen was rated according to the degree of motility as follows:

0 = no motility discernable.

1 = less than 50 per cent of the spermatozoa in motion—motion mostly weak and oscillatory.

2 = more than 50 per cent of the spermatozoa in motion. The motion largely vigorous and rapid. No waves and eddies formed by the movement of the spermatozoa.

3 = from 75 to 85 per cent of the spermatozoa in motion. Motion vigorous, and waves and eddies produced by the movements of the spermatozoa, these waves moving slowly across the field.

4 = approximately 90 per cent of the spermatozoa in rapid, vigorous motion. Waves and eddies form and change with great rapidity.

5 = all of the spermatozoa in very vigorous motion. Movement and changes of the eddies extremely rapid.

It is recognized that in this rough approximation of the percentage of motility there were likely to be some errors, especially in the higher ratings, not only due to the inadequacy of the eye to note a thousand small details at once but also because many non-motile spermatozoa were pushed along by their very vigorously moving neighbors and seemed to be actually moving themselves. This method was found quite useful and satisfactory for relative methods of comparison, however. (4) pH of the semen. This was determined with a Beckman glass electrode pH meter. (5) Volume of the ejaculate and total number of spermatozoa in the ejaculate. The collection tubes were graduated so that the volume could be read directly after collection. The concentration multiplied by the volume gave the total number of spermatozoa. (6) Examination of the stained spermatozoa for morphology. The semen smear was made by either of two methods—(a) a drop of the dilution fluid made for the purpose of counting the spermatozoa in the haemacytometer was spread evenly over a clean microscopic slide and allowed to dry; or (b) a small drop of semen was placed on a clean slide and was spread out in a thin film by gently drawing another slide over the surface. After the slides were thoroughly dried they were stained five minutes with a Rose Bengal stain made as follows:

3 grams Rose Bengal
1 cc. 40% formalin
99 cc. distilled water

After staining, the slides were washed gently in distilled water, allowed to dry, and examined. Three hundred thirty-three spermatozoa from various parts of the smear were examined. The number and kinds of abnormal spermatozoa found were recorded and these figures were multiplied by three to give the number of abnormal spermatozoa per 1000. Most of the examinations were made at 1075 x magnification with a high-dry objective. A few were made with an oil immersion lens at 930 x magnification. In addition to noting the abnormal spermatozoa, observations were made as to the presence of debris, epithelial cells, etc. in the semen. In recording the abnormal spermatozoa the following system was followed. In any one microscope field the tailless spermatozoa were counted first, the coiled tailed next, then the pyriform heads, and then other abnormal forms. No spermatozoon was included in more than one group. Hence a pyriform head that was tailless or coiled tailed was not listed as pyriform, but either as tailless or coiled tailed. For this reason the values for each abnormality are not totally absolute but represent only the abnormal forms that fall in their group after other forms have been separated out. Since many of the tailless and coiled tailed spermatozoa possessed other defects, it can be seen that the values for these two forms will be high in comparison to the others. For this reason, the figure used for comparison of ejaculates was the total abnormal spermatozoa rather than any one type of abnormal spermatozoa.

Insemination of Cows

When possible, cows were inseminated with semen which either had been recently collected or which had been stored and upon which observations had been made. Cervical insemination as recommended by Herman and Ragsdale (1939) was practiced.

OBSERVATIONS AND DATA

Breeding Records of Sires Compared with Examination of Their Semen

The average of the results of all the semen examinations made for each bull was secured. This average was in most cases considered representative of the semen of the bull in question. The bulls were then divided upon the basis of their actual breeding records into four groups, namely, bulls of good fertility, bulls of questionable fertility, bulls of poor fertility, and bulls whose breeding records were unknown. In making this division into groups the bulls used in any one herd necessarily had to be considered as individual cases because of the wide difference in the genital health of the cows in the different herds. In several of the herds vaginitis had been causing breeding difficulty for some time. These herds, in addition to a few with trichomonad infection, account for most of the ratios of service per conception which are above 1.8 for the highly fertile bulls.

The detailed results of the semen examinations for bulls of good fertility are given in Table 1. The bulls were listed in order of age in this table so that the effects of age, if any, could be determined. An inspection of Table 1 shows a wide range of nearly all characteristics of the examination of semen. The samples of semen examined from bulls 24, 39, 25 and 38 were reported to be definitely not representative of these bulls' normal ejaculate, hence they were so indicated. The two ejaculates from bull 39 and those from bulls 24 and 25 were thin and of low motility, while the normal ejaculates of these bulls had been of heavier consistency. One of the ejaculates of bull 38, secured with difficulty, was contaminated with urine and debris, and was definitely abnormal.

The results of examination of the semen of the bulls of questionable, poor, and unknown fertility are listed in Table 2. Although the shortcomings of the simple average in comparing the results of these examinations, because of the wide variation within the groups, is appreciated, it appeared to be the most convenient means of comparison for the data in question.

Three values that stand out very plain in their difference between the good group on the one hand and the questionable and poor groups on the other are: (1) the hours a motility rating of 2 or better was maintained in stored semen, (2) initial motility, and (3) the total number of abnormal spermatozoa per 1000. The average 2 motility duration of 71 hours for the good group is more than twice the 26.9 hours of the poor group and about one and one-third times the 56.8 hours of the questionable group. It is important to note, however, that a bull should not be rated

TABLE 1. RESULTS OF EXAMINATION OF SEMEN OF 42 BULLS WITH GOOD BREEDING RECORDS
Listed in order of age

Bull No.	Age in Years	Breed	No. of Samples Checked	Breeding Record		Services per Conception	Average Appearance of Semen	Average Volume in cc.	Concentration per cu. mm. in 1000's	pH of Semen	Average Initial Motility	Maintenance of 2 Motility hrs.	Abnormal spermatozoa per 1000					
				Cows Inseminated	Cows Settled								Total	Tailless	Coiled tail	Pyriform	Other Head Abnormals	Body Abnormals
56	1.0	Hol.-Fr.	2	3	3	1.00	Normal	1.85	748.0	6.60	4.50	36	81	36	17	18	10	0
46	1.0	" "	2	2	2	1.00	Normal	1.85	1268.0	----	4.50	15	120	15	24	73	5	3
31	1.0	" "	1	6	5	1.25	Yellow	5.00	864.0	7.00	5.00	48	138	42	87	9	0	0
21	1.5	" "	2	13	10	1.30	Yellow	4.50	1048.0	6.20	5.00	102	117	12	86	11	5	3
9	1.5	" "	2	--	--	----	Yellow	4.75	1372.0	6.45	5.00	42	44	15	23	2	3	1
10	1.5	" "	1	--	--	----	Normal	4.00	1472.0	6.50	5.00	30	93	12	51	21	9	0
16	2.0	" "	1	30	16	1.88	Normal	4.50	1232.0	----	5.00	Not stored	228	114	57	45	12	0
40	2.5	" "	2	22	11	2.00	Normal	4.70	1428.0	6.05	5.00	99	86	41	24	6	11	4
39	3.0	Guernsey	2*	55	19	2.90	Thin	2.25	148.0	7.85	0.50	0	177	123	39	15	0	0
54	3.0	Hol.-Fr.	36	19	14	1.36	Normal	3.59	811.9	6.48	3.72	52.4	157	28	110	12	5	2
19	3.0	" "	2	36	22	1.64	Normal	6.00	1055.0	6.85	5.00	78.0	67	18	36	9	3	1
20	3.0	" "	3	28	18	1.72	Yellow	3.57	1934.0	6.08	4.70	16.0	87	30	31	17	6	3
1	3.0	" "	2	55	28	1.96	Normal	4.45	852.0	6.05	4.00	Not stored	98	7	35	56	0	0
4	3.0	" "	1	42	22	1.91	Normal	10.00	1020.0	----	5.00	Not stored	83	6	36	3	18	20
24	3.5	Guernsey	1*	--	--	----	Thin	7.00	224.0	6.50	5.00	6.0	42	6	30	0	6	0
26	3.5	Hol.-Fr.	1	--	--	----	Normal	8.00	1392.0	6.20	5.00	96.0	66	9	45	6	6	0
29	3.5	Jersey	3	399	216	1.85	Yellow	3.23	989.3	6.25	4.00	32.0	218	80	168	3	0	0
7	4.0	Hol.-Fr.	1	4	3	1.33	Normal	5.00	1744.0	----	5.00	Not stored	117	30	57	18	9	3
5	4.0	" "	1	43	22	1.96	Normal	4.20	1330.0	----	4.00	Not stored	90	3	47	6	24	10
48	4.0	Guernsey	34	22	17	1.29	Thick	3.98	1218.2	6.43	3.94	32.7	139	60	73	3	2	1
49	4.0	Hol.-Fr.	28	11	8	1.38	Normal	4.46	1059.9	6.25	4.75	118.4	116	25	85	3	2	1
11	4.0	" "	3	59	33	1.78	Normal	3.75	620.0	6.60	3.50	38.0	81	30	34	16	1	0
14	4.0	Jersey	2	37	29	1.28	Thick	0.50	2144.0	5.95	3.00	22.0	106	32	74	0	0	0
13	4.5	Hol.-Fr.	2	48	31	1.55	Normal	3.00	1672.0	6.05	4.00	28.0	326	15	309	2	0	0
17	5.0	" "	6	21	9	2.33	Normal	7.90	1166.5	6.73	3.50	36.0	136	57	52	16	11	0
50	5.0	" "	41	7	7	1.16	Normal	4.56	684.2	6.50	3.90	74.7	236	38	60	128	7	3
32	5.5	" "	3	44	26	1.69	Normal	4.00	1005.0	6.75	5.00	240.0	133	26	84	15	7	1
35	5.5	Guernsey	3	78	28	2.78	Normal	3.83	993.3	6.73	4.30	48.0	109	46	47	15	1	0
25	5.5	"	1*	--	--	----	Thin	5.00	120.0	6.60	2.00	0	200	85	105	0	10	0
23	6.5	"	1	--	--	----	Normal	4.50	1120.0	6.30	5.00	120	90	9	81	0	0	0
27	7.0	Jersey	2	439	224	1.96	Yellow	2.75	784.0	6.43	4.00	20	273	50	193	20	10	0
42	7.5	Hol.-Fr.	2	37	20	1.85	Normal	7.00	980.0	6.40	4.50	111.0	96	33	30	18	15	0
38	8.0	Guernsey	2*	120	52	2.31	Normal	4.85	784.0	----	3.50	48.0	328.5	19.5	225	73.5	10.5	0
28	8.0	Jersey	3	104	72	1.44	Yellow	4.25	1244.0	7.00	3.50	22.0	294	50	76	158	10	0
3	8.0	Hol.-Fr.	1	13	7	1.86	Normal	4.00	2440.0	----	4.00	Not stored	150	2	29	0	12	107
47	9.0	" "	23	52	35	1.49	Normal	6.02	722.8	6.38	4.14	41.4	170	41	53	70	5	1
36	10.5	Guernsey	3	101	37	2.73	Normal	4.30	640.0	6.30	5.00	88.0	222	35	97	64	22	4
2	11.5	Jersey	1	20	13	1.54	Normal	1.50	390.0	----	5.00	Not stored	174	--	--	--	--	--
41	13.0	Hol.-Fr.	2	38	21	1.81	Normal	6.05	1048.0	6.50	5.00	171.0	99	15	75	1.5	1.5	6
37	13.0	Guernsey	1	75	29	2.72	Normal	6.80	816.0	----	5.00	96.0	153	30	99	9	15	0
15	13.0	Jersey	2	21	8	2.63	Normal	6.00	1096.0	6.20	5.00	264.0	168	26	69	66	7	0
30	----	Hol.-Fr.	1	48	21	2.29	Normal	4.00	968.0	----	5.00	Not stored	57	6	27	3	21	0
Ave.	5.1					1.81		4.56	1010.8	6.47	4.32	71.0	142	33	61	25	8	5

*Samples not considered representative of bull's normal semen.

TABLE 2. RESULTS OF EXAMINATION OF SEMEN OF BULLS WHOSE BREEDING RECORDS ARE POOR, QUESTIONABLE OR UNKNOWN

Bull No.	Age in Years	Breed	No. of Samples Checked	Breeding Record		Services per Conception	Average Appearance of Semen	Average Volume in cc.	Concentration per cu. mm. in 1000's	pH of Semen	Average Initial Motility	Maintenance of 2 Motility hrs.	Abnormal spermatozoa per 1000					
				Cows Inseminated	Cows Settled								Total	Tail-less	Coiled Tail	Pyriform	Other Head Abnormals	Body Abnormals
									Bulls with Poor Breeding Records									
52	3.5	Jersey	18	19	6	3.17	Thick	2.79	1737.8	6.53	1.78	1.0	597	46	549	0	1	1
53	4.5	"	25	22	7	3.14	Normal	5.10	861.9	6.47	4.46	20.7	131	37	70	17	5	2
51	8.0	Guernsey	18	--	-	Very poor	Thin	5.45	295.6	6.71	2.22	32.6	373	112	123	117	16	5
33	11.0	Jersey	1	--	-	" "	Normal	6.50	1016.0	----	2.00	0	690	350	260	30	15	35
18	13.0	Hol.-Fr.	3	11	2	5.50	Sl.Thin	6.87	565	7.18	2.70	80.0	500	57	149	284	1	9
Ave.	8.0					3.93		5.34	895.2	6.72	2.63	26.9	458.2	120.4	230.2	89.6	7.6	10.4
									Bulls with Questionable Breeding Records									
55	4.0	Hol.-Fr.	32	22	9	2.44	Normal	4.78	947.3	6.41	4.29	21.3	91	30	21	35	4	1
34	4.0	" "	2	--	-	----	Normal	3.15	808.0	6.53	4.50	120.0	236	39	178	3	11	5
8	9.0	Jersey	9	--	-	----	Normal	1.27	1123.6	6.57	4.00	29.0	448	36	196	210	2	4
6	13.0	"	1	--	-	----	Normal	2.20	670.0	----	4.00	Not stored	183	102	69	0	12	0
Ave.	7.5					2.44		2.85	887.2	6.50	4.2	56.8	239.5	51.8	116.0	62.0	7.3	2.5
									Bulls whose Breeding Records Were Unknown									
22	---	Guernsey	1	--	-	----	Normal	----	------	----	----	----	144	33	105	6	0	0
45	---	Jersey	1	--	-	----	Normal	4.50	1344	----	5.0	Not stored	207	156	42	6	3	0
44	6.0	"	2	--	-	----	Sl.Thin	3.50	512	----	----	----	233	99	94	30	5	5
43	10.0	"	2	--	-	----	Thin	4.00	272	----	----	----	461	92	303	42	24	0
Ave.	8.0							4.00	709	----	5.0	Not stored	261	95	136	21	8	1

on this value alone, since many of the bulls of good fertility averaged less than 56 hours maintenance of 2 motility, and a few were even below the average of the poor bulls. Also bulls 18 and 34 of the poor and questionable groups respectively averaged well above the 71-hours average of the good group. It also should be called to attention here that the hours which a motility of 2 or better was maintained are determined from the last observation at which a motility rating of 2 was observed. Thus if a sample was rated 1 motility at the second examination (6 hours), it was recorded as having maintained 2 motility 0 hours, although it probably had maintained 2 motility longer. After the first day, when examinations of the semen were made only once every 24 hours, the chance of a time error was even greater. All bulls were treated in the same way, however, so for purposes of comparison the method was adequate. A comparison of motility maintenance in stored semen and breeding records of seven bulls used in the same herd is given on page 29.

Motility.—The motility of the fresh semen showed a striking difference between the good and poor bulls. It was observed that as additional samples from each bull of good fertility were examined, the value for initial motility more nearly approached five. Increased examinations of semen of poor bulls were more likely to give a consistently low rating. Bull 53 was an exceptional case among the poor bulls, since his initial motility rating was very good. The initial motility of the semen of bulls with questionable breeding records was occasionally observed to give high ratings. An examination of Tables 1 and 2 indicates that the initial motility of several ejaculates of a fertile bull should average approximately 3.75 or above. If this value should fall below 3.50, the fertility of the bull is probably low. Apparently a high initial motility rating alone, however, is not a dependable measure of high fertility since all of the bulls of questionable fertility and bull 53 of poor fertility often produced semen with good average initial motility rating. As a rule, however, bulls with good breeding histories, when producing semen normal in appearance and of high spermatozoa concentration and free from debris and urine, were found to have an initial motility rating of approximately 4 or better, when measured at 100°F.

Abnormal Spermatozoa.—The average values of the total abnormal spermatozoa per 1000 showed a striking difference among the three groups of bulls. The semen of the bulls of good, questionable and poor fertility averaged respectively 142, 240 and 458 abnormal spermatozoa per 1000. It is plain that all of the bulls of poor fertility, except bull 53, ejaculated semen containing a high percentage of abnormal spermatozoa, the lowest being 37 per cent. There was more variation within the other two groups, however. The high average percentage of abnormal spermatozoa in the semen of bulls of questionable fertility was obviously caused by the values for two of the bulls, 34 and 8.

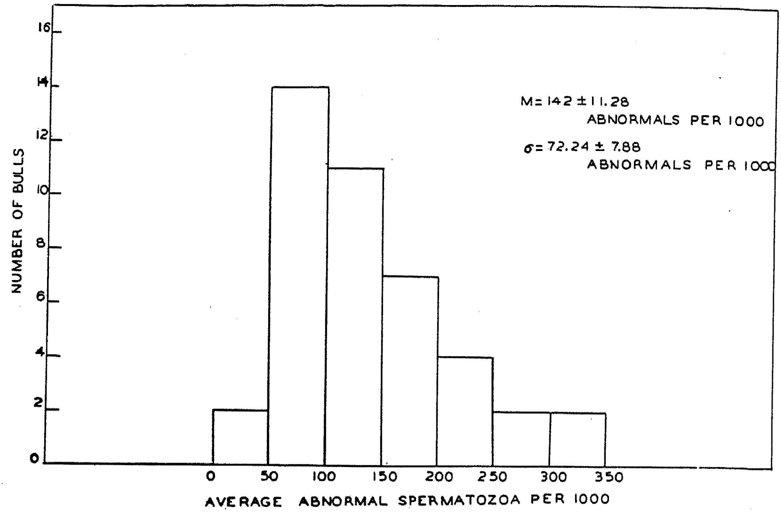

Fig. 1—Distribution of the average number of total abnormal spermatozoa per 1000 for dairy bulls of high fertility.

The distribution of the bulls of good fertility according to the percentage of abnormal spermatozoa in their semen is graphically presented in Fig. 1. This distribution is markedly skewed. Only two of the bulls had semen averaging below 5 per cent and the majority (32 bulls) had semen which averaged 5 to 20 per cent abnormal spermatozoa, while eight bulls had semen containing more than 20 per cent of abnormal spermatozoa. Semen of four bulls contained more than 25 per cent and semen of two bulls more than 30 per cent abnormal spermatozoa. The majority of the abnormal forms for three of the four highest bulls (13, 38, and 27) were coiled tails. The other (bull 28) had about 16 per cent pyriform heads and 29 per cent total abnormal spermatozoa. Semen of two other bulls of good fertility (50 and 46) was also quite high in percentage of pyriform heads.

There seemed to be no one particular type of abnormal spermatozoa associated with reduced fertility. The main distinguishing characteristics between spermatozoa of bulls of good fertility and those of poor fertility were the percentage of total abnormal spermatozoa and the uniformity of size of the heads. Spermatozoa of highly fertile bulls were more uniform in size than those of bulls of low fertility. There were, of course, exceptions to the above statement in that some bulls with semen of abnormal morphology were very fertile and that some bulls of low fertility had semen which appeared to have very few morphological defects. Bulls 53 and 55 were in the latter class.

Microphotographs of representative groups of spermatozoa were made from semen of several of the bulls. The bulls represented in Figs. 2, 3, and 4 were used in the University of Missouri dairy herd which is comprised of cows free of genital diseases; so their breeding records are indicative of their comparative fertility. In Fig. 2 are presented representative microphotographs of spermatozoa of two bulls of good fertility, bulls 54 and 48. The microphotographs of Fig. 2 show much contrast to those of Fig. 3 which are representative of two bulls (51 and 52) of poor fertility and possess many abnormal spermatozoa. In semen of bull 52, the abnormalities were confined almost entirely to a large number of coiled tails. In semen of bull 51, on the other hand, nearly all types of abnormal spermatozoa were found. In addition, the size of the heads was not uniform and epithelial cells and other debris were often observed.

Representative microphotographs of spermatozoa of two bulls (55 and 8) of questionable fertility are shown in Fig. 4. There is a great difference in the morphology of spermatozoa from these two bulls. The average motility rating of their fresh semen and the time which a motility rating of 2 was maintained in stored semen was practically the same for the two bulls, however.

The most common abnormal form encountered, making up a good one-half of the total in all three groups, was coiled tailed spermatozoa. The next most common abnormal type was tailless, followed closely by the pyriform heads. The other head abnormals, including damaged heads, tapering or pointed heads, small heads, large heads, double heads, phantom heads, and undeveloped spermatozoa, were relatively rare. Body abnormalities, including damaged, enlarged, filiform, beaded, or shrunken middle-pieces, double tails, broken necks, and protoplasmic drops at the neck, were quite rare. They were noticed in only a few ejaculates (bulls 3 and 33) in any appreciable quantities. Photographs of the different types of abnormal spermatozoa are presented on pages 55 to 57.

pH Values.—Further examination of Tables 1 and 2 shows that there was little difference between the pH values of the semen of bulls of good and poor fertility. It was practically the same for the more fertile bulls (pH 6.47) and the questionable

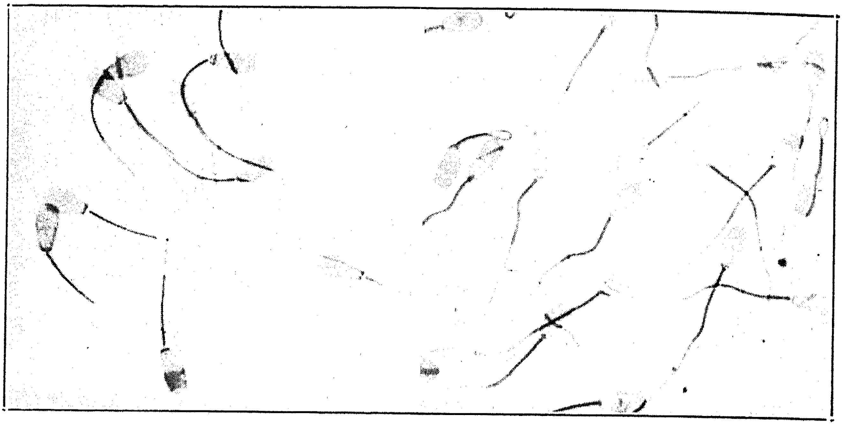

Fig. 2.—Microphotographs (685x) of spermatozoa of two bulls with good breeding records. Left—From semen of bull 54, required 1.36 services per conception. Right—From semen of bull 48, required 1.29 services per conception. Notice the uniformity and fullness of the heads. The percentage of coiled tails for bull 48 was not as high as the picture indicates.

Fig. 3.—Microphotographs (685x) of spermatozoa of two bulls with poor breeding records. Left—Semen of bull 51. It contains tailless, pyriform, coiled tailed, and poor staining spermatozoa plus much debris. Right—Semen of bull 52. The heads appear normal in size, shape, and staining characteristics. Many coiled tails are the main abnormality. This bull required 3.17 services per conception when used on good breeding cows. Notice the abaxial attachment of the middle piece to the head. This was not considered abnormal.

bulls (pH 6.50) but was slightly higher for the poor breeders (pH 6.72). Most of this increase for the poor breeding bulls was due to the values for bulls 51 and 18 which both produced thin, watery semen containing a low concentration of spermatozoa. It was observed that usually semen thin and low in concentration of spermatozoa was higher in pH than normal semen. The pH of the semen, however, did not prove to be a reliable means of evaluating either the fertility of the bull or the concentration

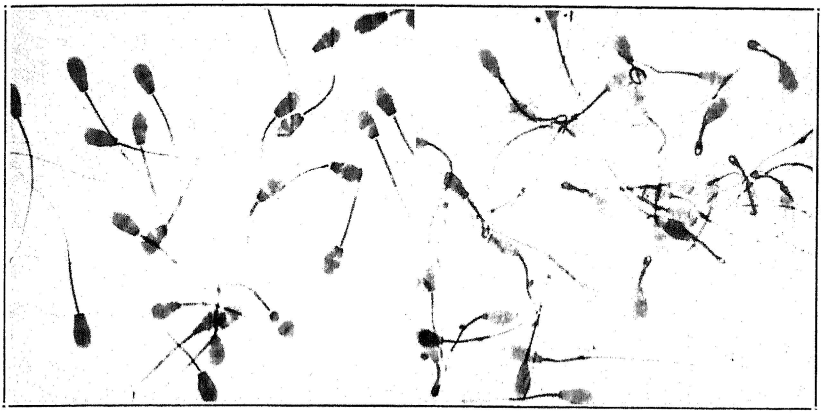

Fig. 4.—Microphotographs (475x) of spermatozoa of two bulls with questionable breeding records. Left—From semen of bull 55. Contains less than 10% abnormal spermatozoa. Service ratio—2.44 services per conception. Right—From semen of bull 8. Contains nearly 50% abnormal spermatozoa—many coiled tails and pyriform heads. The semen had good motility and fair viability.

of the spermatozoa in the semen, even though the averages do indicate that this relation might hold true. Thus, 36 samples of semen from bull 54 averaged pH 6.48 and a concentration of 812,000 spermatozoa per cubic millimeter, while 23 samples from bull 47 averaged pH 6.38 with a concentration of 723,000

Fig. 5.—Microphotographs (475x) of spermatozoa of two bulls 13 years of age. Left—From semen of bull 18. Contains a large percentage of abnormal spermatozoa, mostly pyriform. Bull required 5.5 services per conception. Viability of the spermatozoa was poor. Right—From semen of bull 41. Contained about 10% abnormalities, most of which were coiled tails. Service ratio and viability and appearance of semen were all very good.

spermatozoa per cubic millimeter. Similar variations from the average relationship were observed for other bulls included in this study—Tables 1 and 2.

Concentration of Spermatozoa.—The average number of spermatozoa per cubic millimeter did not vary greatly among the groups of bulls. The value was slightly higher for the bulls of good fertility, however. The gross physical appearance of the semen of the bulls in this study does not correlate in any consistent manner with their breeding records. It is interesting to note, however, that bull 55, a bull of questionable fertility, produced for a time, more than a year previous to this study, thick semen of yellow color. Examination by a veterinarian failed to reveal any abnormalities of the genital organs which might have been responsible for the abnormal color. No yellow tint was noticeable in any of the 32 samples collected from him which are included in this study.

Volume of Semen.—The volume of semen produced per ejaculate showed much individual variation within the groups. It is not conceivable that volume per ejaculate should have much bearing on the fertility of a bull since semen may vary in its number and characteristics of spermatozoa. It may also be observed from Table 1 that the volume of semen does not vary directly with age of the bull. In general, the volume of the ejaculate varied as the size of the bull within any one breed. Thus a few old bulls that were small gave much less semen per ejaculate than younger, larger bulls.

Decline in Motility of Stored Semen.—As mentioned above the rate in decline of motility in the stored semen was roughly correlated with the bull's fertility. Hence, the records of seven bulls which were used in the same herd were compiled for comparison. These records are presented in Table 3. The bulls included are 50, 48, 54, 49, and 47 whose breeding records indicated that they were of good fertility. Bull 55 was of questionable fertility and bull 53 of poor fertility. These bulls are listed in the order of the ratio of services per conception. Although the bulls were obviously not used on the same cows, except in a few instances where rebreeding was necessary, the service ratios are considered comparative because all the cows were known to be free of any genital diseases and those which were afflicted with ovarian cysts as reported by the veterinarian were excluded from the study. It was observed that the average motility ratings of the fresh semen (at 0 hours) of all the bulls except 54, one of the bulls of good fertility, were above 4 and varied but little.

The average motility ratings given in Table 3 are graphically presented in Fig. 6. The motility maintenance curves of the four bulls of highest fertility are shown at the right. The sharp

TABLE 3. AVERAGE RATE OF DECLINE OF MOTILITY OF SEMEN OF SEVEN BULLS USED IN THE SAME HERD COMPARED WITH THEIR BREEDING EFFICIENCY AND THE AVERAGE ABNORMALITY COUNT, CONCENTRATION, AND pH OF THEIR SEMEN.

Bull No.	No. of samples in motility Ave.	Average Motility Ratings at following Hours of Storage -- 40° F. 0	6	16	24	48	72	96	120	144	168	Services per Conception required for Bull*	Average No. of abnormal spermatozoa per 1000*	Concentration of Spermatozoa in semen 1000's per cu. mm.*	pH of Semen *	Average hrs. 2 motility maintained
50	30	4.27	3.07	2.53	2.03	1.73	1.57	1.33	0.97	0.83	0.67	1.16	236	684.2	6.50	74.7
48	29	4.04	2.41	1.83	1.62	1.35	1.17	0.93	0.69	0.45	0.28	1.29	139	1218.2	6.43	32.7
54	30	3.90	2.97	2.37	2.13	1.80	1.60	1.23	1.00	0.80	0.67	1.36	157	811.9	6.48	52.4
49	28	4.93	4.18	4.00	3.96	3.39	3.00	2.36	1.86	1.57	1.00	1.38	116	1059.9	6.25	118.4
47	15	4.87	2.33	1.73	1.53	1.20	1.07	0.87	0.67	0.47	0.33	1.49	170	722.8	6.38	41.4
55	26	4.42	2.46	1.73	1.50	1.04	0.92	0.77	0.58	0.50	0.31	2.44	91	947.3	6.41	21.3
53	17	4.71	2.47	1.77	1.41	1.12	1.00	0.77	0.47	0.35	0.18	3.14	131	861.9	6.47	20.7

*Taken from Tables 1 and 2.

early decline in motility is seen to be characteristic of the semen of the two poorer bulls. The motility of their semen averaged 1.5 and below at the end of 24 hours and was 1.0 or lower by 72 hours, whereas semen of none of the other bulls averaged 1.0 or below until the 96 hours observation.

The average motility ratings of two of the considered more fertile bulls, 47 and 48, however, approached very closely those of the two poorer breeding bulls. Hence, it is clear that judgment of a bull's fertility by this method, as by all others so far presented, must be made with reserve. From other observations

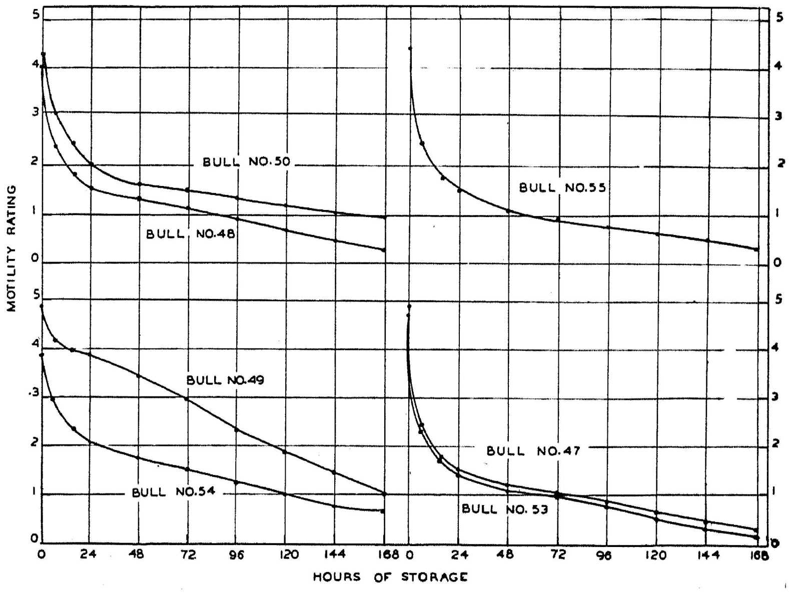

Fig. 6.—Average rate of decline of motility in semen of bulls 50, 48, 54, 49, 47, 55, and 53 stored undiluted at 40° Fahrenheit.

made on the semen of these bulls, however, the apparently poor records of bulls 47 and 48 may be partly explained. The 15 samples of bull 47 were received at weekly intervals during the winter when he, being an old and heavy bull, was not allowed to exercise in the icy lots as often as were the other bulls. His semen collected early in the fall previous to this study and later in the spring following this study has shown better motility maintenance than did the winter samples. The same cannot be said for any of the other bulls. The performance of the semen of bull 48 must be explained in a different manner. The semen of this bull was characteristically thick (see Table 1), quite viscous, and often contained a very high concentration of spermatozoa. In studies with the use of diluents during storage it was found that the semen of this bull reacted extremely favorably to dilution. Very good motility was maintained in the E-Y-B (egg-yolk-buffer) diluted sample after motility had apparently ceased in the undiluted sample. Hence the power of motility was evidently present in the spermatozoa of this bull, but it was repressed by viscosity or some other characteristic of the semen. The semen of bull 55 was also sometimes thick, but it did not react to dilution nearly as well as did the semen of bull 48.

The examples of these two bulls (47 and 48) show some of the fallacies that may enter into any method of judgment of a bull's fertility based on one test. The results secured by finding the average motility at intervals during storage agreed, along with the average time a motility of 2 or better was maintained, more closely with these bulls' breeding records than did any other value. It is shown in Table 3 that there was no consistent correlation between either the percentage of abnormal spermatozoa, the concentration of spermatozoa, or the pH of the semen and the service ratio or motility maintenance. Although the service ratios of the first four bulls probably do not differ significantly, the results with semen of these bulls show the superiority of motility maintenance of the spermatozoa as a rough method of determining a bull's fertility.

Correlation of Analysis of Semen Samples with Their Fertility

In order to determine whether there were any characteristics of the individual samples of semen used in insemination of cows with good breeding histories that could be correlated with their power to produce conception, the data in Tables 4 and 5 were compiled. In Table 4 is listed an analysis of semen samples upon which nearly complete data were available, the use of which resulted in conception. Table 5 lists the samples of semen that failed to settle cows with good breeding histories. Bulls whose samples are included in Table 4 are good breeding bulls 47, 48, 50, and 54, questionable breeding bulls 8 and 55, and poor breeding bulls 52, and 53. Table 5 contains semen samples from only five bulls, poor bulls 52 and 53 make up the majority of the cases followed closely by questionable bull 55. Bull 54 has one sample in Table 5, as also has bull 47. Several of the samples of semen had been stored previous to use. Notes regarding time and method of storage and other pertinent information are given in the column headed "remarks".

From a comparison of the averages of the two tables, apparently one significant difference is that the semen that resulted in conception maintained a motility rating of 2 or better for a mean of 42 hours while a motility of 2 or better was maintained

TABLE 4. ANALYSIS OF SEMEN THAT SETTLED COWS

Bull No.	Concentration per cu. mm. in 1000	Total Spermatozoa used in insemination 1,000,000's	pH of Semen	Motility When Used	Fresh Motility	Hours 2 Motility Maintained	Abnormal Spermatozoa per 1000 Total	Tailless	Coiled Tails	Pyriform	Other Abnormals	Remarks
48	1072	1072	----	3	3	6	123	39	81	3	--	2d ejaculate
"	656	656	6.60	3	3	0	156	114	42	--	--	
"	1792	2688	6.30	1	5	24	105	60	42	3	--	Double insem. Semen 72 hrs. old
47	1000	2000	----	5	5	--	130	20	50	20	40	Same sample
"	1000	2000	----	5	5	--	130	20	50	20	40	" "
"	736	736	----	5	5	--	171	48	36	63	24	Same sample
"	736	736	----	5	5	--	171	48	36	63	24	
"	656	1312	6.00	5	5	--	219	138	12	48	21	
"	760	1520	6.10	5	5	16	81	18	27	27	9	Double insemination
"	520	520	6.25	4	4	72	222	51	135	30	6	16 hrs. apart
"	960	960	6.25	4	4	72	294	42	111	138	3	2d ejaculate
"	1168	1168	5.95	5	5	16	240	45	75	120	--	
"	1352	1352	6.40	4	4	24	120	48	24	48	--	Heifer's 5th service.
"	1088	1088	6.35	4	5	96	78	36	12	27	3	Semen 74 hrs. old
"	328	328	6.70	4	4	4	96	21	30	45	--	E-Y-B diluted 1-3
50	448	?	----	2	2	--	535	40	60	415	20	Bulls 1st service in 3 mos.
"	192	384	----	2	2	--	345	33	51	231	30	2d ejaculate
"	616	924	----	4	4	--	90	54	12	15	9	2d ejaculate
"	1248	1248	5.95	5	5	144	225	15	6	186	18	
"	672	672	6.55	4	5	24	228	15	93	111	9	Shipped 40 mi. 4 hrs. old at use.
"	360	360	6.45	2	5	24	120	51	21	48	--	Semen 24 hrs. old. Undiluted.
"	360	360	6.45	5	5	24	120	51	21	48	--	Same sample as above, fresh.
55	648	648	6.15	5	5	0	108	24	27	57	--	
54	760	760	6.10	5	5	96	96	30	36	27	3	
"	384	384	7.10	3	3	0	144	24	102	9	9	
"	1616	1616	6.15	4	5	72	87	51	21	12	3	Semen 24 hrs. old
"	728	728	6.30	4	5	144	138	15	99	6	18	Semen 44 hrs. old
53	752	752	----	5	5	--	90	27	42	18	3	2d ejaculate. Cow's 3d service
"	264	264	6.75	4	4	24	93	21	63	6	3	
52	1824	1824	----	2	2	0	513	111	399	3	--	
8	600	900	----	5	5	--	216	54	84	75	3	2d ejaculate
"	1216	1216	----	4	4	--	255	42	120	93	--	Same sample
"	1216	730	----	4	4	--	255	42	120	93	--	
Ave.	840.2	997.0	6.34	4.0	4.3	42	182	44	65	64	9	(Total of 33 cows settled)

TABLE 5. ANALYSIS OF SEMEN THAT FAILED TO SETTLE COWS WITH GOOD BREEDING HISTORIES

Bull No.	Concentration per cu. mm. in 1000	Total Spermatozoa used in insemination 1 000 000's	pH of Semen	Motility When Used	Fresh Motility	Hours 2 Motility Maintained	Abnormal Spermatozoa per 1000: Total	Tailless	Coiled Tails	Pyriform	Other Abnormals	Remarks
47	736	736	----	4	5	---	171	48	36	63	24	Semen stored 20 hrs.
55	1104	2208	----	5	5	---	81	9	12	--	60	
55	504	1008	----	--	--	---	39	24	12	--	3	
55	1120	1120	6.45	2	5	48	57	6	18	33	--	Egg-diluted, 48 hrs. old.
55	688	688	6.45	5	5	0	51	18	15	18	--	
54	1328	1328	6.00	5	5	0	78	15	33	27	3	
52	1696	1696	----	4	4	---	744	45	696	--	3	
52	1104	1104	----	4	4	---	612	33	573	--	6	
53	1392	1392	6.65	5	5	6	81	33	42	6	--	
"	344	344	6.45	5	5	6	129	33	84	9	3	Double insemination
"	672	672	6.55	5	5	16	87	15	51	12	9	with 2 samples
"	344	344	6.45	5	5	6	129	33	84	9	3	Double insemination
"	672	672	6.55	5	5	16	87	15	51	12	9	with 2 samples
"	800	800	----	3	3	--	168	21	147	--	--	
"	592	592	----	5	5	--	51	48	3	--	--	Very little fluid from cervix
"	1416	1416	6.20	1	5	6	186	60	84	42	--	Semen 24 hrs. old.
"	720	720	6.35	5	5	16	327	75	195	57	--	
"	904	904	----	5	5	--	84	57	21	--	6	
"	928	928	6.75	5	5	24	87	27	33	3	24	
"	592	592	----	5	5	--	51	48	3	--	--	
"	---	---	6.40	--	--	48	79	21	24	9	18	
"	800	800	6.40	5	5	6	108	15	78	6	9	
Ave.	878.9	955.4	6.44	4.4	4.8	15.2	158	32	104	14	8	(Total of 23 cows failing to settle)

a mean of only 15.2 hours in semen that did not result in conception. Although this difference appears significant at first inspection, if the samples of bulls 52, 53 and 55 (the predominating ones in Table 5) which settled cows are compared with those of the same bulls which failed to produce conception, it is seen that the difference is not so great.

It will be noted that the sperm concentration, total spermatozoa used in insemination, and pH of the semen exhibit very little differences in the two tables. The average number of abnormal spermatozoa per 1000 is higher in the fertile semen (182) than in the semen which didn't result in conception (158) but these averages are neither such as to classify the bulls as non-breeders, according to investigators reported in the review of literature. The majority of abnormal spermatozoa in Table 5 was coiled tails, while the fertile semen was high in pyriform heads and coiled tails and also contained a few more tailless than did the semen which failed to beget. Bulls 53 and 55 had semen which was low in percentage of abnormalities, while bulls 50, 47 and 8, which account for most of the cases in Table 4, had semen with much higher abnormality counts.

Evidently semen somewhat high in percentage of abnormal spermatozoa may at times settle cows. Of the 33 cows settled listed in Table 4, two were bred with semen containing over 50 per cent abnormal spermatozoa, one with semen with 34.5 per cent and nine with semen having between 20 and 26 per cent abnormal spermatozoa. This makes more than one-third of the cows in Table 4 settled with semen containing more than 20 per cent abnormal spermatozoa.

Semen of very low motility has also been effective in producing conceptions. One of the fertile samples of semen rated a motility of 1, four rated 2, and three rated 3 motility at the time of insemination. On the other hand, of the semen that failed to settle cows, one sample rated a motility of 1, one rated 2, and only one rated 3 motility.

From the data presented in Tables 4 and 5 it will be observed that it is extremely difficult to point out definite characteristics of semen from apparently healthy bulls that will produce conception as opposed to the characteristics of semen which will not result in pregnancy when used in artificial insemination. The data do show, however, that use of semen of bulls with poor breeding histories has occasionally resulted in conception. Likewise a single insemination with semen from very fertile bulls has sometimes failed to settle cows with good breeding histories. There is always the possibility that the fault does not lie entirely with the semen. The operation of the genital system of the cow is not understood well enough to be able to absolve the cow of all blame. The ability of the semen to produce conception is more closely correlated with the breeding record of the bull, obviously, than any characteristic of the semen that has been measured here. Hence, it seems that the method of evaluating semen which is in best agreement with the service ratios of different bulls from which the semen was obtained will likewise be the most valuable over a large number of cases in predicting the conception power of any one semen sample.

Variations in the Semen of Different Ejaculates from the Same Bull

It became apparent early in this investigation that bulls do not produce semen of uniform quality and quantity at different collections. In order to study the variation which occurred in each of several different bulls, all the examinations which had been made of semen from eight bulls used in the University of Missouri dairy herd were tabulated, and are presented in Tables 6 to 13 inclusive. The samples are listed in the order in which they were collected so that a progressive picture is obtained in every case. Collections of semen were made from most of the bulls regularly twice a week.

The record of the semen examinations for bull 47 is given in Table 6. As mentioned previously, this was an old and heavy bull and he was not allowed to exercise in the icy lots as often as were some of the younger bulls. For this reason semen collections were made from him approximately once a week. As examination of Table 6 shows an interesting progressive change in the characteristics of semen produced by this bull during the winter. The first very cold, icy weather occurred after the December 21 sample was collected. Thereafter it was noticed that the quality of the semen decreased. Initial motility was often poor, the percentage of abnormal spermatozoa low, and survival time of good motility was lessened. With the coming of warmer spring weather, the semen of bull 47 became more uniform in quality and of much better quality. This was the only one of the ten bulls used at the University of Missouri herd which showed this apparent seasonal variation in semen quality.

Although many of the ejaculates from bull 48 (Table 7) were thick and viscous, there was much variation in the consistency and characteristics of his semen. This semen, that which was rather watery in appearance and which contained a low concentration of spermatozoa, nearly always exhibited a pH which was higher than normal. This fact was observed in the semen of several other bulls, also; hence it was thought that there might be some correlation between pH and concentration. A scatter diagram was made accordingly. It revealed that if any such correlation existed, it was within exceedingly wide limits of

TABLE 6. RECORD OF SEMEN EXAMINATIONS OF BULL 47* Age 9.0 years

Date of Collection	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	pH	Initial Motility Rating	Maintenance of [2] Motility in hrs.	Abnormal spermatozoa per 1000					
							Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
10-19-39	Normal	9	1000	----	5	---	130	20	50	20	30	10
11-12-39	Normal	7	736	----	5	---	171	48	36	63	24	0
11-18-39	Normal	9.5	656	6.00	5	---	219	138	12	48	12	9
12- 8-39	Normal	5.5	760	----	5	16	81	18	27	27	9	0
12-16-39	Normal	10.5	1088	6.35	5	96	78	36	12	27	0	3
12-21-39	Normal	6.5	712	----	5	196	93	12	66	15	0	0
1-20-40	Thin	4.0	368	6.50	1	0	117	27	39	51	0	0
1-24-40	Thin	3.5	400	6.45	1	0	120	27	18	75	0	0
1-27-40	Normal	5.0	624	6.65	5	6	108	27	21	60	0	0
1-31-40	Thin	6.0	224	6.40	3	48	123	33	54	27	9	0
2- 3-40	Normal	7.0	1352	6.40	4	24	120	48	24	48	0	0
2-10-40	Thick	6.5	600	6.30	5	120	222	39	66	117	0	0
2-17-40	Very thin	4.5	520	6.25	4	72	222	51	135	30	3	3
2-27-40	Normal	5.5	1080	6.25	5	6	120	18	66	36	0	0
3- 2-40	Normal	6.0	1536	6.40	5	24	183	114	12	54	3	0
3- 9-40	Thin	4.0	400	6.50	4	0	216	24	39	147	6	0
3-12-40	Thin	5.2	488	6.00	4	24	270	24	99	141	6	0
3-21-40	Thin	7.0	328	6.70	--	4	96	21	30	45	0	0
3-25-40	Thin	3.2	224	6.90	2	0	306	51	117	135	0	3
3-25-40	Normal	6.5	960	6.25	4	72	294	42	111	138	3	0
3-30-40	Fair	5.5	1168	5.95	5	0	240	45	75	120	0	0
4- 6-40	Fair	6.5	416	6.60	5	24	219	42	84	90	3	0
4-13-40	Normal	4.5	984	6.35	4	96	165	54	18	93	0	0
Averages		6.02	722.78	6.38	4.14	41.4	170	42	52	70	5	1

*Breeding Efficiency - (52 services, 35 pregnancies) = 1.49.

TABLE 7. RECORD OF SEMEN EXAMINATIONS OF BULL 48* Age 4.0 years

Date of Collection	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	pH	Initial Motility Rating	Maintenance of 2 Motility hrs.	Abnormal spermatozoa per 1000					
							Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
12-16-39	Fair	2.5	920	----	2	---	129	45	75	0	0	9
1- 5-40	Normal	7.3	1648	6.60	3	---	66	42	15	0	9	0
1- 5-40	Fair	4.1	960	6.45	5	---	102	27	75	0	0	0
1- 6-40	Fair	6.4	520	6.80	3	---	294	9	285	0	0	0
1-10-40	Thick	3.8	1512	6.75	3	16	96	12	66	6	9	3
1-10-40	Normal	7.0	1072	----	3	0	123	39	81	3	0	0
1-13-40	Thick	2.6	1008	6.10	5	16	87	15	54	6	9	3
1-17-40	Normal	2.9	552	6.75	3	6	195	21	174	0	0	0
1-20-4C	Thick	4.1	1568	6.65	2	0	108	12	93	3	0	0
1-24-40	Thick	5.8	1288	6.65	5	48	81	30	51	0	0	0
1-27-40	Normal	3.1	688	6.50	4	6	114	24	90	0	0	0
1-31-40	Very thick	5.7	1576	6.25	5	120	180	60	117	0	3	0
2- 3-40	Thin	0.8	1128	----	2	6	264	72	186	6	0	0
2- 6-40	Thick	5.5	1864	6.40	5	16	120	54	66	0	0	0
2-10-40	Thick	6.2	1120	5.95	5	120	153	81	69	3	0	0
2-13-40	Normal	5.3	1104	6.80	3	24	207	123	78	6	0	0
2-17-40	Normal	3.0	656	6.60	3	0	156	114	42	0	0	0
2-20-40	Fair	1.5	832	6.80	1	0	129	69	51	9	0	0
2-20-40	Normal	4.5	1280	6.30	5	0	240	129	108	3	0	0
2-24-40	Normal	3.5	1112	6.20	3	6	162	117	33	12	0	0
2-24-40	Normal	3.0	1064	6.30	5	72	117	78	36	0	3	0
2-27-40	Thick	5.0	848	6.40	4	168	189	99	84	0	3	3
2-29-40	Normal	2.0	808	6.70	5	24	174	138	33	0	3	0
3- 2-40	Very thick	3.0	1912	6.65	4	6	135	84	39	6	6	0
3- 5-40	Very thick	5.0	1952	6.35	4	48	123	63	54	3	3	0
3- 9-40	Thick	3.0	1792	6.30	5	24	105	60	42	3	0	0
3-12-40	Thick	4.5	928	6.20	5	0	117	63	45	3	0	6
3-19-40	Thick	3.5	1672	6.30	5	48	114	39	75	0	0	0
3-19-40	Normal	4.0	1200	6.25	5	96	108	48	54	6	0	0
3-30-40	Thin	1.5	524	7.00	4	---	153	99	42	6	6	0
3-30-40	Normal	4.0	1376	5.70	5	---	114	66	36	0	6	0
4- 2-40	Normal	4.5	1120	6.05	5	16	33	15	15	3	0	6
4- 6-40	Thick	2.0	1456	6.60	5	6	129	39	75	9	6	0
4- 9-40	Thick	4.6	2360	6.10	3	24	117	66	51	0	0	0
Averages		3.98	1218.24	6.43	3.94	32.7	139	60	73	3	2	1

*Breeding Efficiency - (22 services, 17 pregnancies) = 1.29.

variation; hence it could serve little useful purpose of predicting one value if the other were known.

It is interesting to note that wide variation in semen characteristics was exhibited from one ejaculate to the other for practically every bull. This was especially true for the bulls of good fertility. The semen from bulls of poor fertility, however, showed much less variation. Their semen was uniformly poor in quality. This fact is illustrated in Table 10 which gives the results of semen examinations for bull 51. It is shown that only once did initial motility in his semen rate 5 and that motility of 2 was maintained in most instances less than six hours. In fact, the initial motility rating in a majority of the cases was not above 2. The percentage of abnormal spermatozoa varied, but it was nearly always high, that is, about 30 per cent. The semen of bull 52, another bull of low fertility, showed even less variation than that of bull 51. It was very uniform in that it was low in volume (about two cc.), highly concentrated, thick, and viscous. It always contained 40 per cent or more abnormal spermatozoa most of which consisted of coiled tails; and the initial motility rating, seldom ranking over 2, was nearly always 1 after six hours of storage. The production of consistently poor quality of semen seemed to be characteristic of these two bulls of poor fertility. Semen of bull 53 (Table 11), also of poor fertility, on the other hand, varied just as much in characteristics as did the semen of bulls of good fertility. Most of the semen from this bull appeared initially to be of good quality, too. Although the percentage of abnormal spermatozoa varied from 5.1 per cent to 32.7 per cent, it was usually quite low. Maintenance of 2 motility did not vary as greatly as it did in some of the semen from bulls of good fertility. Although it was never observed to be less than six hours, it never exceeded 72 hours. No plausible explanation for the low viability of semen from this bull has been made. Although he did not show an excessive amount of aggressiveness at the breeding chute, when the gate separating him from the cow was drawn back bull 53 quickly mounted and ejaculated. Semen of other bulls used no more regularly than was No. 53 did not show the uniformly low time of survival that his semen did.

Semen examinations of bull 54, listed in Table 12, show that his semen was extremely variable in nearly all characteristics. This bull was small and he was always quite eager and fast at service. Often the first ejaculate would be very thin and of poor quality but a second one taken immediately following the first proved to be much higher, in most cases, in motility, viability, and concentration. This difference in quality of first and second ejaculates was noted often for some bulls but seldom for others, as will be shown later. This occurred in spite of the

TABLE 8. RECORD OF SEMEN EXAMINATIONS OF BULL 49* 4.0 years

Date of Collection	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	pH	Initial Motility Rating	Maintenance of 2 Motility hrs.	Abnormal Spermatozoa per 1000					
							Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
1-10-40	Normal	4.5	520	6.40	5	96	129	39	84	3	0	3
1-13-40	Thick	3.5	608	6.15	5	48	237	27	186	15	9	0
1-17-40	Normal	4.6	944	6.25	5	6	87	30	48	3	3	3
1-20-40	Thick	7.0	1744	6.60	3	6	48	9	33	3	3	0
1-24-40	Normal	3.7	992	6.70	3	240	372	6	366	0	0	0
1-27-40	Normal	3.5	336	6.40	5	72	96	42	36	6	0	12
1-31-40	Normal	11.0	728	6.45	2	96	546	54	489	0	0	3
2- 3-40	Thick	5.0	1624	5.95	5	144	129	33	93	3	0	0
2- 6-40	Normal	4.5	1272	6.10	5	72	84	39	39	3	3	0
2-10-40	Thick	3.5	880	6.20	5	120	72	39	24	3	6	0
2-13-40	Normal	3.1	816	6.40	5	120	114	45	66	0	3	0
2-17-40	Normal	1.7	1000	6.60	5	168	48	15	30	0	0	3
2-20-40	Normal	3.9	816	6.20	5	216	78	27	48	0	0	3
2-24-40	Normal	3.5	728	5.95	5	160	69	12	48	6	3	0
2-24-40	Normal	4.0	816	6.05	5	144	84	21	57	6	0	0
2-27-40	Normal	3.3	1296	6.05	5	144	114	60	51	0	3	0
3- 2-40	Normal	2.8	800	6.25	5	144	126	18	108	0	0	0
3- 2-40	Normal	4.0	1280	6.20	5	168	30	18	6	3	3	0
3- 5-40	Normal	3.0	1344	6.25	5	48	51	9	39	3	0	0
3- 9-40	Thick	4.5	960	6.20	5	120	90	18	63	3	3	3
3-12-40	Normal	5.0	1140	6.10	5	144	117	33	78	0	0	6
3-19-40	Thick	6.0	1408	6.20	5	72	63	3	45	12	3	0
3-26-40	Normal	4.0	1640	6.25	5	72	144	0	144	0	0	0
3-30-40	Normal	5.0	880	6.10	5	72	87	36	45	0	6	0
4- 2-40	Normal	3.7	616	6.40	5	72	60	18	42	0	0	0
4- 6-40	Normal	6.0	2032	6.05	5	168	36	3	15	6	6	6
4- 9-40	Normal	6.5	1392	6.25	5	288	81	6	69	6	0	0
4-13-40	Normal	4.0	1064	6.30	5	96	60	27	24	9	0	0
Averages		4.46	1059.86	6.25	4.75	118.4	116	25	85	3	2	1

*Breeding Efficiency (11 series, 8 pregnancies) = 1.38.

TABLE 9. RECORD OF SEMEN EXAMINATIONS OF BULL 50* Age 5.0 years

Date of Collection	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	pH	Initial Motility Rating	Maintenance of 2 Motility hrs.	Abnormal spermatozoa per 1000					
							Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
11-15-39	Fair	4.5	752	----	3	---	345	102	15	213	15	0
11-15-39	Fair	4.7	448	----	2	---	535	40	60	415	10	10
11-15-39	Thin	2.0	256	----	-	---	350	70	--	250	30	0
11-17-39	Fair	6.0	192	----	2	---	345	33	51	231	21	9
11-20-39	Thin	4.5	264	----	2	---	408	114	30	258	6	0
11-20-39	Normal	4.5	728	----	4	---	393	45	126	192	27	3
12- 2-39	Normal	3.5	920	----	4	---	291	42	42	198	6	3
12-11-39	Thin	3.7	424	----	4	---	60	15	12	24	9	0
12-11-39	Normal	6.5	616	----	4	---	90	54	12	15	6	3
1- 6-40	Normal	6.3	512	----	4	162	300	12	54	210	24	0
1-11-40	Fair	6.0	976	----	5	102	141	12	48	69	12	0
1-15-40	Normal	4.0	1126	----	5	216	159	9	54	87	0	9
1-18-40	Normal	5.0	960	----	3	168	312	12	246	45	9	0
1-25-40	Normal	6.3	808	----	5	168	42	9	6	24	3	0
1-31-40	Normal	5.5	1248	5.95	5	144	225	15	6	186	18	0
2- 5-40	Fair	3.0	544	6.25	4	120	357	18	246	87	0	6
2- 8-40	Fair	5.7	464	7.00	5	24	165	78	39	27	12	9
2-15-40	Normal	8.0	752	7.80	3	0	90	12	21	45	12	0
2-19-40	Normal	4.4	972	6.25	5	72	57	12	15	30	0	0
2-22-40	Normal	4.5	192	6.45	4	168	414	15	234	144	15	6
2-27-40	Normal	0.5	1280	6.65	3	---	246	21	135	81	9	0
2-29-40	Thin	3.2	680	6.70	5	6	117	18	33	63	3	0
2-29-40	Normal	7.5	1040	6.40	5	48	126	12	36	75	3	0
3- 4-40	Thin	4.5	592	6.45	5	16	129	12	72	45	0	0
3- 5-40	Clear	2.5	56	6.55	2	0	135	21	24	66	15	9
3- 5-40	Fair	5.0	600	6.55	5	144	246	21	105	114	6	0
3- 7-40	Normal	6.0	1040	6.40	5	120	213	3	33	168	6	3
3-11-40	Normal	4.0	896	6.40	4	24	426	63	87	276	0	0
3-14-40	Normal	4.0	724	6.55	4	72	249	24	69	147	6	3
3-14-40	Normal	9.0	800	6.50	4	96	294	18	135	141	0	0
3-18-40	Normal	2.5	1096	6.20	5	144	234	30	72	129	3	0
3-21-40	Thin	4.0	672	6.55	5	24	228	15	93	111	6	3
2-25-40	Normal	2.6	1160	6.35	2	16	330	90	72	168	0	0
3-28-40	Normal	3.0	1160	6.20	5	24	183	24	36	123	0	0
3-28-40	Normal	7.5	704	6.30	5	24	237	99	12	126	0	0
4- 1-40	Very thin	3.5	160	6.50	3	0	141	90	15	30	6	0
4- 5-40	Thin	3.0	144	6.50	1	0	126	54	6	66	0	0
4- 5-40	Fair	5.2	360	6.45	5	24	120	51	21	48	0	0
4- 9-40	Fair	3.0	576	----	5	20	231	15	51	132	3	30
4-13-40	Thin	3.0	304	6.60	4	24	273	48	18	207	0	0
4-13-40	Normal	5.0	856	6.45	5	144	312	126	27	159	0	0
Averages		4.56	684.24	6.50	3.90	74.65	236	38	60	128	7	3

*Breeding Efficiency (7 services, 6 pregnancies) = 1.16

TABLE 10. RECORD OF SEMEN EXAMINATIONS OF BULL 51* Age 9.0 years

Date of Collection	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	pH	Initial Motility Rating	Maintenance of Motility in hrs.	Abnormal spermatozoa per 1000: Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
1- 6-40	Thin	4.4	112	6.80	1	0	463	320	80	60	3	0
1-11-40	Thin	4.2	352	6.65	3	102	360	54	270	24	6	6
1-15-40	Fair	7.0	640	6.90	3	6	320	100	75	140	5	0
1-18-40	Normal	7.0	792	6.90	3	6	246	45	114	78	9	0
1-20-40	Fair	8.0	520	6.85	2	0	246	30	42	168	3	3
1-22-40	Thin	4.8	376	6.80	3	144	459	60	318	69	6	6
1-25-40	Thin	5.0	328	6.55	1	0	261	21	150	81	3	6
1-29-40	Very thin	6.5	96	6.60	1	0	192	60	57	69	0	6
2- 1-40	Thin	5.0	416	6.50	4	144	396	72	204	102	18	0
2- 5-40	Thin	7.0	424	6.75	5	120	336	75	156	87	12	6
2- 8-40	Thin	6.8	456	6.75	3	48	336	30	162	135	3	6
2-15-40	Thin	2.5	320	6.70	2	16	320	30	155	120	10	5
2-19-40	Very thin	4.5	80	6.70	1	0	205	45	120	25	15	0
2-22-40	Clear	5.0	16	6.60	2	0	360	150	60	120	30	0
2-29-40	Very thin	4.2	96	6.95	2	0	500	205	40	185	40	30
3- 4-40	Thin	6.0	128	6.70	2	0	555	70	140	290	55	0
3- 7-40	Thin	5.7	128	6.60	1	0	565	160	65	260	70	10
3-11-40	Clear	4.5	40	6.50	1	0	605	495	0	100	10	0
Averages		5.45	295.55	6.71	2.22	32.56	373.0	112	123	117	16	5

*Breeding Efficiency (not used for insemination).

TABLE 11. RECORD OF SEMEN EXAMINATION OF BULL 53* Age 4.5 years

Date of Collection	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	pH	Initial Motility Rating	Maintenance of 2: Motility in hrs.	Abnormal spermatozoa per 1000: Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
12-10-39	Thin	5.0	550	----	4	--	75	27	27	12	9	0
12-10-39	Normal	6.0	904	----	5	--	84	57	21	0	3	3
12-16-39	Fair	6.7	592	----	5	--	51	48	3	0	0	0
12-22-39	Normal	6.0	1264	----	1	--	111	3	108	0	0	0
12-28-39	Normal	5.5	800	----	3	--	168	21	147	0	0	0
1-11-40	Normal	4.0	984	6.60	3	6	204	24	63	111	6	0
1-15-40	Normal	5.3	928	6.75	5	24	87	27	33	3	24	0
1-22-40	Normal	6.3	1392	6.65	5	6	81	33	42	6	0	0
1-27-40	Thin	5.5	264	6.75	4	24	93	21	63	6	3	0
2- 7-40	Thin	4.2	344	6.45	5	6	129	33	84	9	0	3
2- 8-40	Normal	5.5	672	6.55	5	16	87	15	51	12	6	3
2-10-40	Thick	4.6	912	6.15	4	24	141	21	90	21	3	6
2-17-40	Normal	6.0	560	6.70	4	72	177	24	129	12	0	12
2-19-40	Normal	6.0	720	6.35	5	16	327	75	195	57	0	0
2-24-40	Normal	5.7	920	6.50	5	24	231	90	84	36	15	6
2-29-40	Normal	4.8	752	----	5	--	90	27	42	18	3	0
3- 4-40	Thick	4.0	968	6.40	5	6	111	27	69	12	0	3
3- 7-40	Normal	3.5	888	6.50	5	6	114	36	54	3	18	3
3-11-40	Normal	5.5	1232	6.40	5	16	150	84	48	15	3	0
3-13-40	Normal	---	---	----	5	48	72	21	24	9	15	3
3-18-40	Normal	2.7	1040	6.15	5	16	93	27	57	0	9	0
3-25-40	Normal	6.5	1416	6.20	5	6	186	60	84	42	0	0
3-28-40	Normal	5.5	800	6.40	5	6	108	15	78	6	6	3
4- 6-40	Normal	4.0	920	6.50	5	24	156	42	108	3	3	0
4-15 40	Normal	3.5	864	6.55	5	48	144	63	51	18	3	9
Averages		5.10	861.92	6.48	4.46	20.7	131	37	70	17	5	2

*Breeding Efficiency (22 services, 7 pregnancies) = 3.14.

TABLE 12. RECORD OF SEMEN EXAMINATIONS OF BULL 54* Age 3.0 years

Date of Collection	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	pH	Initial Motility Rating	Maintenance of 2 Motility hrs.	Abnormal spermatozoa per 1000					
							Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
12-15-39	Normal	5.5	1264	6.00	5	--	21	12	3	3	3	0
12-18-39	Fair	4.0	616	6.20	5	--	63	48	9	6	0	0
12-18-39	Thin	4.5	152	6.65	1	0	87	72	9	6	0	0
1- 6-40	Thin	3.0	328	7.00	1	0	204	21	171	9	3	0
1-10-40	Normal	4.4	1400	6.60	5	0	81	6	45	27	3	0
1-13-40	Thick	4.6	1016	6.30	5	6	234	66	135	21	9	3
1-17-40	Thin	3.0	168	6.55	1	0	39	9	9	18	3	0
1-20-40	Thin	3.5	496	6.70	2	0	99	24	63	9	0	3
1-24-40	Normal	3.7	976	6.50	3	168	336	9	327	0	0	0
1-27-40	Normal	4.2	624	6.60	4	192	273	18	252	3	0	0
1-31-40	Normal	6.5	760	6.10	5	96	96	30	36	27	3	0
2- 3-40	Thin	3.8	728	6.30	5	144	138	15	99	6	3	15
2- 6-40	Thin	8.5	384	7.10	3	0	144	24	102	9	6	3
2-10-40	Thin	2.2	304	6.65	3	6	51	21	15	9	6	0
2-13-40	Normal	3.2	936	6.70	5	72	60	15	36	9	0	0
2-17-40	Normal	3.0	768	6.50	5	24	66	15	30	15	3	3
2-20-40	Very thin	2.3	80	6.75	1	0	100	65	25	10	0	0
2-20-40	Normal	3.2	928	6.55	5	48	120	18	63	33	0	6
2-24-40	Thick	2.0	1760	6.30	2	72	381	18	348	0	15	0
2-27-40	Thin	2.8	784	6.80	4	16	210	15	174	12	3	6
3- 2-40	Thin	2.5	320	6.80	2	0	168	27	126	12	3	0
3- 2-40	Normal	3.8	896	6.60	5	24	273	6	249	12	6	0
3- 5-40	Normal	5.0	704	6.40	5	96	111	9	69	24	6	3
3- 5-40	Thick	3.0	1400	6.50	5	96	129	30	36	57	3	3
3- 9-40	Thin	2.7	576	6.40	5	0	135	78	21	33	3	0
3-12-40	Thin	2.5	592	6.35	5	168	225	93	126	0	3	3
3-19-40	Thin	2.5	384	6.40	3	72	360	9	342	9	0	0
3-19-40	Thin	3.0	600	6.60	4	120	231	6	213	9	3	0
3-26-40	Thin	2.6	424	6.40	1	0	120	12	99	0	6	3
3-26-40	Normal	3.5	1384	6.00	4	96	270	9	255	0	0	6
3-30-40	Normal	5.0	1280	6.00	5	0	126	63	48	6	6	3
4- 1-40	Normal	3.7	1328	6.00	5	0	78	15	33	27	0	3
4- 2-40	Very thin	2.5	---	6.55	1	0	99	27	39	9	24	0
4- 6-40	Normal	2.5	1976	6.60	5	120	360	36	315	6	3	0
4- 9-40	Normal	2.6	1616	6.15	5	72	87	51	21	12	3	0
4-13-40	Thin	4.0	464	6.50	5	72	78	6	39	9	0	24
Averages		3.59	811.89	6.48	3.72	52.4	157	28	110	12	5	2

*Breeding Efficiency (19 services, 14 pregnancies) = 1.36

TABLE 13. RECORD OF SEMEN EXAMINATIONS OF BULL 55* Age 4.0 years

Date of Collection	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	pH	Initial Motility Rating	Maintenance of 2 Motility hrs.	Abnormal spermatozoa per 1000					
							Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
10-23-39	Normal	4.5	1104	----	5	--	81	9	12	--	60	0
11-15-39	Normal	7.5	760	----	5	0	105	57	9	36	3	0
12-12-39	Normal	7.0	1352	6.10	4	--	42	24	15	3	0	0
12-21-39	Fair	5.0	528	----	2	--	21	18	3	--	0	0
12-30-39	Fair	7.0	504	----	--	--	39	24	12	--	3	0
1-11-40	Normal	4.4	1040	6.60	2	0	102	63	15	24	0	0
1-15-40	Normal	3.5	648	6.15	5	0	108	24	27	57	0	0
1-18-40	Thin	3.4	184	6.70	1	0	102	75	12	9	6	0
1-22-40	Normal	5.5	920	6.40	5	6	63	30	3	24	3	3
1-25-40	Normal	3.2	1000	6.80	2	0	120	21	18	78	3	0
1-29-40	Very thin	4.5	320	6.90	1	0	108	57	36	12	0	3
2- 1-40	Normal	5.8	672	6.30	5	24	132	54	18	45	12	3
2- 5-40	Normal	5.5	1336	6.20	5	96	69	15	18	36	0	0
2- 8-40	Thick	5.0	1496	6.25	5	24	57	15	9	33	0	0
2-12-40	Thick	5.8	1152	6.50	5	6	60	12	33	15	0	0
2-15-40	Normal	4.3	1120	6.45	5	48	57	6	18	33	0	0
2-19-40	Thick	8.0	1424	6.25	5	96	198	21	72	99	3	3
2-22-40	Thick	3.0	800	6.30	5	48	81	21	27	27	6	0
2-27-40	Thick	3.5	1096	6.15	5	24	132	30	39	51	9	3
2-29-40	Normal	4.0	784	6.60	5	0	45	18	15	6	6	0
3- 4-40	Normal	3.4	688	6.45	5	0	51	18	15	18	0	0
3- 7-40	Normal	4.0	784	6.50	5	24	99	21	18	60	0	0
3-11-40	Thin	4.3	760	6.40	5	0	171	93	45	30	3	0
3-14-40	Normal	3.0	1176	6.50	5	24	105	39	15	51	0	0
3-18-40	Normal	4.0	1216	6.35	5	0	75	21	18	33	3	0
3-21-40	Thin	5.0	536	6.60	4	6	120	12	36	69	3	0
3-25-40	Normal	4.0	880	6.55	3	0	78	24	24	27	0	3
3-28-40	Normal	4.5	1232	6.40	5	6	72	15	18	36	0	3
4- 1-40	Thick	5.0	1320	6.00	5	6	123	15	42	57	9	0
4- 6-40	Normal	2.8	1200	6.30	5	24	126	39	30	54	3	0
4- 9-40	Normal	7.7	1296	6.30	5	72	87	39	3	39	6	0
4-13-40	Normal	5.0	984	6.50	4	48	93	24	18	51	0	0
Averages		4.78	947.25	6.41	4.29	21.33	91	30	21	35	4	1

*Breeding Efficiency (22 services, 9 pregnancies) = 2.44

fact that before each ejaculate the bull was held back until he appeared to be ready to serve as evidenced by dripping from the preputial sheath and eagerness to mount.

Semen from bull 55, as shown in Table 13, did not exhibit great variations but showed poor viability in storage as well as poor fertility. Semen of this bull was more nearly uniform in percentage of abnormal spermatozoa than semen from any of the other bulls, including the bulls of poorer fertility, never exceeding 20 per cent of abnormal spermatozoa and usually less than 15 per cent.

Semen from bull 50 (Table 9) showed marked variation in all characteristics examined. The first few ejaculates were taken after the bull had been confined in a box stall for two months and had not been mated for some time previous to that. The changes apparently resulting from such treatment will be pointed out later.

In order to show effectively the extent of variation which occurs in separate consecutive ejaculates of the same bull, the data for bull 49, given in Table 8, were organized in column diagrams. The data on this bull's semen were used because they were more complete than those for any of the other bulls. It must be kept in mind that the following graphs do not represent semen of an average bull, but simply the variation which occurred in the semen of one bull. Characteristics of the semen of different bulls do not all follow the same pattern as those of a so-called average bull but seem to be subject to variations characteristic of the individual bull. Bull 49 consistently gave semen of very high quality. Even though it was of high quality, however, it was subject to a great deal of variation as is shown below.

The characteristic of semen which varied the least among different ejaculates of any of the bulls was the initial motility rating. It was usually uniformly good or uniformly bad. The semen of bull 49 varied less in this respect than the semen from any of the other bulls. Motility ratings of his semen are given in Fig. 7. It is noted that with the exception of three samples all rated 5.

The pH of the semen showed the least absolute variation next to the initial motility rating. The variations in pH of semen from bull 49 are shown in Fig. 8. Very rarely were semen samples found which were below a pH of 6.00 and the samples of pH above 7.0 were equally rare. Some of the thin samples of semen of low concentration were of a higher than normal pH. In order to illustrate the variability of this relationship, Fig. 9, which shows the variations in concentration of the spermatozoa in consecutive semen samples of bull 49, was placed below Fig. 8. A comparison of the two figures shows little consistent correlation between the two values.

The variations in volume of semen produced by bull 49 are shown in Fig. 10. Volume of semen per ejaculate is also subject to wide variation. A comparison of Figs. 9 and 10 shows that there was little correlation between volume and concentration. Both may be low at one time, as in collection 12 or 17, and there may be an inverse relationship as in collections 16 and 19.

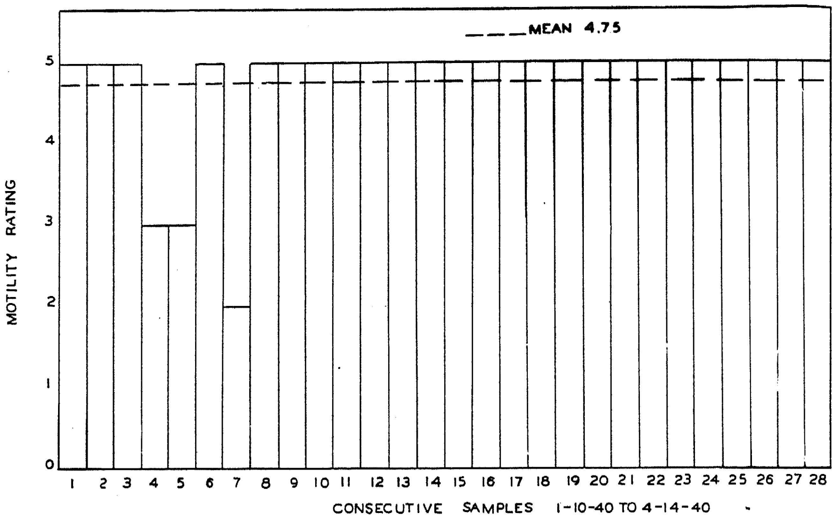

Fig. 7.—Initial motility ratings of consecutive samples of semen from bull 49.

The variation in production of abnormal spermatozoa in semen of bull 49 is pictured in Fig. 11. With the exception of three cases, this variation is not very great. Practically all of the observations fall below the mean of 116 per 1000. The very high percentage of abnormalities in collection 7 makes it appear to have been contaminated with urine or water. No other evidences of such could be found, however. The property of the semen which caused the high percentage of abnormal spermatozoa and also the low initial motility (see Fig 7) was not determined. Semen of most of the other bulls showed much greater variation in percentage of abnormalities than did semen of bull 49.

The variation in percentage of abnormal spermatozoa in consecutive ejaculates was usually caused by an increase in concentration of only one or two types of abnormalities. The type which was subject to increase seemed to be characteristic for the individual bull. Thus, as shown in Table 8, the increased

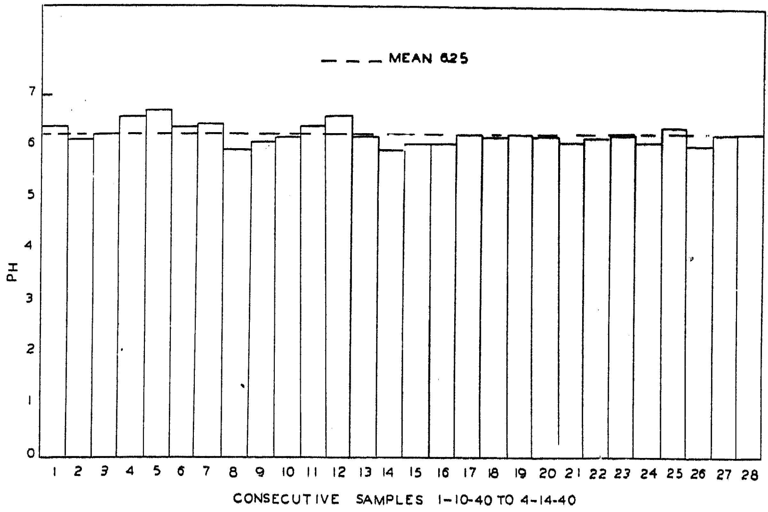

Fig. 8.—pH values of the fresh semen samples of bull 49. Showing variation in consecutive samples.

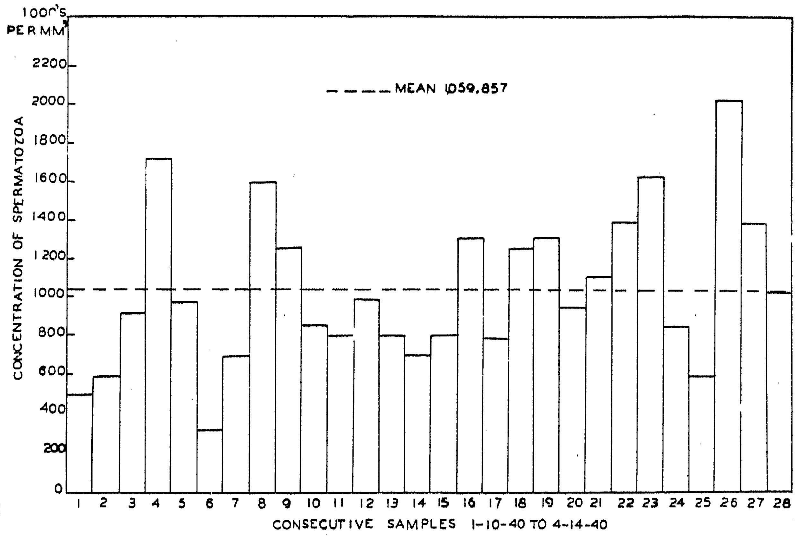

Fig. 9.—Concentration of spermatozoa per cubic millimeter in consecutive samples of semen from bull 49.

percentage of abnormalities in semen of bull 49 was caused almost entirely by an increase in coiled tails with little change in the shape of the heads. Semen of bull 50, on the other hand, varied greatly from time to time in the percentage of pyriform heads. This is illustrated by microphotographs from two col-

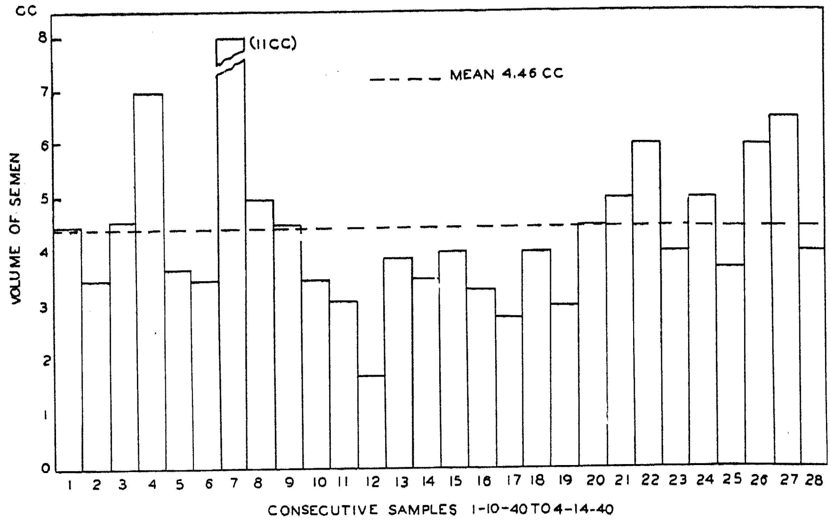

Fig. 10.—Volume of semen produced per ejaculate in consecutive collections from bull 49.

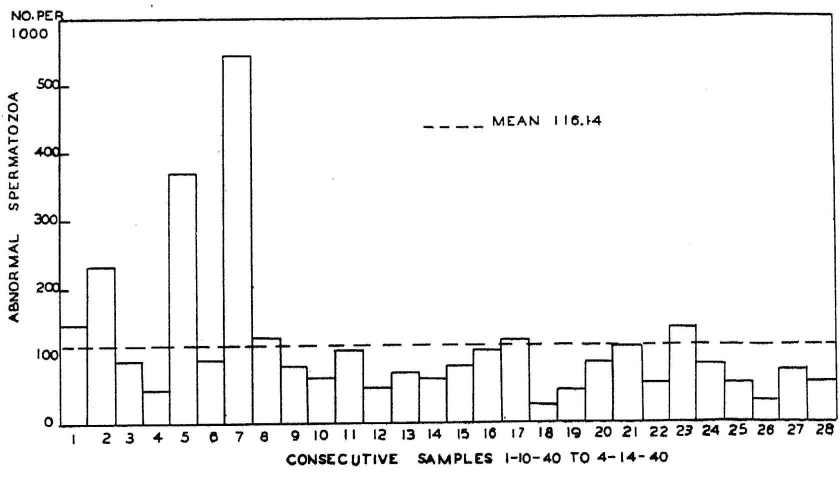

Fig. 11.—Abnormal spermatozoa per 1000 in consecutive samples of semen collected from bull 49.

lections in Fig. 12. Semen from bull 50 also varied greatly at times in the percentage of coiled tailed spermatozoa it contained (shown in Table 9).

The time a motility rating of 2 or more was maintained is given in Fig. 13. This characteristic of the semen was quite variable, but not extremely so, for bull 49. Semen from many of the other bulls showed much greater variation in this respect. An interesting fact shown by a comparison of Figs. 11 and 12 is that a high percentage of abnormal spermatozoa did not greatly harm the viability of the spermatozoa of this bull of good fertility.

Fig. 12.—Microphotographs showing the variation in morphology of the spermatozoa of bull 50. Left.—Sample collected December 2, 1939. Contains a very large percentage of pyriform heads (685x). Right.—Sample collected February 19, 1940. Contained only 5.7% abnormal spermatozoa and 3.0% pyriform heads. The spermatozoa of this bull varied considerably in the shape of the heads.

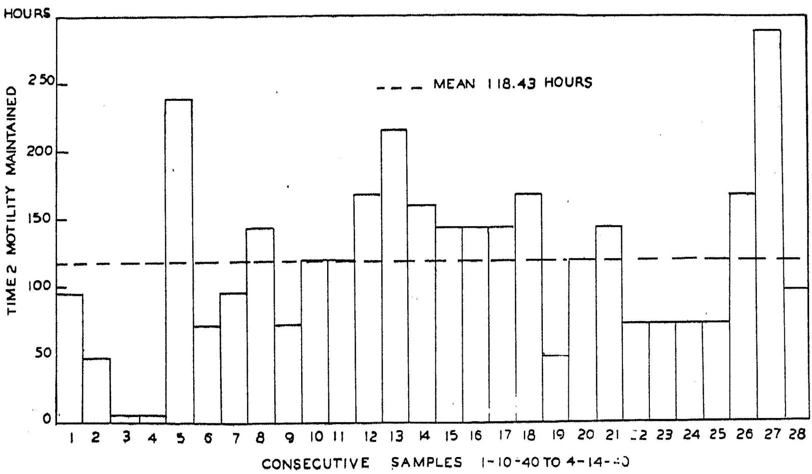

Fig. 13.—Hours of duration of motility of 2 or better in consecutive samples of semen from bull 49.

The foregoing tables, figures, and discussion have shown the extent of variation in semen characteristics which occurred in ejaculates of eight bulls used regularly. Besides the day to day variation in semen of the same bull, the characteristics of the semen showed additional variations. One is the difference in semen properties of the first and second ejaculates.

From many of the bulls from which semen was collected a second ejaculate was secured immediately (after five minutes) following the first. The results of examination of these samples of semen are given in Table 14. There are 37 comparisons (74 samples) from 17 different bulls included in the table. Although there was some variation in the response among the different bulls, the averages are fairly typical of the differences which were found between the first and second ejaculates of bulls being used moderately or lightly. The average volume of the second ejaculate was increased (from 3.5 to 4.56 cc.) over that of the first ejaculate. At the same time the concentration of spermatozoa in the second ejaculate was also higher than that in the first. The pH of the fresh semen was slightly lower on the average in the second ejaculate. The variation in pH was subject to extremely wide individual variation, however. There were just as many comparisons in which the pH of the second ejaculate was higher than that of the first as there were in which the opposite was true. Initial motility of the first ejaculates was lower (average 3.54) than that of second (average 4.35). However, in two single cases the reverse was true and often no difference in motility of the two ejaculates was noticed. The percentage of abnormal spermatozoa in the second ejaculate averaged slightly higher than in the first. The greater part of this increase was due to an increase in coiled tails. Most of the average increase in coiled tailed spermatozoa was caused by the comparison of ejaculates of one bull. This bull, No. 34, had 237 more coiled tails per 1000 in the second ejaculate than in the first. Excluding the comparisons of ejaculates of bull 34, the difference in percentage of abnormal spermatozoa between the first and second ejaculates was slight. Only the three major classifications of abnormal spermatozoa were listed in Table 14; hence, in some cases the sum of these three does not equal the total abnormal spermatozoa.

It was observed that some bulls were subject to greater variation regarding their first and second ejaculates than were others. Some of the bulls, such as bulls 49, 21, 32 and 8, showed little difference in the quality of the first and second ejaculates. The main difference in semen of these bulls was that the second ejaculate was usually of larger volume and often also of greater concentration. The semen of these bulls was characteristically of good quality at the first ejaculation. In some of the other bulls, however, such as bulls 54 and 50, the first ejaculate was often of thin, watery appearance and low in concentration and motility, while the second ejaculate was of good, normal

TABLE 14. COMPARISON OF QUALITY OF SEMEN OF FIRST AND SECOND EJACULATES

Bull No.	Volume in cc.		Concentration: 1000's/cu.mm.		pH		Motility		Abnormal spermatozoa per 1000: Total		Tailless		Coiled Tails		Pyriform		No. of Compared Samples
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	
47	3.2	6.5	224	960	6.90	6.25	2	4	306	294	51	42	117	111	135	138	1
17	15.0	6.0	888	1664	7.10	6.00	1	4	147	156	90	54	36	42	18	30	1
20	4.5	4.0	2024	1088	5.90	6.25	5	4	93	108	30	57	36	36	24	9	1
21	4.5	4.5	1232	864	6.30	6.10	5	5	132	102	12	12	93	78	21	0	1
35	2.0	6.0	1316	944	6.75	6.70	4	4	108	81	18	30	69	36	21	15	1
34	2.3	4.0	896	720	6.35	6.70	5	4	111	360	30	48	60	297	6	0	1
9	3.5	6.0	1280	1464	6.40	6.50	5	5	63	24	18	12	36	9	3	0	1
11	3.0	4.5	192	1048	6.80	6.40	2	5	87	60	9	12	42	39	33	9	1
32	3.5	4.0	856	1296	----	----	5	5	120	81	51	12	45	30	18	27	1
8	0.9	1.63	1205	851	6.65	6.45	3.6	3.6	404	467	25	46	200	148	243	274	3
48	3.51	4.43	1217	1159	6.61	6.16	3.2	4.7	120	134	63	65	47	65	6	2	6
50	3.55	6.31	526	662	6.42	6.37	3.3	4.3	203	255	47	43	28	63	120	141	8
54	3.15	3.50	421	893	6.49	6.48	3.2	4.0	154	163	28	23	112	138	117	20	6
49	3.15	4.00	764	1048	6.10	6.13	5.0	5.0	98	67	15	20	78	32	3	5	2
53	5.0	6.0	550	904	----	----	4.0	5.0	75	81	27	57	27	21	12	0	1
1	3.7	5.2	872	832	6.00	6.10	4.0	4.0	96	99	12	3	33	36	51	60	1
46	2.0	1.7	1400	1136	----	----	4.0	5.0	111	129	21	9	24	24	54	93	1
All Bulls	3.50	4.56	835.3	950.7	6.50	6.35	3.54	4.35	166	190	38	38	69	81	59	67	

consistency and of much better motility. Bulls of the latter type usually served very readily and quickly. It is possible that this quickness of service may have accounted for the poor quality semen. In a few cases in which these bulls had been forced back without ejaculating after they had mounted the cow the first time, a sample of good quality semen was secured at their second mounting.

From an examination of Table 14 it appears that a pH of 6.80 or above in the first ejaculate may be associated with abnormally low concentration of spermatozoa and abnormally high dilution of the semen by secretion of the accessory sex glands. This effect is noticeable in the cases of bulls 11, 47 and 17, especially. The first ejaculate of bull 17 was secured after a two month's sexual rest. This probably accounts for its very large volume and poor motility.

The progressive seminal pictures of two bulls which were collected from regularly after a prolonged sexual rest are given in Table 15. The sample secured from bull 8, February 22, was his first service in approximately two months. The November 15 collection from bull 50 was his first service in three and one-half months. In addition to lack of service, bull 50 had been kept confined in a box stall for more than three months and he was quite fat. Bull 8, however, was not in good condition. The most outstanding change was a decline in the percentage of abnormal spermatozoa with succeeding ejaculates. The initial motility rating tended to increase also. The concentration of spermatozoa did not vary uniformly from the first to the ninth ejaculate.

TABLE 15. PROGRESSIVE CHANGES IN THE SEMINAL PICTURE OF BULLS IMMEDIATELY FOLLOWING A LONG SEXUAL REST PERIOD

Date	Appearance of Semen	Volume in cc.	Concentration 1000's/cu.mm.	Initial Motility Rating	Abnormal spermatozoa per 1000: Total	Tail-less	Coiled Tails	Pyriform	Other head Abnormals	Body Abnormals
				Bull No. 8						
2-22-40	Normal	0.7	1000	5	582	6	261	312	0	3
2-22-40	Normal	1.5	1104	4	513	51	144	315	0	3
3- 2-40	Thick	0.7	1312	5	531	36	330	147	12	6
3- 9-40	Thick	0.5	1128	1	600	51	228	315	6	0
3- 9-40	Normal	1.8	848	2	672	33	207	432	0	0
3-19-40	Normal	1.5	1488	5	429	33	279	99	3	15
4- 1-40	Normal	1.5	1416	5	231	18	111	102	0	0
4- 1-40	Thin	1.6	600	5	216	54	84	75	0	3
4- 5-40	Normal	1.6	1216	4	255	42	120	93	0	0
				Bull No. 50						
11-15-39	Fair	4.5	752	3	345	102	15	213	15	0
11-15-39	Fair	4.7	448	2	535	40	60	415	10	10
11-15-39	Thin	2.0	256	0	350	70	0	250	30	0
11-17-39	Fair	6.0	192	2	345	33	51	231	21	9
11-20-39	Thin	4.5	264	2	408	114	30	258	6	0
11-20-39	Normal	4.5	728	4	393	45	126	192	27	3
12- 2-39	Normal	3.5	920	4	291	42	42	198	6	3
12-11-39	Thin	3.7	424	4	60	15	12	24	9	0
12-11-39	Normal	6.5	616	4	90	54	12	15	6	3

The Volume of Ejaculate

The volume of 334 ejaculates collected from 50 bulls ranged from 2:5 to 5.5 cc. with a mean of 4.38±1.02 cc. The standard deviation was 1.86±0.07 cc. Most of the ejaculates above 6.0 cc. were abnormally high and those below 2.0 cc. were abnormally low in volume. Bulls did not consistently produce ejaculates at either extreme of the volumes recorded. (Fig. 14).

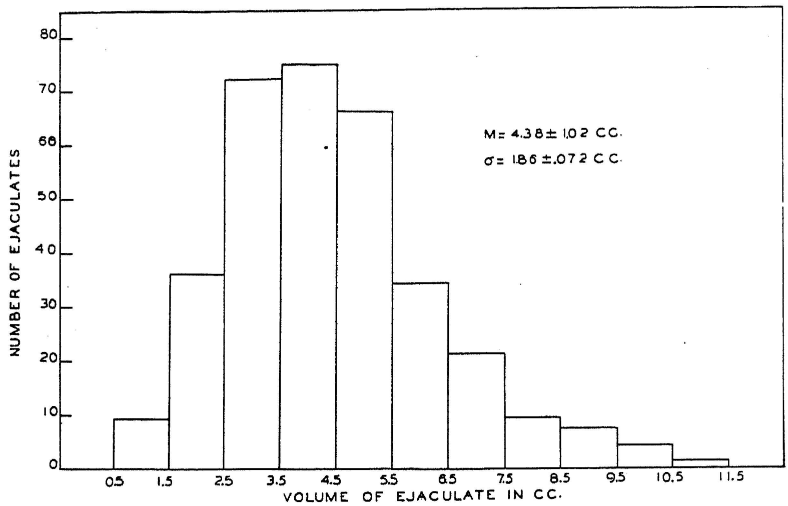

Fig. 14.—Distribution of 334 ejaculates from 50 bulls according to volume of semen per ejaculate.

The average rate of loss of motility of spermatozoa in semen stored undiluted at 40°F. is shown in Fig. 20. This curve was drawn from the average daily motility ratings of 216 stored samples of semen representing 32 bulls. It includes samples from bulls of both good and poor fertility. However, only four of the 32 bulls were considered to be of poor fertility. Motility of the average sample of semen declined rapidly for the first 24 hours. It had a rating of 1 at 120 hours of storage. The average time a motility rating of 2 or better was maintained in semen of bulls with good fertility has been shown (Table 1) to be 71 hours which is approximately 3 days.

Types of Abnormal Bovine Spermatozoa Observed

Abnormal spermatozoa were found in every sample of semen examined, and this seemed to be a normal occurrence in the semen of all bulls. Bulls varied greatly in the percentage of abnormal spermatozoa produced; also as to the type of abnormalities. The semen of some bulls was characteristically high in one type of abnormal spermatozoa.

Some of the abnormal types of spermatozoa observed are presented in the following microphotographs.

The microphotograph at the left side in Fig. 15 shows a group of pyriform spermatozoa. This type was differentiated from the tapering head shown in Plate 1 by the portion of the head which was constricted. If the anterior portion, making up the cytoplasmic part of the head, were defective, making a pointed head, it was called tapering. If the constriction and deficiency occurred at the posterior part of the head, however, which involves the nuclear portion, the head was described as being pyriform. Pyriform heads were often observed without tails; so it would appear that the tails are easily separated from this type of head. Spermatozoa with pyriform heads are capable of motion, however.

Fig. 15.—Types of abnormal spermatozoa. Microphotographs (685x) of semen smears. Left.—Pyriform heads. The nuclear portion of the head is markedly constricted. Tailless heads are often found with pyriform heads, the tailless ones also being pyriform. Note the long, narrow head between the two tailless. Right.—Beaded middle piece on spermatozoa marked *b*. Spermatozoa *a* has a broken neck. These are rare abnormalities in the bull semen observed in this study.

The microphotograph at the right side in Fig. 15 shows two types of abnormalities which were not observed frequently. The broken-necked spermatozoa in this photograph are not thought to be artifacts since they were also observed in the fresh unstained semen. They were motionless. They were probably of the stage preceding tailless heads. Damages of the middle pieces were rarely observed. Three types of middle piece beads were observed. One, shown in Fig. 16, had two beads, one at the anterior and one at the posterior part of the middle-piece. Another type, shown in Plate 1, had only a bead at the anterior end of the middle-piece. The third type had a similar bead at the posterior end of the middle-piece. Other abnormal middle-pieces observed were filiform, in which the middle-piece was represented by a mere thread of material, and enlarged middle-pieces in which the entire middle-piece was enlarged.

Fig. 16.—Microphotographs showing types of abnormal spermatozoa. Left.—Coiled tails (475x). Notice various types of coiled tails from a small coil at end of the tail to a tight coil involving even the middle-piece. Also the looping back at the middle piece type of loosely coiled tail. Most of these heads are pyriform also. Note the enlarged middle-piece in the lower left corner. Right.—Enlarged head (795x). It is one-half larger than the normal sized heads around it. The middle-piece appears to be divided.

Double middle-pieces, shown in Fig. 16, were rarely found, usually only where the spermatozoa heads were larger than normal. Hence the question arises whether or not the enlarged heads which were occasionally observed were not the product of incomplete division of the spermatids.

Many types of coiled tailed spermatozoa were observed. Characteristic types are pictured in Fig. 16. Some of the coiled tails consisted of a simple looping back of the tail at the middle-piece. In others the tail looped back and forth around the middle-piece forming a coil. In some spermatozoa the coil seemed to start at the middle-piece close to the head and the tail was wound tightly around the head. This type of spermatozoa was never observed in motion, but the type in which the tail was coiled around the middle piece often showed motility. In another type, shown quite well at left in Fig. 16, the coiling of the tail started at the tip and proceeded coiling tightly up the tail. In some this type of coiling had proceeded only a short way; in others it had progressed to the middle piece; and in a few the coil was situated directly at the base of the head.

A few tailless spermatozoa heads were a common occurrence in semen of practically all the bulls examined. As shown in Plate 1, the break of the tail from the head practically always occurred at the neck, not at the end of the middle-piece.

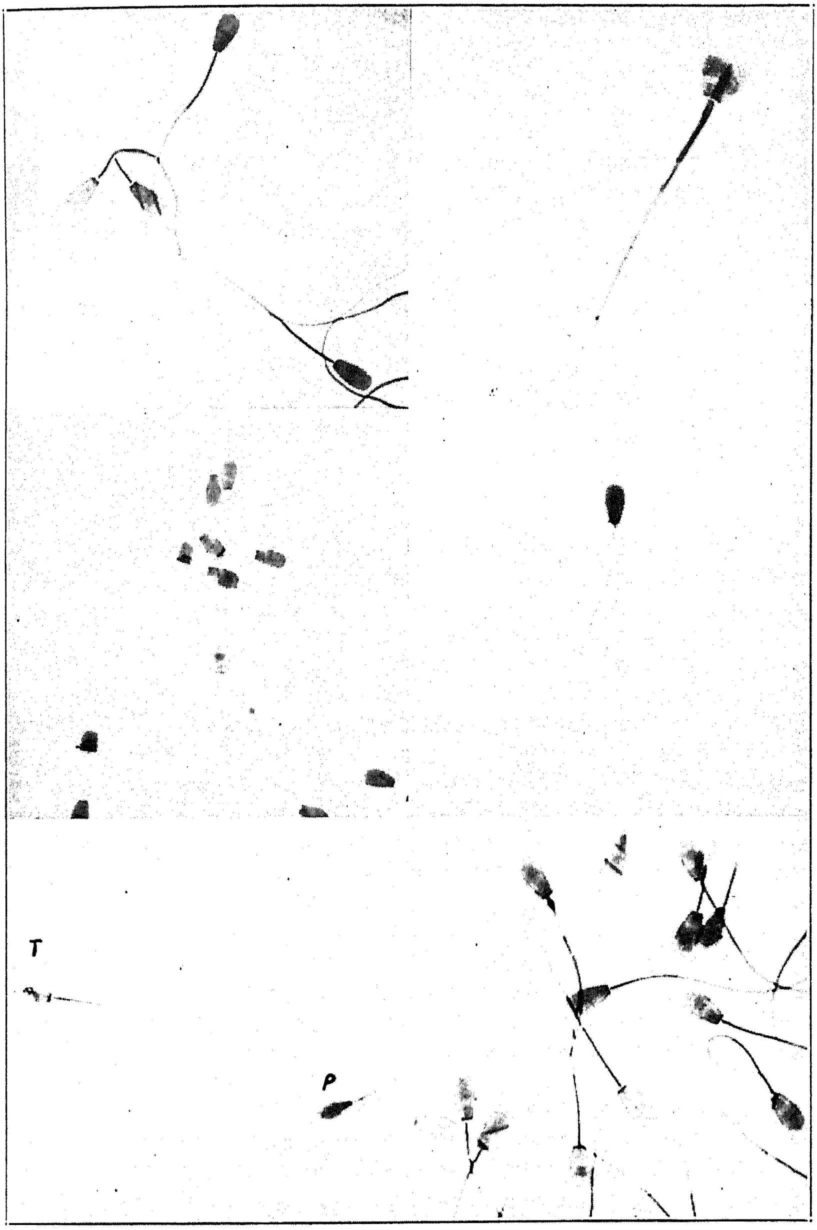

Plate I

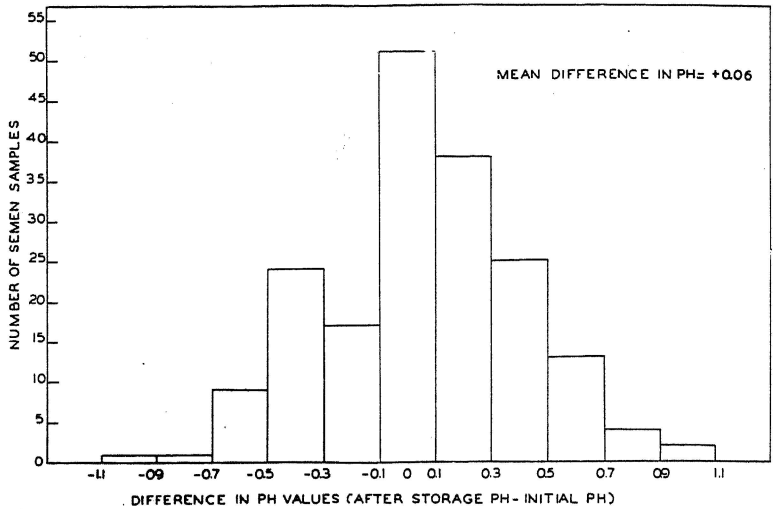

Fig. 17.—Distribution of the differences in pH of the semen after storage from its initial pH for 185 samples of semen. Stored until motility rated 1 for 3 to 4 days.

Double spermatozoa were very rarely observed. Some of the forms which might be called double are shown in Plate 1. These were never observed in the unstained preparation.

Primary spermatocytes were observed in semen of only one bull. Undeveloped spermatozoa were likewise rare, but were more common than the primary germ cells. Undeveloped cells were large and relatively shapeless masses with no division of staining properties in the cell to show nuclear and cytoplasmic aspects. They had a short undeveloped tail which was usually curved around the cell.

Some sperm heads of abnormal size were observed. Most of these forms, both enlarged and small were normal in shape, proportions, and staining properties. Their only abnormality was their atypical size. Both have been observed in motion in fresh semen.

Studies on the Storage of Semen

Changes in Morphology of Spermatozoa During Storage.—A portion of every sample of semen which was large enough to bear division was stored undiluted, as has been explained before, at 40° Fahrenheit. When the motility rating of the stored semen became 1, which appeared to be less than 50 per cent motility, and had remained so for three days, the pH of the semen was again taken, a smear was made for staining purposes, and the semen was discarded. A few of the samples were kept four days after the motility rating had reached 1. Those samples which rated a motility of 0 at any time were not stored longer.

After 100 of the samples of semen from 16 different bulls had been examined for morphological abnormalities before and after storage, comparisons were made to determine the differences and the significance of them. The average storage time was 146.2 hours and it varied from 72 hours to 360 hours. The average number of abnormal spermatozoa of the 100 samples before storage was 179.2 with a standard deviation of 122.1, per 1000. The corresponding value after storage was 173.4 with a standard deviation of 127.6. The mean difference was 5.8 which was found to be non-significant.

The results of this study are not intended to imply that no changes took place in the heads of the spermatozoa. If any changes did take place, however, it was not possible to observe them in the stained spermatozoa by the microscopical techniques followed in this study.

Changes in pH of Semen in Storage at 40°F.—The pH value of the semen before storage was compared with the pH value of the same sample of semen after storage in more than 200 cases. Table 16 lists 185 samples from 11 bulls for which two or more comparisons were made. The semen of seven of the

TABLE 16. AVERAGE pH CHANGES WHICH OCCURRED IN SEMEN OF DIFFERENT BULLS DURING STORAGE (UNDILUTED) AT 40°F.

Bull No.	No. of Comparisons	Initial pH Ave.	Ave. pH After Storage	Difference in pH	Average Time Stored: Hrs.
52	10	6.53	6.56	+ .03	77.2
55	22	6.41	6.74	+ .33	106.1
51	17	6.71	6.75	+ .04	115.8
53	15	6.47	6.71	+ .24	102.3
50	22	6.48	6.53	+ .05	132.0
47	17	6.37	6.56	+ .19	120.2
48	24	6.43	6.64	+ .21	120.0
54	30	6.43	6.35	- .08	137.6
49	23	6.26	6.04	- .22	194.6
8	3	6.48	6.23	- .25	112.0
34	2	6.53	6.25	- .28	216.0
Average all cases		6.45	6.51	+ .06	128.2

bulls, showed a rise in pH during storage, while four declined in pH during storage. As the storage period was increased the pH of the semen changed either decreasing or increasing in relation to the length of the storage period. The longest storage time of any semen on which pH comparisons were made was 312 hours. Everyone of the samples of semen held that long dropped in pH 0.3 or more. Many samples held only 96 hours exhibited just as great declines in pH values, however, and many increased in pH in similar proportion.

The distribution of the 185 samples of semen as to the difference between pH after storage and initial pH is plotted in Fig. 17. It is shown that the greatest number of samples of semen changed less than 0.3 in pH value and that very few changed more than 0.5.

A study of these variations of pH in storage have indicated that spermatozoa are not killed in storage simply by the depression of pH of the media. Much of the semen which survived the longest, such as that from bull 49, was lower in pH initially than were most samples of semen when their motility was practically all gone. Since the semen was discarded and the final pH readings were made at a given motility rating of the semen and not at a given time interval, it would be reasonable to assume that the ending pH values all should have been very close together if motility had been inhibited mainly by a shift in pH. Obviously, this was not the case. In fact, the ending pH values were more at variance than were the initial ones.

Rate of Loss of Motility in Stored Semen.—Motility ratings were made at regular intervals on all samples of stored semen. During the first twenty-four hours of storage the semen was examined for motility at 6, 16 and 24 hours. Thereafter the stored samples of semen were examined at 24-hour intervals. The motility ratings of 216 samples of semen up to the seventh day of storage were tabulated and the average rating at each examination was determined. These are presented in Fig. 19. It is noted that the greatest loss of motility occurred during the first 24 hours. From 24 hours to 48 hours the decline in motility was very slight, and the decline from 24 to 168 hours appeared to be roughly linear. The average initial motility rating was above 4, but after six hours of storage the average motility had dropped to about 3, and by 24 hours the average rating was 2.24. The average motility rating then declined at the rate of about 0.25 per 24 hours to 168 hours. If the curve were extrapolated to the zero point, the time of storage would be over 200 hours or about nine days. At this time there would be no motility left in an average sample of semen. Actually, as has been pointed

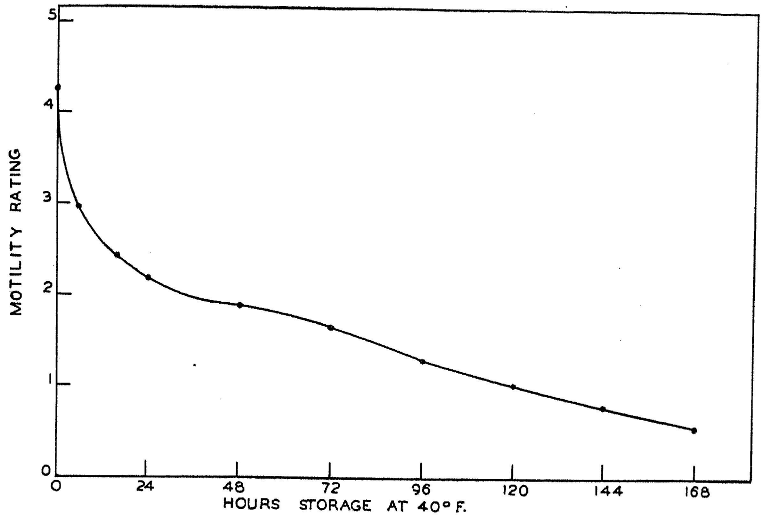

Fig. 18.—Average rate of decline of motility in 216 samples of semen stored undiluted at 40° Fahrenheit.

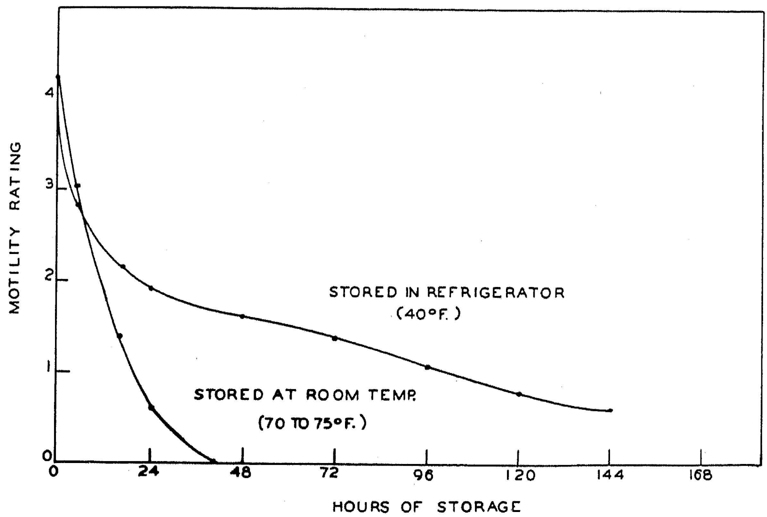

Fig. 19.—Comparison of the rate of loss of motility in semen stored at room temperature with that in semen from the same samples stored in refrigerator. Average of 37 samples from 10 bulls.

out previously, the viability of samples of semen is a very variable factor.

The temperature of holding was observed to have a marked effect upon the rate of loss of motility in the semen. Thirty-seven samples of semen representing 10 bulls were stored at room temperature (70 to 75° Fahrenheit) and examined at 6, 16, 24, 40 and 48 hours or until motility had ceased. The average ratings for the respective examination times are plotted in Fig. 19. For comparison the average ratings of the same samples of semen stored at 40° Fahrenheit were computed and are shown in the same graph. At the six-hour examination the semen stored at room temperature showed slightly better activity than did that stored in the refrigerator. At 16 hours, however, motility in many of the samples stored at room temperature was very low while in those stored at 40° Fahrenheit the decline had been slight. After 24 hours storage at room temperature, motility was absent in 16 of the 37 samples and only one of the rest rated 2. At 40 hours spermatozoa in all samples except one were dead. This one rated 1 still and it had been rated only 2 initially. The samples which showed excellent initial motility and which retained motility very well in storage were observed to die just as fast at room temperature as did those of poorer quality. Hence the maintenance of motility in semen at room temperature was not useful in predicting motility maintenance in the same sample stored.

Motility Maintenance in Diluted and Undiluted Semen.— The effect of dilution of the semen with a few of the common diluents in use upon motility and survival of the spermatozoa was studied. The first diluents studied were solutions of glucose. Two of these were simply 3 and 5 per cent solutions of pure glucose and a third one was Milovanov's SGC-2 dilutor made up according to the formula given by Walton (1933). It was found that all three of these diluting media were detrimental to the sperm. More vigorous motility was maintained in the undiluted semen than in that diluted where each of these diluents was used. This adverse effect of these diluting media was observed in every sample in which they were used. Hence their use was early discontinued. Figure 20 presents the ratings of three of the samples of semen diluted with SGC-2 compared with motility of the same samples of semen stored undiluted. Although the time of survival was nearly the same in the SGC-2 dilutor as in the undiluted semen, the percentage of motile spermatozoa was less.

The egg-yolk-buffer proposed by Phillips (1939) was also used. Eighteen samples of semen, collected from eight bulls, which were stored after dilution with this diluent (hereafter it will be designated as E-Y-B dilutor) were compared with the same samples of semen stored undiluted. The average motility ratings for each method of storage of these samples are plotted

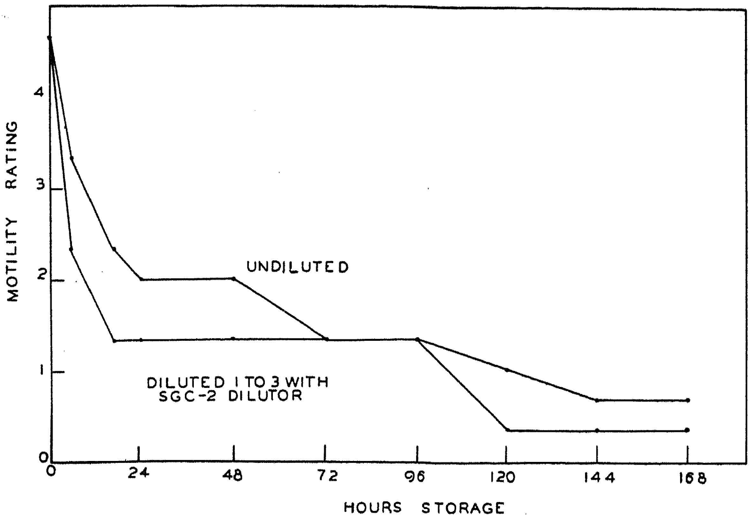

Fig. 20.—Comparison of motility maintenance in semen diluted 1 to 3 with SGC-2 dilutor and semen stored undiluted.

in Fig. 21. Dilution with E-Y-B dilutor caused the maintenance of more vigorous motility in semen than was observed in semen of the same samples stored undiluted. This beneficial effect

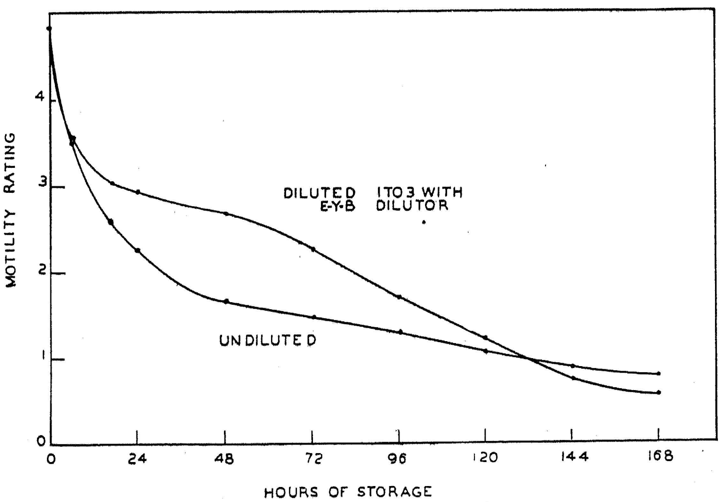

Fig. 21.—Comparison of motility maintenance in semen diluted 1 to 3 with E-Y-B dilutor and semen stored undiluted.

of dilutor on motility of the semen was observed up to the fifth day. After the fifth day of storage motility in the diluted semen dropped markedly while motility in the undiluted semen was maintained fairly unformly so that it averaged higher than did motility in the diluted semen. In most cases the diluted semen was observed to cease motility at about the same time or a little earlier than did the undiluted semen. Hence it would appear that the use of this dilutor would enhance the vitality of the semen up to the fourth or fifth day, but for periods of longer storage it would give little benefit.

The semen of individual bulls varied as to the effect of E-Y-B dilutor observed. The use of E-Y-B dilutor seemed to give very little, if any, beneficial effect on motility in semen of bulls 49, 50, 51 and 47. In semen of bulls 48 and 55, however, dilution with E-Y-B dilutor gave very striking favorable results. The semen of bulls 48 and 55 was very much alike in that it was thicker and more viscous in storage than the normal semen of the other bulls. Since semen of these two bulls was also benefitted alike by dilution with the E-Y-B dilutor while semen of the other bulls was not strikingly benefitted by the use of this diluent, it seems that the properties of the semen, such as viscosity, may determine whether or not dilution will enhance the viability of semen in storage.

Effects of Removing Fluid Portion of Semen

Since the secretions of the accessory sex glands had been shown to be harmful to survival of the spermatozoa, a few samples of semen were stored after removal of the sperm fluid. Separation was accomplished by centrifuging the fresh semen at 1300 RPM for 15 minutes and drawing off the fluid with a pipette.

Storage of the concentrated spermatozoa without dilution was not successful. Such semen was diluted at examination with physiological saline or 5 per cent glucose solution. After six hours storage less than 50 per cent of the spermatozoa were in motion and at 48 hours no motion could be observed whatever.

Dilution of the centrifuged spermatozoa with four volumes of 3 per cent glucose or SGC-2 dilutor to one volume of concentrated sperm likewise proved of little value for the maintenance of motility. Motility rating of semen treated in this manner was 1 at six hours while the same samples stored in their normal fluid had motility ratings of 3 and 4 at six hours. A low motility rating was maintained for three to five days in the centrifuged-glucose diluted semen. It perished before the same semen stored naturally did, however.

The dilution of the concentrated sperm with four volumes of E-Y-B dilutor gave a very decided benefit to the spermatozoa as measured by motility comparisons with the semen of the same sampled stored in its natural fluid. Motility ratings of semen used in fourteen such comparisons were tabulated. The averages of the motility ratings at each examination are graphically presented in Fig. 22. It can be seen that the effect of dilution with E-Y-B dilutor after centrifuging is very similar to the effect of dilution of the entire semen with the same dilutor but to a much greater degree. Very good motility was maintained for 48 hours in most of the samples diluted after centrifuging. In every instance dilution after centrifuging gave

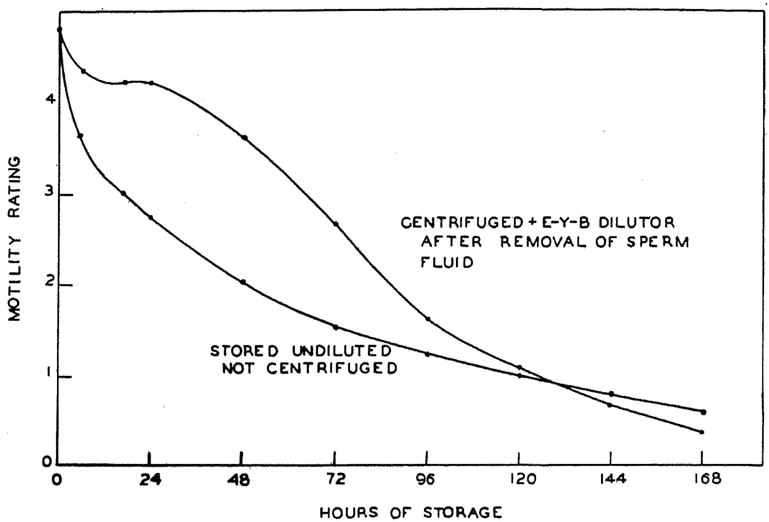

Fig. 22.—Comparison of motility maintenance in semen stored undiluted and semen stored after replacing sperm fluid with E-Y-B dilutor.

a longer maintenance of a high rate of motility than was observed in the same samples stored untreated. This was not the case in all samples merely diluted without removal of the sperm fluid. In a few instances samples were divided three ways. One part was held undiluted, one was diluted with E-Y-B dilutor, and the third was centrifuged and diluted with E-Y-B dilutor. In these cases it was found that the centrifuged diluted samples retained much better motility than the others for the first three days, as shown in Figs. 21 and 22. About the fifth day of storage, however, the motility ratings of the treated semen were practically the same as those of the untreated semen and thereafter the treated semen died more rapidly. It was characteristic of the centrifuged E-Y-B diluted semen to maintain a high rating of motility for several days then suddenly drop to a very low rating. The undiluted semen, on the other hand, usually changed more gradually from a high degree of motility to a lower one.

The results of these experiments show that replacing the sperm fluid with the E-Y-B dilutor, which had previously been shown to exert some beneficial effect on whole semen, resulted in a high rating of motility for the first three days of storage. Dilution with glucose dilutors did not prove beneficial. It would appear that, taking motility of stored semen as a criterion of fertility, the certainty of good breeding results from the use of semen stored for three days or less could be improved by centrifuging the semen and replacing the sperm fluid with E-Y-B dilutor. If the semen is to be stored for four or five days or longer, however, it appears that for most bulls storage of their whole semen undiluted is as good or better than any of the other methods tried.

Results of Interchanging Spermatozoa and Sperm Fluid of Different Bulls.—It was observed that motility of the semen of some bulls was retained only a short time, whereas that from other bulls was persistently high in motility. A brief experiment was conducted to see whether or not this loss of motility in some semen and persistence of motility in other semen was due to some property of the sperm fluid. Four samples of semen, two of which were suspected to be of good viability, were centrifuged in the manner as has been mentioned before. The clear fluid was then drawn off by pipette and put in storage vials. The concentrated sperm material was then mixed with the different samples of sperm fluid such that the spermatozoa of the two samples of good viability were put in fluid from the two samples of poor viability and vice versa. The motility ratings of these different mixtures are graphically presented in Fig. 23. Sample No. 50A was the first ejaculate from bull 50 and sample No. 50B was his second ejaculate. Sample No. 55 was from bull 55 and sample No. 49 was from bull 49. All samples were secured the same day at the same time. The solid lines in each graph represent spermatozoa of a sample in fluid of that sample. The dotted lines represent spermatozoa of the same sample in fluid of either a poorer or a better quality sample whichever the case may have been. It was noted that the motility of spermatozoa of any one sample generally was very much the same regardless of the sperm fluid in which it was mixed. Spermatozoa of samples No. 50A and No. 50B showed the same motility in foreign sperm fluid as in their own sperm fluid. Spermatozoa of bulls 49 and 55 showed slightly better motility in their own sperm fluid than in foreign fluid. The lowering of motility did not appear to be due to the effect of the foreign fluid, however. Some detrimental effect from the centrifuging and mixing may have been the cause of the lowered motility observed in these samples.

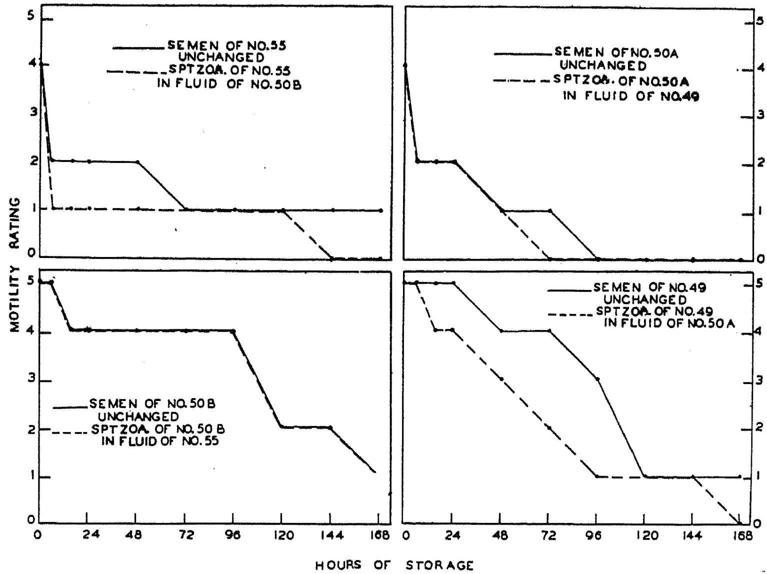

Fig. 23.—Comparisons of motility maintenance of spermatozoa in their natural fluid and in the sperm fluid of different bulls.

This experiment in general, however, indicated that the motility of spermatozoa after ejaculation was more dependent on a property of the spermatozoa themselves than upon the type of sperm fluid in which the spermatozoa were suspended. Spermatozoa of good quality semen showed good motility in sperm fluid from poor quality semen and spermatozoa from poor quality of semen showed poor motility even when put in the normal fluid of the spermatozoa of good quality.

Results of Insemination Using Stored Semen.—Whenever possible stored semen was used in the insemination of cows in the University of Missouri and a few cooperating herds. The results of inseminations made with stored semen during the winter of 1939-40 along with the motility of the semen at the time it was used, the percentage of abnormal spermatozoa of each semen sample, and the hours of storage are given in Table 17. A few of the stored samples of semen were diluted with E-Y-B dilutor. These are indicated with asterisks. Most of the stored semen used, however, had been stored undiluted. Any sample of semen was considered stored if it had been cooled to 40° Fahrenheit immediately after collection and had been stored for four hours or more before use. This terminology was considered correct for semen handled in this manner because

TABLE 17. PROPERTIES OF SEMEN USED IN INSEMINATION AFTER IT HAD BEEN STORED AT 40°F--DIVIDED AS TO THE RESULT OF THE INSEMINATION.

Conceptions resulting from stored semen					Failures from use of stored semen				
Bull No.	Date of Insemination	Motility in Semen When Used	Abnormal Spermatozoa per 1000	Hours Semen in Storage	Bull No.	Date of Insemination	Motility in Semen When Used	Abnormal Spermatozoa per 1000	Hours Semen in Storage
47	12-19-39	3	78	74*	47	11-13-39	4	171	20
47	12-29-39	2	93	198*	47	12-26-39	2	93	125*
48	1- 5-40	3	102	4	55	12-23-39	1	21	55*
48	3-12-40	1	105	68	55	2-17-40	4	57	48*
48	3-12-40	3	117	11	49	2- 7-40	4	84	24
48	3-31-39	3	---	10	49	2- 9-40	4	84	72
54	2- 5-40	4	138	44	49	2-15-40	3	72	120
54	4-10-40	4	87	24	49	3- 7-40	4	51	56**
49	1-30-40	3	96	72	53	3-26-40	1	186	20
49	3-20-40	4	63	16**	50	3-18-40	2	294	96
49	3-21-40	5	63	48	50	3-21-40	4	228	4
53	3-28-40	1	108	24					
50	4-6-40	2	120	24					
50	3-21-40	4	228	4					
40	3- 2-40	3	60	115					
Average		3	104	49.0			3	122	58.2

*Diluted 1-3 with E-Y-B diluent.

**Centrifuged and sperm fluid replaced by 2 volumes E-Y-B diluent.

of the fact that motility of the average sample of semen declined very rapidly during the first 24 hours of storage (see Fig. 19).

Fifteen samples of stored semen used resulted in conception. The average time of storage was 49 hours and it varied from 4 to 198 hours. The motility of the semen when used varied from a rating of 1 to 5 with a mean of 3. Eleven of the inseminations made with stored semen failed to produce conception. The average storage period of these samples was 58.2 hours with a range of 4 to 125 hours. The average motility rating was likewise 3 and it varied from 1 to 4. There was very little difference in the average percentage of abnormal spermatozoa.

Two of the samples of semen were represented in both groups, that is one cow was settled by use of the sample and one cow failed to conceive after use of the same sample of semen. From these results it appears that the genital health of the cow and/or the time of insemination with respect to ovulation are quite important in the use of stored semen in artificial insemination. Some of the cows in one of the cooperating herds were afflicted with vaginitis. As many cows known to have vaginitis were settled with stored semen, however, as there were which failed to settle from insemination with stored semen.

The inseminations and conceptions from semen according to the length of time it was stored are given in Table 18. The average motility ratings according to the time of storage are also given. Table 18 shows that a majority of the inseminations (21 out of 26) were with semen less than 89 hours old. Only two conceptions were secured from four inseminations with semen more than 100 hours old; only one conception resulted from three inseminations with semen over 120 hours old, and this one was with semen 198 hours old. The average services per conception for all the stored semen used was 1.73.

From these results it appears that semen that is stored undiluted up to 100 hours and which was of good motility (rating 2 or 3) was effective in producing conceptions. Conceptions with very good quality semen can be expected above 100 hours storage.

Stored semen of poor motility (rating 1) was effective in settling cows in two instances and failed in two instances.

In the summer of 1938 an artificial breeding association was organized in cooperation with the Farm Security Administration near Hughesville, Missouri, some 75 miles from Columbia, Missouri. In the beginning, before the organization had secured bulls, semen was collected every other day from bulls in the University of Missouri dairy herd and used to inseminate cows in this organization. Cows inseminated on any one day were those which had first been observed in estrum either the day before or the day of the insemination. All of the semen used

TABLE 18. RESULTS OF INSEMINATIONS WITH STORED SEMEN ACCORDING TO THE TIME OF STORAGE

Hours Semen was Stored	Average Motility when Semen was used	Number of Inseminations	Number of * Conceptions
Under 20	3.5	6	5
20 - 39	2.7	6	3
40 - 59	3.6	5	2
60 - 79	2.8	4	3
80 - 99	2.0	1	0
100 -119	3.0	1	1
120 -200	2.3	3	1
Totals & Average	3.0	26	15

*Average of 1.73 inseminations per conception.

TABLE 19. RESULTS OF THE USE IN AN ARTIFICIAL INSEMINATION RING OF DILUTED AND UNDILUTED SEMEN AFTER STORAGE FOR 4 TO 8 HOURS AT 45° FAHRENHEIT

Treatment of Semen	Semen Diluted				All Diluted Semen	Un-diluted Semen	Totals
	1:0.5	1:1	1:3	1:4			
Cows settled	3*	2	5°	2*	12	25	37
Cows not settled	0	14	6°	0	20	54	74
Total inseminations	3	16	11	2	32	79	111
Inseminations per conception	1.00	8.00	2.20	1.00	2.67	3.16	3.00

*Cows in each group bred from same sample of semen, respectively.

°Both with semen of Bull No. 2

in this manner came under the category of stored semen since it was never used before four or five hours after collection and was held at a temperature of about 45° Fahrenheit. Some of the semen had been diluted with SCG-2 diluter.

The results of inseminations with this stored semen are summarized in Table 19. From 111 inseminations 37 conceptions resulted, making a ratio of 3 services per conception. The diluted semen seems to have been slightly more effective than the undiluted semen. They required respectively 2.67 and 3.16 services per conception. This is not considered a significant difference, however, because a greater proportion of the cows bred the day after they came in heat (which in some cases may be a disadvantageous time) were bred with undiluted semen than were bred with diluted semen. Five of the twelve cows settled by the use of diluted semen were inseminatd from two samples, both from very fertile bulls. The high service rate for semen diluted 1:1 is largely due to the fact that most of the samples of semen were from bull 52 which was later proved to be of poor fertility. Semen from this bull made up many of the samples used undiluted, also. Another factor which caused the service rate to be high was the poor health and condition of many of the cows. Many were settled only after five or more services and some were given up as non-breeders.

The results of this work showed that little, if any, advantage as to fertility of the stored semen was to be gained by dilution with the diluent used. The health and management of the cows appeared to be a very important factor in the use of stored semen also, since in herds where the cows were adequately fed and of sound genital health the service rate was very low.

Some Results From Use of Fresh Semen Under Field Conditions.—After the artificial insemination association at Hughesville, Missouri had secured bulls, the practice of using the uncooled semen for insemination within an hour or two after collection was followed. The records of this organization have been analyzed and the results are presented in Table 20.

It is shown that the services required per conception when cows were inseminated the same day as first observed in heat were 1.81. When cows were inseminated the day after heat was first observed 2.06 services were required per conception. Three cows were settled out of six inseminated the second day after they had first been observed in heat. Cows which were known to be non-breeders have been eliminated from the data.

It appears from these records that better results are obtained from inseminating cows the same day they are observed in heat rather than the day after or later. However, a relatively

TABLE 20. RESULTS OF ARTIFICIALLY BREEDING COWS WITH FRESH SEMEN AT VARIOUS STAGES OF ESTRUM

Stage of Estrus Cycle at which cow was inseminated	Number of Insemina-tions	Number of Cows Settled	Average Insem-ination per Conception
4-12 hours. Same day as observed in heat	901	496	1.81
12-36 hours. Next day after heat first observed	180	87	2.06
30-60 hours. Second day after heat was first observed	6	3	2.00

TABLE 21. RELATION BETWEEN AMOUNT OF FRESH SEMEN USED AND THE INSEMINATIONS REQUIRED PER CONCEPTION FOR BULLS NO. 27, NO. 28, AND NO. 29. PERIOD OF JULY, 1938 TO APRIL, 1940

Amount of Semen Used	Number of Inseminations	Number of Cows Settled	Inseminations Per Conception
0.1	2	2	1.00
0.2	65	24	2.70
0.3	3	0	----
0.4	203	105	1.93
0.5	86	45	1.91
0.6	169	96	1.76
0.7	2	2	1.00
0.8	66	37	1.78
0.9	0	0	----
1.0	324	182	1.78
1.1 to 2.0	10	7	1.43
Totals & Average	930	500	1.86

large percentage of the cows inseminated the day after they first appear in heat can be expected to conceive.

The dosage of undiluted semen used in artificial insemination has varied from 0.1 cc. to 2.0 cc. To show the effect of using different amounts of semen in the insemination dose the data from the cooperative artificial breeding association at Hughesville, Missouri were analyzed. The results are presented in Table 21. The largest number of inseminations were made using 1.0 cc. of semen. The next largest number were made with 0.4 cc. of semen. The records in Table 21 show that the service rate is practically independent of the amount of semen used in insemination when quantities of 0.4 cc. or more are used.

DISCUSSION OF RESULTS

Artificial insemination has placed a great responsibility on a few bulls. In natural service a single ejaculation is used to impregnate one cow. In artificial breeding, however, a single ejaculate may be used for 5 to 20 cows. If a single cow bred naturally fails to conceive, the loss is not great; but if a number of cows inseminated from an ejaculate fail to conceive because of deficiency in the semen, the loss becomes of serious proportions. In addition to this a large quantity (as in natural service) of poor quality semen may contain enough good spermatozoa to enable fertilization to occur; but the chances of fertilization with a small quantity (as in artificial insemination) of poor quality semen are greatly reduced. For these reasons the variations in quantity and quality of semen produced by an individual bull are of importance and must be taken into account in the operation of an artificial breeding association.

This study has shown that the dairy bull semen produced varies widely in its respective characteristics from one collection to the next. Initial motility varied less than the other characteristics of the semen examined. The duration of good motility was quite variable, however. The most outstanding difference between semen from bulls of good fertility and semen from bulls of poor fertility was the longer time of survival of good motility in the former. This is a reasonable relationship because of the fact that time is consumed in the journey of the spermatozoa from the cervix to the Fallopian tubes and also in the release of the ovum. The spermatozoa which cannot survive the necessary length of time obviously will be of no value for impregnation. Since the factor of survival is normally variable from one ejaculate to the next for any given bull, it appears that it will be difficult to guarantee uniform results in artificial insemination, especially if the semen has been stored. The use of bulls whose semen has a high average time of survival of good motility should make results in an artificial insemination association more certain, however. Frequent examination of the semen and observation of the survival time under storage conditions should be routine. The breeding record of the bull in question, either by natural or artificial means continues to be a highly reliable index to his general fertility.

Artificially increasing the survival of good motility in bull semen by the use of diluents such as the E-Y-B dilutor shows some promise. Further use of this method supported by pregnancy results with the same serum diluted and not diluted will be necessary to prove the advantage of it over the use of undiluted semen.

The exact causes for the variation in behavior of the spermatozoa under storage conditions have not been determined. However, the results of storage, dilution, and interchanging of semen may strongly suggest that variations in length of the survival time of spermatozoa are due to intrinsic properties of the spermatozoa themselves. The hypothesis that decline in sperm activity is due to (1) a depletion of nutrients from the sperm fluid or (2) autointoxication does not satisfactorily explain these variations. The variation in the duration of motility and the rate of decline of motility in semen which seemed the same in other respects lends support to the theory advanced by Gray (1931), that the decline in motility is determined by intracellular units of the spermatozoa themselves which are not the same for all the units of the population. If this theory is true, the effect of dilution to increase sperm survival is probably not one of nutrition but one of furnishing for the spermatozoa a most favorable environment so that their "store of energy" will be used most efficiently. The findings of Bernstein (1933c) that nutrient substances in the diluent did not cause increased sperm survival likewise refute the idea of nutrition of the spermatozoa. Further experimentation is necessary to prove whether or not the spermatozoa assimilate substances from the surrounding fluids for the production of energy.

The variation in morphology of the spermatozoa of the same bull was not as great as was the variation of motility maintenance. The importance of morphology of the spermatozoa to fertility seemed to be secondary in most cases. That is, regardless of the percentage of abnormal spermatozoa, if the semen lacked high viability, it was of low fertility; but semen that was high in percentage of abnormal spermatozoa was often fertile, apparently because it had high viability. Semen that was very high in percentage of abnormalities, however, was usually of poor motility and viability. Hence, the use of the average percentage of abnormal spermatozoa produced as a basis of judgment of the bull's fertility is to some extent justified. From this study it appears that the upper limit of percentage of abnormal spermatozoa compatible with good or fair fertility is approximately 30 per cent. Semen which is below 30 per cent in abnormal spermatozoa is not necessarily fertile, however, because other factors important to fertility, such as motility and viability of the spermatozoa, varied independently of morphology. Semen of some bulls contained spermatozoa which were of excellent morphology and high initial motility but which were very low in viability. For these reasons the importance of abnormal morphology or initial motility for the evaluation of semen to be used in artificial insemination is limited. As a rule, the time of survival of samples of low initial motility was very short, hence it is not advisable to use such semen for artificial insemination.

The cause of the variations in production of abnormal spermatozoa in normal ejaculates is largely due to processes occurring in the testicles, according to Lagerlöf (1934). Sciuchetti (1938) found the same variation in abnormal spermatozoa in the contents of the vas deferens as in the ampulla, which indicated that no noticeable change in morphology occurred in the ampulla. It was observed that contaminating the semen with urine or water often produced an excessive percentage of loosely coiled tails. These were different than the tightly coiled tails observed characteristically in the semen of some bulls and which were undoubtedly due to genitalian abnormality. The significance of the different types of abnormalities could not be determined from the data in this study. Williams (1920) stressed the serious defect of a large percentage of pyriform heads. The results of this study, however, indicate that pyriform heads are of no more significance than other abnormal types encountered. Williams (1920), Williams and Savage (1925 & 1927) and Lagerlöf (1934) evidently did not consider coiled tailed spermatozoa abnormal since they did not describe abnormalities with coiled tails. This probably accounts for the fact that they considered bulls which produced more than 170 to 180 abnormal spermatozoa per 1000 (meaning abnormal heads) of low fertility. Coiled tails were considered abnormal by McKenzie and Berliner (1937) and McKenzie and Phillips (1934) and also by Moench (1931) and Mason (1929). In the observations of this study it was noticed that the coiled tails unaccompanied by abnormal heads made up the predominating type of abnormal spermatozoa for some bulls of low fertility. For this reason, coiled tails were considered abnormal and this explains the fact that some sires having above 17 to 18 per cent abnormal sperm were fertile, whereas Williams and Lagerlöf, apparently basing their judgment on head types alone classed such bulls as low in fertility.

The variations in pH of the fresh semen are not well understood. Since semen of high pH was usually also of low concentration of spermatozoa it seems that the pH may vary with the extent of secretion of one or more of the accessory sex glands. The results of this work agree in the large part with Kuhne (1936) in that the semen of pH 6.60 was most often of better quality than that of higher pH. A few samples of semen of pH above 6.60 were of good quality, however, and a great many of lower pH were of poor quality. Hence, the significance of pH for evaluation of the semen to be used in artificial insemination seems slight. A rise in pH due to contamination with urine or other fluids at collection is also accompanied by low initial motility; so in such cases the pH determination is not necessary to show the poor quality of the semen.

Variations, such as volume and concentration of spermatozoa, were regularly observed in successive collections from the same bull as well as between the different bulls. The bulls were handled as nearly as possible, in the same manner every time, but many of the variable characteristics of bull semen and the reactions responsible are apparently regulated by mechanisms beyond control of the operator. No doubt they are to some extent due to various forms of stimula concerned with involuntary muscle action.

The work on the use of diluents in storage and their effect on the viability and fertility of the semen has not been extensive enough to be conclusive. The results indicate, however, that semen of good quality can be stored undiluted and successfully impregnate cows after three to five days of storage at 40° Fahrenheit. Longer periods of storage have not been tried except in one instance (198 hours) which was successful—this sample had been diluted with E-Y-B dilutor. The E-Y-B dilutor was the only diluent tried which gave any beneficial effect whatsoever over the use of undiluted semen. The benefit to be derived from the use of this diluent seemed to be dependent upon the properties of the semen. When used with semen that was normally of very good viability it gave little if any benefit; but its use with semen which tended to lose motility rapidly in some cases greatly increased the persistency of motility and time of survival of the spermatozoa. From these results it would appear that the main benefit of the E-Y-B dilutor was to counteract an unfavorable environment and substitute for it a favorable one in some samples of semen. In other samples the natural environment of the sperm fluid was not improved upon and the spermatozoa maintained motility as well and longer in the undiluted semen than they did in the diluted semen.

From the standpoint of evaluating the fertility of a given sample of semen before use, the length of the time the spermatozoa survive in storage with a high rating for motility seems most valuable. The average time of spermatozoal survival with good motility in several samples secured from a bull at regular intervals offers a good index of the fertility of that bull, and can well be applied in practical applications of artificial breeding. Semen from highly fertile bulls will produce conception more often than semen from bulls of poor fertility. Thus, the actual breeding records also furnish a most excellent, and the most dependable index of fertility. These two factors may be recommended for evaluating any sample of semen which appears normal in measurable characteristics at the time of collection. The more samples of semen examined from any one bull, the more accurate will be the forecasting of that bull's fertility. Because of the variation in samples secured from the same bull, it appears that the maintenance of fertility in any one sample for a long period of time is to some extent a matter of chance and not accurately predictable at the time of collection. Another basis for predicting the life of a semen sample is its initial motility. If initial motility is very low, the duration of motility in the semen will be short.

SUMMARY

1. A critical examination of semen from 342 separate ejaculates representing 55 dairy bulls has been made. The fertility of 51 of the bulls has been determined from their breeding records and compared with the examination of their semen.
2. The semen samples were found to vary widely in all properties studied. The variation was observed in different ejaculates of the same bull as well as in ejaculates of different bulls. The variations in initial motility and pH were not great. The greatest variations observed were in the length of time vigorous motility persisted and in percentage of abnormal spermatozoa.
3. The time of survival with good motility was found to be a good index of fertility of the semen. The longer vigorous motility was maintained in a sample of semen, the greater was the chance of that semen producing conception.
4. The pH, initial motility, and percentage of abnormal spermatozoa were found to be related to fertility of the semen to only a limited extent. Semen of high pH, 7.00 or higher, was usually of very low viability. Semen of poor initial motility survived only a short time. A normal pH, good initial motility and low percentage of abnormal spermatozoa were an indication but not assurance of good fertility.

5. Morphologically abnormal spermatozoa were found in every sample of semen examined. Bulls which produced semen averaging 30 per cent of abnormal spermatozoa were usually of poor fertility, but not all samples of semen containing over 30 per cent abnormal spermatozoa were infertile. The most common types of abnormal spermatozoa, in order of their occurrence, were coiled tails, tailless, and pyriform. In the semen observed in this study no one of the three main types of abnormal spermatozoa seemed of more significance to infertility than other types.

6. Survival time of semen which was normally of good quality was not increased by dilution with any of the common diluents used. Survival of semen from a few bulls was materially increased by dilution with egg-yolk-buffer dilutor. Such semen normally became thick and viscous in storage and the dilutor apparently prevented this or other deleterious effects.

7. Fertility of good quality semen was maintained from 3 to 5 days when stored undiluted at 40° Fahrenheit.

8. The fertility of a bull cannot be accurately estimated from a single semen examination. Three or more semen samples examined several days, or even weeks apart, with accompanying records of the bull's actual breeding record, provides the most accurate method of evaluating fertility.

BIBLIOGRAPHY

Addis, M. 1937. *Modifications of spermatozoa in infertility.* Nuovo Ercol. 42; 455-464. Abst. in An. Breed. Abst. 7:218.

Bartlett, J. W. and E. J. Perry 1939. *Lessons learned from eighteen months' experience with cooperative artificial breeding of dairy cattle in New Jersey.* Proc. of Am. Soc. An. Prod. 32: 243.

Bederke, G. 1933. *Studies on the influence of various methods of storage on vitality of dog sperm.* Arch. Tierernährg. Tierz., 9: 585-622. Abst. in An. Breed. Abst. 1: 276.

Bernstein, A. 1933a. *Glucose metabolism in semen.* Orenburg Vet. Inst.—Studies of Physiol. of Spermatozoa, 9. Abst. in Physiol. Abst. 26: 331.

Bernstein, A. 1933b. *Problems of artificial insemination.* Probl. Zhivotn. No. 1: 77-88. Abst. in An. Breed. Abst. 1: 82.

Bernstein, A. 1933c. *Nutrient media and equilibrated salt solutions as diluents for semen.* Orenburg Vet. Inst. Studies on Physiology of Spermatozoa 116. Abst. in Physiol. Abst. 20: 322.

Bernstein, A. and H. Lasarev 1933. *Effect of blood on the activity of spermatozoa.* Orenburg Vet. Inst. Studies on Physiology of Spermatozoa 73. Abst. in Physiol. Abst. 20: 332-3.

Bernstein, A. D. and V. V. Petropavlovsky 1937. *The action of non-electrolytes upon the survival of sperm.* III. Bul. Biol. Med. exp. URSS. 3: 39-40. Abst. in An. Breed. Abst. 7: 177.

Bernstein, A. and J. Slovochotov 1933. Orenburg Vet. Inst. *Studies on the Physiology of Spermatozoa* BF. Abst. in Physiol. Abst. 20: 332.

Birillo, I. M. and L. H. Puhaljskii 1936. *Problems of prolonged storage of bull and ram sperm.* Prob. Zhivotn. No. 10, 24-40. Abst. in An. Breed. Abst. 5: 219-220.

Bretschneider, W. 1936. *Biological studies on bull spermatozoa.* Inaug. Diss., Univ. Leipzig. Abst. in Exp. Sta. Rec. 77: 612.

Comstock, R. E. 1939. *A study of the mammalian sperm cell. I. Variations in the glycolytic powers of spermatozoa and their relation to motility and its duration.* J. Exp. Zool. 81: 142-164.

Comstock, R. E. and W. W. Green 1939. *Methods for semen evaluation. I. Density, respiration, glycolysis of semen.* Proc. Am. Soc. An. Prod. 32: 213.

Davis, H. P. 1938. *Some factors affecting artificial insemination in cattle.* Proc. Am. Soc. An. Prod. 31: 246.

Davis, H. P. 1939. *Influence of massage obtained semen samples from bulls upon the inseminations required for conception, the period of gestation, and the sex ratio.* Celebrazioni Spallanzaniane 11-14 Aprile, 1939, xvii Regia Universita Di Pavia.

Davis, H. P. and N. K. Williams 1939. *Evaluating bovine semen. I. Influence of the number of ejaculates upon various physical and chemical characteristics and the relationship between those factors.* Proc. Am. Soc. of An. Prod. 32: 232.

Donham, C. R. and B. T. Simms 1931. *Fertility studies in the bull. I. Studies of the genitalia of bulls obtained from the abattoir.* Jour. Am. Vet. Med. Assn. 78: 658.

Donham, C. R., B. T. Simms, and J. N. Shaw 1931. *Fertility studies in the bull. II. The relation of the microscopic findings in semen to its fertility.* Jour. Am. Vet. Med. Assn. 78: 665.

Dubincik, J. 1934. *The influence of physico-chemical factors on vitality of spermatozoa.* Ginekologija, No. 3: 79-86. Abst. in An. Breed. Abst. 4: 256.

Generales, K. D. J., Jr., 1938. *Recent biometrical investigations of sperm and fertility.* Stuttgart. Ferdinand Euke. Abst. in An. Breed. Abst. 7: 197.

Gilman, H. L. 1922. *Genital infections in the bull.* Rept. of the N. Y. State Vet. Coll. 1920-21, p. 108.

Gilman, H. L. 1923. *A study of some factors influencing sterility and fertility in the bull.* Rept. of N. Y. Sta. Vet. Coll. 1921-1922, p. 68.

Gladcinova, E. F. 1937. *The influence of marked drops in temperature on survival of spermatozoa.* Usp. Zooteh. Nauk. 4: 56-64. Abst. in An. Breed. Abst. 7: 373.

Gray, J. 1931. *The senescence of spermatozoa.* II. Jour. Exp. Biol. 8: 202.

Hatziolos, B. 1937. *Studies on on the conservation of bull sperm for the purpose of artificial insemination.* Z. Zucht. 38: 199-254. Abst. in Exp. Sta. Rec. 79: 465.

Herman, H. A. 1939. *Some factors affecting the efficiency of artificial insemination of dairy cows.* Proc. Am. Soc. An. Prod. 32: 245.

Herman, H. A. and A. C. Ragsdale 1939. *Artificial insemination of dairy cows.* Mo. Ag. Exp. Sta. Bul. 407.

Ivanov, E. E. 1931. *On the question of the energy of the movements of the spermatozoa.* An. Breeding and Breeding Biol. including An. Feeding 20: 404. (Translated from German).

Ivanov, E. E. 1936. *Respiratory quotient of spermatozoa.* Bul. soc. chim. biol. 18: 1613-22. Abst. in Chem. Abst. 31: 1477[2].

Ivanov, I. I. 1935. *The action of monohaloacetic acids on the glycolysis and mobility of spermatozoa.* J. Physiol. 18: 950.

Ivanov, I. I. 1936. *Significance of non-carbohydrate metabolism for the maintenance of motility in spermatozoa.* Biochemistry 1: 245-54. Abst. in Chem. Abst. 30: 7645[3].

Kelley, P. L. 1940. *How should we manage the herd sire?* Hoard's Dairyman 85: 40.

Kersin, I. A. 1937. *Artificial insemination of sheep and cattle on collective farms in 1936.* Probl. Zhivotn. No. 3, 7-19. Abst. in An. Breed. Abst. 5: 252.

Kingman, H. E. 1936. *Artificial insemination.* J. Amer. Vet. Med. Assn. 88: 126.

Kirillov, V. 1934. *The sexual life of the bull.* First all-union Conf. artif. insemin. 1935. Abst. in An. Breed. Abst. 3: 255.

Kirillov. V. and V. Morozov. 1933. *The influence of sexual activity of bulls on their reproductive ability.* Probl. Zhivotn. No. 5, 90-95. Abst. in An. Breed. Abst. 1: 237.

Kirillov, V. S. and V. A. Morozov, 1936. *Duration of the vitality of spermatozoa of a bull in an epididymis isolated from the testiculae.* Inst. Zhivotn Usp. Zootekh. Nauk. 2: 19 Abst. in Exp. Sta. Rec. 77: 323.

Komarov, N. I. and E. F. Gladcinova 1937. *Storing bull sperm.* Probl. Zhivotn. No. 8, pp. 62-68. Abst. in An. Breed. Abst. 6: 106.

Kozlova, V. M. 1937. *Can cows be inseminated with doses of 0.1 cm.[3]. of undiluted sperm?* Probl. Zhivotn. No. 12, 137-139. Abst. in An. Breed. Abst. 7: 121.

Kufarev, A. P. 1935. *Insemination of cows with undiluted sperm.* Probl. Zhivotn. No. 6, pp. 107-108. Abst. in An. Breed. Abst. 4: 35.

Kuhne, W. 1936. *Untersuchungen von Bullensperma auf Beschaffenheit und Eignung fur die kunstliche Besamung.* Dissertation. Univ. Giessen.

Kust, 1936. *Artificial insemination in cattle.* Bert. tierarztl. Wschr. 52: 805-808. Abst. in An. Breed. Abst. 5: 142.

Lagerlőf, N. 1934. *Morphologishe Untersuchungen über Veranderungen im Spermabild und den Hoden bei Bullen mit verminderter oder aufgehobener Fertilitat.* Acta. Pathologica et Microbiologica Scandinavica, Supplementum, XIX.

Lardy, H. A. and P. H. Phillips 1939. *Preservation of spermatozoa.* Proc. Am. Soc. An. Prod. 32: 219.

Lewis, L. L. 1911. *The vitality of the reproductive cells.* Okla. Ag. Exp. Sta. Bul. 96.

McKenzie, F. F. 1939. *Artificial insemination in livestock.* The Cattleman, Vol. 26, No. 4, p. 53.

McKenzie, F. F. and V. Berliner 1937. *The reproductive capacity of rams.* Mo. Ag. Exp. Sta. Res. Bul. 265.

McKenzie, F. F. and R. W. Phillips 1933. *Determining fertility in the boar.* Mo. Ag. Exp. Sta. Bul. 328.

McKenzie, F. F. and R. W. Phillips, 1934. *Measuring fertility in the ram.* Jour. Amer. Vet. Med. Assn. 84: 189.

MacLeod, J. 1939. *The metabolism of human spermatozoa.* Proc. Soc. Exp. Biol. Med. 42: 153.

Mason, W. 1929. *Sterility with special reference to the spermatozoa.* Amer. Jour. Obst. and Gynec. 17: 376.

Miller, F. W. 1934. *The technic of artificial insemination with dairy cattle.* Mimeo. BDIM—738. Bureau of Dairy Industry, U. S. D. A.

Milovanov, V. K. 1933. *Three years work on dilutors for sperm of livestock.* Probl. Zhivotn. No. 4: 95-100. Abst. in An. Breed. Abst. 1: 153.

Milovanov, V. and Kh. Khabibulin 1933. *Anabiosis of spermatozoa and its utilization in socialistic animal breeding.* Probl. Zhivotn., No. 5: 83-90. Abst. n An. Breed. Abst. 1: 225.

Milovanov, V. K. and O. A. Selivanova 1932. *Dilutors for sperm of livestock.* Probl. Zhivotn. No. 2: 75-86. Abst. in An. Breed. Abst. 1: 153.

Moench, G. L. 1929. *The investigation of the relation of sperm morphology to fertility by means of microdissection.* Amer. Journ. Obst. and Gynec. 18: 53.

Moench, G. L. and H. Holt, 1931. *Sperm morphology in relation to fertility.* Amer. Obst. and Gynec. 22: 199.

Nesmejanova, L. N. 1938. *The K, Na, and Ca, content of the sperm and accessory secretions of the bull and ram.* Lab. Artif. Insem. Inst. Anim. Breed. Moscow. Abst. in An. Breed. Abst. 7: 204.

Phillips, R. W. 1935. *The physiology of spermatozoa.* Proc. of Am. Soc. An. Prod. 28: 222-236.

Perry,, E. J. and J. W. Bartlett 1939. *Artificial insemination of dairy cows.* N. J. Ag. Exp. Sta. Exten. Bul. 200.

Petrov, E. F., and M. S. Schneerson 1933. *The keeping of the sperm fluid in the dilutor GFK-4.* Proc. Leningr. Inst. Dairy Cat. Breed. 1: 92-99. Abst. in An. Breed. Abst. 2: 107.

Polowzow, W. 1928. *Die spermatozoenproduction beim Pferde I. Mitteibung: Über den Einfluss der haufigen Kohabitationem auf die Spermienproduction.* Pfluger's Arch. f. d. ges Physiol. 218: 374-385.

Reynolds, E. 1916. *Fertility and sterility.* Jour. of Amer. Med. Assn. 67, 1193.

Rumjancev, N. V. and N. A. Flegmatov 1935. *On the influence of secretions of the female genital organs on spermatozoa.* Trud. Mosk. zoovet. Inst. 2: 163-175. Abst. in An. Breed. Abst. 6: 255.

Savage, A., W. W. Williams, and N. M. Fowler 1927. *A statistical study of the head length variability of bovine spermatozoa and its application to the determination of fertility.* Trans. Roy. Soc. Can. 21: 425.

Savage, A., W. L. Williams, and N. M Fowler 1930 *A study of the head length variability of equine spermatozoa.* Can. Journ. of Res. 3: 327.

Schneerson, S. S. 1936. *The importance of determining the pH of sperm for artificial insemination of cattle.* Sborn. Trud. zooteh, Kaf. s. h. Skol. Kirov. 1: 142-147. Abst. in An. Breed. Abst. 4: 419.

Sciuchetti, A. 1938. *Investigation of fertility of some stud bulls.* Z. Tierz. 41: 161-224. Abst. in An. Breed. Abst. 7: 121-122.

Shergin, N. P. 1935. *The acidity of the sperm.* Prob. An. Husb. 12: 100-122. Abst. in Chem. Abst. 30: 2622.

Shergin, N. P. 1937. *Glycolysis in the sperm of farm animals.* Probl. Zivotn. 12: 126-132. Abst. in An. Breed. Abst. 7: 181.

Shergin, N. P. 1939. *Respiration of sperm of farm animals.* Dokl. Akad. seljskohoz. Nauk No. 2/3 pp. 60-64. Abst. in An. Breed. Abst. 7: 181.

Smirnov-Ugrjumov, D. V. and M. A. Laptev 1938. *The feeding of stud bulls during mating season.* Probl. Zhivotn. No. 2: 64-72. Abst. in An. Breed. Abst. 6: 293.

Steensma, R. 1938. *Storage of ram and bull semen.* Master's Thesis. University of Missouri.

Strocenko, E. 1934. *The influence of drugs on the viability of spermatozoa.* Ovcevodstvo, No. 9: 37-38. Abst. in An. Breed. Abst. 3: 108.

Volskov, P. A. 1935. *Sexual activity and sperm quality in the horse.* Konevodstvo. No. 2: 45-49. Abst. in An. Breed. Abst. 3: 245.

Vorobjev, S. M. and M. S. Schneerson 1933. *Results of artificial insemination on Farm No. 2 of the Institute.* Proc. Leningr. Inst. Dairy Cat. Breed. 1: 100-106. Abst. in An. Breed. Abst. 2: 113.

Walton, A. 1933. *The technique of artificial insemination.* Imperial Bureau of Animal Genetics. Oliver & Boyd, Edinburgh, Scotland.

Walton, A. and J. Edwards 1938. *Criteria of fertility in the bull. I. The exhaustion test.* Proc. Amer. Soc. An. Prod. 31: 254.

Weber, H. 1936. *Zur physiologie des Bullenspermas (Im. Hinblick auf die kunstliche Besamung.)* Vet. Med. Dissertation, Leipzig.

Webster, W. M. 1932. *Bovine sterility in New Zealand.* Australian Vet. Jour. 8: 199-222. Abst. in An. Breed. Abst. 1: 96.

Williams, W. L. 1921. *The diseases of the genital organs of domestic animals.* First edition. Published by author. Ithaca, New York.

Williams, W. L. 1932. *The pathology of reproduction. The role of the male in the pathology of reproduction.* Vet. Med. 27: 288.

Williams, W. L. 1939. *Diseases of the genital organs of domestic animals.* Second Edition published by W. L. Williams, Ithaca, New York.

Williams, W. W. 1920a. *Technique of collecting semen for laboratory examination with a review of several diseased bulls.* Cornell Vet. 10: 87-94.

Williams, W. W. 1920b. *Diseases of the bull interfering with reproduction.* Jour. Amer. Vet. Med. Assn. 11: 29.

Williams, W. W. and A. Savage 1925. *Observations on the seminal micropathology of bulls.* Cornell Vet. 15: 353.

Williams, W. W. and A. Savage 1927. *Methods of determining the reproductive health and fertility of bulls.* A review with additional notes. Cornell Vet. 17: 374-385.

Windstosser, K. 1935. *Respiration of mammalian spermatozoa.* Klin. Wschr. 14: 193.196. Abst. in An. Breed. Abst. 3: 339.

Winters, L. M., R. E. Comstock, C. L. Cole, W. W. Green, and J. J. Bulik 1938. *Artificial insemination of farm animals.* Minn. Agr. Exp. Sta. Bul. 336.