BOVINE SEMEN CHARACTERS AND THEIR RELATION TO FERTILIZING CAPACITY IN ARTIFICIAL INSEMINATION

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TABLE OF CONTENTS

BOVINE SEMEN CHARACTERS AND THEIR RELATION TO FERTILIZING CAPACITY IN ARTIFICIAL INSEMINATION

Introduction

The problem of livestock improvement covers a wide field of studies and have been attacked effectively by the proper use of breeding methods. The advantages in using artificial insemination as a method of livestock breeding have been discussed by many workers including in recent years Walton (106), Smith (91), Cole (25), Bartlett and Perry (13), Lambert and McKenzie (50), and Hammond (37).

It is true that artificial insemination is, as Waddington (104) stated, the most important new biological technique at present passing into general practice. Its great advantage from the economic standpoint lies particularly with its allowing the dairy breeders to use well-bred sires at low cost. Its importance in animal production has emphasized the great need for better methods of semen evaluation and of measuring male fertility. During recent years, great attention has been paid to reveal the relationship between semen characteristics and the fertilizing capacity of the spermatozoa. Yet, the literature reveals that although investigators have their preferences in one technique over another, there is still no one really satisfactory criterion of semen evaluation that has

large predictive value.

The investigation was therefore designed with the hope of appraising the present methods of semen evaluation that are of greatest value so that semen may more quickly and more economically be screened to the end that only semen of acceptable quality may be used, thus insuring a relatively high and economical conception rate.

Review of Literature

1. Cyto-physiological Aspects of Spermatozoa

Spermatozoa were first observed by Ham in 1677 (36), and Spallanzani (92) was the first man to show that the presence of sperm in semen was essential in fertilization. Subsequently, Kölliker (47) discovered that sperm arise from the cells of the testis and Barry (12) observed the conjugation of the sperm and the ovum in the rabbit, and later Newport (72) recorded its occurrence in the frog. It was not until late in the nineteenth century that the significance of the process of fertilization was realized. It was largely through the work of Hertwig, Strasburger, and Van Beneden (citation from Weisman, A.I. Spermatozoa and Sterility. Paul H. Hoeber, N. Y. 1941. 314 pp.) that most biologists came to believe that the union of the nuclei of the gametes was the eseential act in the initiation of new life.

During the last twenty years immense strides have been made in the study of the chemistry and physiology of semen and spermatozoa. The use of the electron microscope makes it possible to reveal more fine details about these minute cells (90,14,41,17, 80 and 81).

In the studies of bovine semen Lardy and Phillips (51) found ascorbic acid content in bovine semen was 2-8 mg per ml, and Van Dermark and Salisbury (103) found that thiamin, riboflavin,

miacin and pantothenic acid content was 0.89, 2.09, 3.63 and 3.71 γ/ml. respectively. Jurgens (44) compared the vitamin content of sperm during their various stages of maturation. He found that the ascorbic acid was decreased as the sperm passed through the testis and was finally ejaculated.

The interesting finding in the sugar chemistry of semen is that Mann (64) revealed that in the seminal plasma of the bull, ram and boar, the greatest amount of reducing sugar is d(-) fructose.

Sarkar, et al (85) have studied the amino acid content in bovine semen. He found a high content of argirine and a low content of tryptophane and methionine as compared with other amino acids. Human and bovine semen have also been studied for their enzyme content. The presence of the following enzymes have been demonstrated: Succinic dehydrogenase (62); diastase (45,68); diaminoxidase (117); acid phosphotase (49,35); 5-nucleotidase (65); and thrombokinase (33). However, the absence of oxidase or peroxidase in human semen has been reported by Kurzrok and Miller (48) and confirmed by Tosic and Walton (97) in bovine semen.

Recent research in sperm physiology has involved a study of the presence of the cytochrome system (62,118,65) and hyaluronidase in semen, its origin and role in relationship to the metabolic activity and the fertilizing capacity of the spermatozoa (40,67,31,84,58,22,43).

The respiratory activity of semen fractions and the effects of various factors on sperm metabolism have been studied by Winchester and McKenzie (114,115). The debate on sperm metabolism has turned on whether the energy required for their normal function is derived from oxidation, from glycolysis (59) or from some other process (42,51). It is now known that the metabolism of mammalian spermatozoa is a glycolytic character, and in the absence of glycolyzable sugars the oxidative utilization of phospholipid reserve evidently furnishes energy (63).

Factors affecting sperm metabolism have also been studied (52,53,54,38,114,115,63). By the using of freshly ejaculated bull semen diluted with egg-yolk medium, Tosic and Walter (96) were able to demonstrate that the sperm produce aerobically a respiratory inhibitor which was later (97) identified as hydrogeny peroxide and was shown to be the metabolic products of the spermatozoa upon a substrate present in the egg yolk and its dialyzable portion. Upon further investigation into this problem, Tosic (98) postulated that the formation of hydrogen peroxide by living spermatozoa is an enzymatic dehydrogenation and deamination proceeding in the presence of molecular oxygen which acts as a hydrogen acceptor.

2. Physiology of Reproduction in Normal Bulls

(1) Sexual Development

Phillips and Andrews (75) found that in bull calves a few

spermatocytes may be found in seminiferous tubules at sixty-three days of age and the spermatocytes became abundant when the animal was 181 days old, and at 224 days the sperm were fully formed.

(2) Activity of Bovine Spermatozoa in the Female Genital Tract

It has been found in cattle (16) that after service the first sperm enter the cervix in less than 40 minutes, and sperm are at the ovarian end of the fallopian tubes in 4 hours. Motility is maintained for 30 hours and after 40 hours it is slight or absent; they also found that in case the insemination is performed outside the heat period the survival of the sperm is only 10 to 15 hours.

(3) Volume of Semen per Ejaculate

In bulls, the volume of semen per ejaculate ranges from 0.5 to 12 ml., with an average of about 4 ml. (8). It may vary from time to time in the same bull and significant differences between bulls have been reported (30). Type and breed differences may also exist (4) in that dairy bulls have given larger ejaculates than beef bulls and the ejaculates have been found to be larger in Holsteins than in Ayrshires.

Anderson (3) reported that the volume of semen per ejaculate is usually smaller in young bulls than in adult ones and Herman and Swanson (38) found a positive relation between the size

of ejaculate and the size of the bull.

(4) The Concentration of Bovine Semen

According to many workers (55,69,28,3,4), the sperm count in bovine semen averages about 600,000 - 1,000,000 per mm^3. A highly significant difference in semen concentration between bulls has also been reported (30).

(5) Initial Motility of Spermatozoa

Many workers (3,4,5,28) agreed that the motility of sperm from normal and fertile bulls is usually high. Herman and Swanson (38) found that the initial motility of bovine spermatozoa varied less than other semen characteristics and for most bulls the sperm have vigorous motility. There was a striking difference between good and poor bulls. However, it is not uncommon to get specimens of poor motility, from time to time, from normal, fertile bulls; and not all bulls which produce semen of good initial motility are of high fertility. Highly significant differences have been noted between bulls in the initial motility of spermatozoa (28,30).

(6) The pH of Fresh Semen

The pH of semen depends upon the amount of accessory secretions and the number and the metabolic activity of the sperm. Webster (108) found that the pH of bull semen lay mostly between 7-7.5 and later (109) he found a pH of 6-7.5. According to Milovanov (69) the pH of bull semen is 6.5 - 6.8 and with increased

amounts of accessory secretions it may become 7 - 7.5. Difference due to methods of collection has also been reported (27). In normal fertile bulls the pH of semen usually lies on the acid side, (4,7), though the range may be quite wide, (88,28).

(7) Respiration of Bovine Spermatozoa

The oxygen uptake of marine spermatozoa has been much studied since Warburg's original determination in 1908 and these methods have been applied to mammalian spermatozoa by Redenz (79) and others, (26,59,60,61,83,114).

Walton and Edwards (107) found the average initial respiration rate in bovine spermatozoa range from 0.127 - 0.286 mm^3 of oxygen per hour per million sperm.

Shergin (89) found that the oxygen consumption of the spermatozoa of various farm animals varied with the change in temperature, pH and the concentration of semen. However, the oxygen consumption per sperm cell was the same in rams, bulls, boars and stallions, i.e. 29 mm^3 of oxygen were consumed by 1 gm. of sperm in one half hour at 38° C.

3. Semen Characteristics in Relation to the Fertilizing Capacity of Sperm.

Much experimental work in the field of artificial breeding has been directed at establishing tests for determining potential fertility of semen samples before they are used for breeding purposes. A number of items have been reported to be associated

with fertility. Among those criteria, a high proportion of normally formed, vigorously motile sperm has been generally accepted as necessary for good fertility. Different methods have also been devised to correlate the resistance of the sperm to adverse conditions and its fertilizing capacity (69,15,56). The semen characteristics which have been considered as important to fertility are briefly reviewed as follows:

(1) Volume of Semen per Ejaculate and Fertility

Lagerlöf (55) has noted that the amount of semen per ejaculate is usually normal in bulls with impaired fertility, although in epididymitis the volume is usually reduced.

(2) Concentration of Semen and Fertility

Anderson (3,4) found that sperm are few or absent in epididymitis and in atrophy or hypoplasia of testis.

Lagerlöf (55) reported that the number of sperm was reduced or absent in cases of infectious changes or with hypoplasia or fibrosis of the testis, and in mild forms of degenerative changes of testis the number of sperm remained normal but it was greatly reduced in cases of marked degenerative changes.

Swanson and Herman (95) found that there was a slight tendency for the more concentrated semen to produce a larger percentage of conceptions but the correlation coefficient between the conception rate and the semen concentration was 0.63, being just short of significance.

However, the importance of sperm concentration on the degree and speed of dispersion of follicle cell mass in rabbit ova have been mentioned by Pincus and Enzmann (77) and the failure of dispersion of the surrounding follicle cells has been observed by these authors in rabbits and by Gilchrist and Pincus (32) in the rat and by Pincus (76) in the mouse.

(3) Motility of Sperm in Fresh Semen and Fertility

The initial motility of sperm has been found to be seriously affected by the abnormal condition of the genital organs and it tends to be poor where the other semen characters depart from normal (9).

Davis (27), and Herman and Swanson (39) considered the initial motility of the sperm as one of the best signs of its viability.

Donham, Simms and Shaw (29) found a definite correlation between motility and fertility in that the semen below normal in motility was less than half as effective in producing conception as semen with high motility, (90 or above).

Williams (110) and Comstock (26) believed that initial motility of the sperm is a useful criterion in measuring fertility.

Herman and Swanson (39) found no correlation between initial motility and fertility, but later (95) they contradicted this and found a significant curvilinear correlation between

conception rate and motility (r = 0.97).

Anderson (9) considered 70% motility as the arbitrary border line and he found no difference in fertility when the initial motility varied from 70% to 100%.

(4) Motility of Sperm in Stored Semen and Fertility

In the study of ram semen, Chabibullin (21) found marked variations between motility and conception rate, however, Underbjerg and Davis (101,102) present evidence that in stored semen good motility is not necessarily associated with good fertility.

(5) pH of Fresh Semen and Fertility

As the H-ion concentration is one of the important environmental factors affecting the cellular activity, pH of the semen has therefore been regarded as one of the criteria for the appraisal of semen quality.

Schneerson (87) noted that the pH of semen from fertile bulls should be below 6.6, while Davis and Williams (28) found that the majority of ejaculates from 11 fertile bulls was alkaline in pH.

Webster (109) found a relationship between pH and fertility; he suggested that the probable fertile range is from 6 - 7.5, with sample of pH 7 - 7.5 of very doubtful or low fertility.

In the study of bovine semen Anderson (6) found that an alkaline reaction in semen was characteristic of typical cases of epididymitis and of bulls with small testes.

Swanson and Herman (95) found that the pH was practically the same for the more fertile bulls (pH 6.47) and bulls of questionable fertility, (pH 6.50), and although it was slightly higher for the poor breeders, the correlation between the pH of fresh semen and the conception rate was non-significant.

(6) Morphology of Spermatozoa and Fertility

According to Anderson (3,4), the chronic pathological changes in testis and epididymis are in most cases associated with the increase in the percentage of abnormal sperm; and the switch in the incidence of the different types of abnormality may indicate an unfavorable condition of the epididymis.

Lagerlöf (55) indicated that the degenerative changes in the fully formed and previously normal testis caused an increase in the percentage of abnormal types, but the relative incidence of the different types remained much the same. In case the testes are in a state of incomplete development, the incidence of various types of abnormal sperm was found to be different.

Although breeding inefficiency was not always detectable by sperm abnormalities, many workers considered sperm morphology as one of the reliable indices in measuring the fertilizing capacity

of the sperm. Williams (111) first demonstrated the relation of sperm morphology to fertility and the work on this subject since then indicates a direct relation between the presence of abnormal sperm and sterility.

Williams and Savage (112) considered the morphology of the sperm head as the most useful information as to the fitness of the germ cells for reproduction. They showed (113) that in the bull the fertility was diminished when the abnormal sperm exceeded 17%.

Trimberger and Davis (99) found that bulls producing semen with more than 50% abnormal cells had very poor breeding records, and many workers (55,2,4,93,39) seemed to agree that semen from bulls of good breeding efficiency average well below 20% of abnormal sperm.

Herman and Swanson (39) found that the semen of bulls with poor breeding records had relatively greater number of coiled tails and tailless forms than those from the bulls with good breeding records, but they found no particular type of abnormality that seemed to be associated with reduced fertility. The main distinction between bulls of good and poor fertility was the percentage of total abnormal sperm. Some bulls with sperm of abnormal morphology, however, were very fertile. In 1944 (95) they found that the correlation between abnormal percentage and

conception rate was non-significant (r = 0.12).

Lagerlöf (55) believed that sperm with protoplasmic droplets on the neck were immature and the presence of more than 2 - 3% was regarded as indicating pathological changes in the genital organs and reduced fertility.

(7) Resistance of the Sperm to Cold Shock and Fertility

The effects of temperature on survival of spermatozoa has been noted by Walton (105) and Chang and Walton (23). Milovanov (69) found the deleterious effects of "temperature shock" on sperm. The resistance of the spermatozoa to various unfavorable environmental conditions has been suggested by many workers as a means for the evaluation of semen. Lasley and Bogart (56) found that in range cattle, the percentage of live spermatozoa in non-diluted semen after a cold shock (0°C for 10 minutes) was not significantly correlated with fertility (r = -0.063), but on the other hand, the percentage of live sperm surviving a cold temperature shock in semen diluted with egg-yolk buffer was significantly correlated with fertility (r = 0.831).

(8) Respiration of Sperm and Fertility

Walton and Edwards (107) found a definite correlation between the number of services per conception and the respiration of bovine spermatozoa, in that the higher the respiration

initially and at 2 hours after semen collection the fewer the services that were required per conception.

(9) Longevity of Sperm and Fertility

Margolin, Bartlett and Lepard (66) in the studies of bovine semen, found a highly significant correlation between longevity and conception ratio ($r = 0.6964 = 0.0212$).

(10) Head Length of Spermatozoa and Fertility

Savage, Williams and Fowler (86) found that the coefficient of variation in head length of bovine spermatozoa is a good indication of fertility and in normal bulls the coefficient should not exceed 4.

Lagerlöf (55) considered that bulls with a coefficient of 4 and under as probably fertile, values from 4 to 4.4 were inconclusive, and those with a value of 4.5 and over as of reduced fertility.

(11) Catalase number of Semen and Fertility

In the study using thybromol catalase test in routine examination of bull semen, Blom and Christensen (18) found that bull semen collected in normal clean manner has a catalase number below 300, a number of 300 - 400 must be regarded as suspicious and the semen should not be used for insemination if the catalase number is above 400.

(12) Some Work on Human Semen Characters and Fertility.

The investigations of Hotchkiss (41) included the semen of 200 fertile men and showed that no one factor can serve as a

reliable index to human fertility. They found no uniform or important information from the tests of viscosity, pH and sugar content of the semen. The criteria they suggested for semen evaluation were the volume and concentration of the semen, sperm motility and the percentage of abnormal sperm.

Material and Methods

1. Animals

This experiment was in cooperation with the Oregon Dairy Breeders Association and only the surplus semen left from shipping for using in artificial insemination was included in this study. Semen from 15 bulls of three different breeds — Guernsey, Holstein and Jersey — was studied for a period of eight months.

The bulls were kept in the Association and were in good condition throughout the period of study. Each bull got 4 to 8 pounds of concentrates a day, depending upon age and conditions together with good quality alfalfa hay and kale ad lib. They were fed at 7 A.M. and again at 5 P.M. Well water was supplied by means of automatic drinking cups. They were kept in individual stalls and 1 - 2 hours exercise at the walk was given by means of a circular exciser each day when weather conditions permitted. The feed concentrates consisted of the following:

Ground Oats.....	50%	Soybean Oil Meal.....	2.5%
Ground Wheat.....	20%	Meat Meal.....	1.0%
Mill Run.....	12%	Dried Skim Milk.....	1.5%
Linseed Oil Meal...	5%	Steamed Bonemeal.....	1.0%
Fish Meal.....	5%	Iodized Salt.....	1.75%
		Irradiated Yeast.....	1.0%

2. Sanitation of Equipment.

All glassware used in handling the semen was washed with a wetting agent (Calgonite), rinsed with hot tap water and sterilized by dry heat in an electric oven at 121°C for a period

of not less than three hours. After each collection of semen, the inner rubber tube and cone of the artificial vagina were rinsed with tap water, immersed in Roccal solution (1 oz. of 10% Roccal solution in 1 gallon of water) for about 1 hour, washed with a high quality powdered soap ("Dreft") and rinsed with hot tap water. They were then dried quickly by an electric fan in a closet blowing over warm light bulbs.

3. Collection of semen

The area around the sheath of the bull was washed with Roccal solution before the collection of semen. The semen was collected every other day at about 5 A.M. A collection was made from any one bull each six days. The semen was collected by means of artificial vagina according to the method described by Lambert and McKenzie (50).

4. Handling of Semen for Shipment

Immediately after collection, the initial motility of the sperm was examined microscopically (100 X) on a warm stage at 37.75° C (100° F.). Only the semen which had a motility rating of 9 or above was used for artificial breeding, and only the surplus semen left from shipment for breeding was subjected to laboratory tests.

4 - 5 ml. of semen were first diluted in 1:1 dilution with fresh egg-yolk citrate buffer and left at room temperature for about 10 minutes, after which it was further diluted to

1:40 in a beaker with the same buffer. It was then colored differently for different breeds according to the method described by Almquist (1). The semen was tubed in pyrex test tubes, each of which contained about 8 ml. of diluted semen. The tubes were then corked, labeled and put in water bath at 6 - 10° C for about 15 minutes, and then left in this water bath and put in the refrigerator at 3°C (38°F.) for a period of about 1 hour until packing. In packing, 8 - 10 tubes of semen were fastened around an ice can, wrapped with two layers of carton paper bags, and packed for shipping by bus to various county breeding laboratories for use in artificial insemination. The longest time required for the semen to reach the fartherest county laboratory was about fourteen hours.

5. Artificial Insemination of the Cows

Artificial insemination was performed by the inseminators of the various counties. 1 ml. of the diluted semen (1:40) was deposited by using a sterilized glass syringe into the uterus of the cow, which, according to the owner's report, had recently shown the symptoms of estrus. The semen used was as fresh as possible, generally ranging in age from the day of collection to three days.

6. Laboratory Procedures

(1) Volume of Semen per Ejaculate (ml.)

The volume of semen per ejaculate was measured by the use of graduated collection tubes.

(2) Concentration of Semen (Number of Sperm /mm^3)

It was measured by the haemocytometer technique, a total number of 80 small squares in the counting chamber was counted for each sample.

(3) Motility of Spermatozoa in Fresh Semen

It was examined immediately after collection under microscope (100 X) on a warm stage at 37.75°C (100°F.). The motility was expressed in terms of grades and swirls; the grade, the activity of the moving sperm, was graded from 0 to 10 where 10 denoted the best motility; the swirls, the gross appearance of the moving mass of sperm in the microscopic field, was also estimated and classified as excellent, good, fair and poor.

(4) Motility of Sperm After Storage for Six Days

Semen was diluted to 1:40 with fresh egg-yolk citrate buffer and kept in refrigerator at 6°C. The six-days' motility was examined under high magnification (430 X) microscopically on a warm stage at 37.75°C. and graded from 0 to 10 as for initial motility; the percentage of sperm that were motile was also estimated.

(5) Percentage of Live Sperm in Fresh Semen

Live sperm was counted by the use of fast green eosin stain, modifying the methods described by Lasley, Easley, and McKenzie (57). A suitable drop of fresh semen was placed on a

clean slide, mixed with a drop of the stain and made into a thin film by a drawing out with another slide; the stained slide was then dried very quickly on an oven. The sperm with the entire head stained red were counted as dead ones.

(6) Percentage of Morphologically Abnormal Spermatozoa

India ink semen smear was made for each fresh sample of semen (a drop of India ink mixed with a drop of fresh semen and spread into a smear and dried). The specimens were examined under oil immersion lens (970 X) for abnormalities. The various types of morphologically abnormal sperm were recorded and expressed in percentages.

(7) Resistance of Spermatozoa to Cold Shock

0.2 ml. of fresh semen was put in a pyrex test tube with 0.8 ml. of fresh egg-yolk citrate buffer, the tubes were then kept in ice-water at 0°C. for 10 minutes, the dead sperm were then counted by the fast green-eosin stain in the same way as that for the percentage of live sperm in fresh semen.

(8) Longevity of the Spermatozoa

Semen diluted 1:40 with fresh egg-yolk citrate buffer was kept at 5°C. in the refrigerator, microscopic examinations on a warm stage at 37.75°C. (100°F.) were made daily until no motile sperm could be found under the high power objective (430 X).

7. Reagents Used

(1) Semen dilutor — The Fresh Egg-Yolk Citrate Buffer.

3.2 gm. of Crystalline sodium citrate ($Na_3C_6H_5O_7.2H_2O$)

were dissolved in 100 ml. of glass distilled water, boiled and cooled, and then mixed with equal parts of fresh egg-yolk.

(2) Fast Green-Eosin Stain (19)

2 gm. fast green and 0.8 gm. eosin-Y were dissolved in 100 ml. of M/8 phosphate buffer (K_2HPO4) pH 7.35.

(3) Coloring of Semen (1)

In order to reduce errors in handling the semen of different breeds, the semen of Holsteins was colored with emerald shade green color and that of Jerseys with strawberry shade red color. These aqueous solutions of coal tar dyes used were certified by the U.S. Food and Drug Administration and require no further preparation before use. They were obtained from the Warner-Jackinson Manufacturing Company, Missouri. In coloring of the semen, 1 drop of the dye solution was added for each 50 ml. of diluted semen.

Results and Discussion

1. Semen Characteristics of Bulls Above and Below Average Fertility

The data of various semen characters included 342 samples tested and the breeding results based on the non-return rate of 6419 inseminations were treated statistically according to the methods described by Pearson and Bennett (73).

The fertility of the bull was based on the non-return to estrus of the cow 50-60 days after insemination. The non-return rate so obtained averaged 59.52%. When this average figure was used to group bulls into high and low fertility, it was found that on the average the group with "above average" fertility had: (1) a larger volume of semen per ejaculate, (2) a higher percentage of live sperm after cold shock, (3) a lower percentage of tailless sperm and percentage of sperm with middle-piece enlargements and protoplasmic droplets (Chart 2). It was also noticed (Chart 4) that the group with "above average" fertility had a higher percentage of samples showing better motility after six-day storage. The percentage of live sperm in fresh semen and the longevity of spermatozoa were practically the same in these two groups (Chart 4).

2. Volume per Ejaculate cf. N.R.%

Generally speaking, the results showed a slight tendency for semen with a greater average volume per ejaculate to be of better quality. However, some individual ejaculates of small volume did

have an "above average" fertility potential. Single ejaculate volumes have therefore been not too dependable predictive value, but trends up or down over a period of weeks might conceivably reflect the physical condition of the bull. Lagerlöf (55) has noted that the amount is usually smaller than normal in case of epididymitis. It seems, therefore, that the volume of semen per ejaculate may be more indicative of the possible existence of pathological conditions in the reproductive tracts of the bull rather than of the fertility level of a particular animal, or of the semen quality of any given ejaculate.

3. Concentration of Semen cf. N.R.%.

The concentration of semen in this experiment varied from 440,000 to 2,740,000 per mm^3, with an average of 1,320,000 per mm^3. The results show a tendency for the more concentrated semen to produce better results in artificial breeding, and our findings agree with the reports of Herman and Swanson (39) in that the difference was not statistically significant.

Pincus and Enzmann (77) in their <u>in vitro</u> studies of rabbit ova, found that both the degree and speed of dispersion of the follicle cell mass is roughly proportional to the concentration of the sperm suspension used and in case the sperm concentration failed to effect a complete dispersion of the follicle cell mass, it also failed to cause second polar body formation. They also found that when freshly ovulated ova are placed <u>in vitro</u> with sperm suspension, there is a rapid dispersion of the surrounding follicle cells which

does not occur in control cultures of ova in sperm free media. Similar phenomena have been observed by Gilchrist and Pincus (32) in the rat and by Pincus (76) in the mouse. Based on these findings and the recent studies on hyaluronidase in semen (43) and its possible role in fertilization (40,67,31,84,58), one reason why in practically all animals a large number of sperm must be present in order to produce fertilization is quite possibly due to the carrying (by the spermatozoa) of a substance essential for fertilization. Another reason for the necessity of large number of sperm to fertilize one egg is quite evidently the necessity of providing sufficient opportunity for sperm and egg to encounter each other within their respective life span. However, in this study, significant differences in breeding results have not yet been observed from the using of semen in various concentrations. This may be due to the fact that the sperm dosage used in insemination was far beyond the minimal requirements in producing pregnancy so that even the least concentrated semen used in this study still contained a sufficient number of spermatozoa capable of producing fertilization. Thus, the probable greater efficiency of the denser semen over that of the more diluted ones showed up only slightly.

Concerning semen concentration, we are likely to believe that in otherwise identical conditions, the more concentrated semen will give more opportunity to produce better breeding results.

This however, may be true only to a certain extent, since too concentrated semen or too large a sperm dosage may bring adverse results in fertilization or the subsequent development of the zygote as already pointed out by Pincus and Enzmann (77) that polyspermy may prevent the second polar division in rabbit ova fertilized in vitro, although in the hen's egg, polyspermy seemed to be the normal condition (74).

4. Motility of Sperm in Fresh Semen cf. N.R.%

The results show that semen containing sperm of better fresh motility based on swirls gave on the average better breeding results than semen which contained sperm rather poor in fresh motility. The breeding performance with excellent swirls was 60%. With good swirls, 57%, and with fair swirls, 51%, and the difference was found to be statistically significant. The same was true with the various types of motility based on grades except that in this case, the difference between results was short of significance. As only semen samples which had atleast a motility grade of 9 or above have been used in this study, the difference between the fresh motility of the semen used was actually fairly slight. However, even so, the difference between the breeding results is still significant. This fact may indicate the great importance of sperm motility in fresh semen in the measuring of male fertility.

Sperm motility may be an indication of the physical state or the vitality of the spermatozoa, and also it may be related to the aging process and in turn to the fertilizing potential of the cell. In the study of cock sperm Munro (71) demonstrated that the grade of motility is lowest in the testes, greater in the epididymis and highest in vas defereus; in addition, he found that the fertility was parallel to the motility. The relation of motility and aging process was studied in bull, ram, rat and guinea pig by Young (116). He found that sperm are very low in motility when they leave the epididymis. It seems to be reasonable to assume that the weak motile sperm are those lacking of maturity or they are mature sperm of physiological weakness. Their effects on reducing fertility may be due to their lacking the opportunity to meet the ovum at the right place of fertilization, or due to their lack of power of penetration into the ovum, and it also may be possible that they can fertilize the egg but due to their physiological weakness, they fail to set the zygote on the right way for normal development.

5. Motility of Sperm After Six-Days' Storage cf. N.R.%

In examining the results, it was found that there was a notable correlation between the motility of the sperm in semen after six days' storage and its fertilizing capacity. Samples showing better six-day motility gave on the average a higher non-return rate than those which contained sperm of poor six-day motility and

the difference was found to be statistically significant. This confirms the observation of Williams (110) that the duration of certain motility in stored semen is a good index of fertility. However, this criterion alone is not a dependable predictive value to any given ejaculate. Results here are in agreement with the idea of Herman and Swanson (39) in that the fertility of a bull should not be rated on this value alone. They found that many bulls of good fertility averaged less than 56 hours maintenance of grade "2" motility, and a few even below the average of poor bulls.

Another question considered in this study in connection with this criterion, is that in artificial insemination, semen is mostly used immediately after collection or 1 - 3 days afterwards and very little semen is used after a storage of six days. The motility of sperm after six-days' storage is therefore not very valuable in predicting the breeding efficiency of semen at the time to decide its use or discard, but we may use it to predict the breeding results shortly after the semen has been used and before the cows have had time to return into estrus, or go on over (become pregnant).

Although motility after a storage period of 6 days could not be useful in evaluating semen prior to its use, it may have value in rating bulls on their levels of fertility. To make this

determination, bulls with non-return rates above the average were compared with bulls having non-return rates below average by computing the average motility after a storage period of 6 days for the bulls in each group.

It is concluded from Chart 4 that:

(a) The bulls with above average fertility had on the average, a high percentage of semen samples with sperm of better than average motility after six-days' storage; and on the other hand, bulls with below average fertility had a higher percentage of semen samples with sperm showing poor motility after six-days' storage.

(b) The predictive value of the six-day motility test although usually high, may vary with individual bulls. Thus, Jersey 16 bull, for example, had the lowest fertility among the bulls studied, also had the highest percentage of samples containing sperm of poor "six-day motility", while Jersey 8 bull, although with a poor breeding record, had fairly high percentage of samples showing good six-day motility (Chart 3).

6. Morphologically Abnormal Sperm cf. N.R.%

(1) Percentage of tailless sperm and percentage of coiled tail sperm cf. N.R.%:

The results show a slight tendency for semen which contained less tailless or coiled tail sperm to give better breeding results than those with higher percentage of these types of abnormalities. But the differences in both cases were not statistically

significant.

(2) Percentage of spermatozoa with protoplasmic droplets and middle-piece enlargement cf. N.R.%.

Herman and Swanson (39) found no particular type of abnormal sperm seemed to be associated with reduced fertility. But the results of this experiment show that the bulls with less than 1 - 9 sperm with protoplasmic droplets and middle piece enlargement per 100 had significantly better breeding results than those with more than 9 per 100. The breeding performance with none of these types of abnormalities was 60%, with 1 - 9 per 100, 60%, more than 9 per 100, 57%. The difference was statistically significant.

As the head of the spermatozoon represents the nucleus and contains the chromatin material, there is no doubt about its importance to fertilization and to the subsequent development of the zygote. However, no matter what fertilizing potential may be carried on by a sperm, fertilization still cannot occur unless the sperm can reach the ovum for conjugation. Thus, the part of the sperm which is essential to motility is therefore also important to fertilization. According to Cody (24) the middle piece appears to be the motor of the sperm since when the head is severed from the middle piece and tail, the latter continues to swim in a straight line while injury to tail piece caused the loss of motility. Popa and Marza (78) indicated that the proximal centriole may be the source of power for motility. Asplund (11) in determining the total content of

contracting substance in human sperm by titration on rabbit intestine in vitro found no correlation between the total content of contract substance and sperm motility. Based on these findings, the middle piece is important at least to sperm motility, and the presence of tailless sperm seems to reduce fertility; and if the sperm dosage used in artificial insemination were reduced to the extent just sufficient to produce pregnancy, the inefficiency of the tailless sperm in fertilization might be revealed much more distinctly.

Concerning the protoplasmic droplets, it appears not necessary to consider their appearance as indicators of physiological maturity of the spermatozoön, for they occur even in the first ejaculate of the boar experiencing moderate sexual activity (82); and a large number of matings in a short time does not cause the increase in the number of this type of abnormalities (46,55,34). Gunn and co-workers (34) agree with Lagerlöf (55) that protoplasmic droplets result from disturbance of spermatogenesis as they have been found in connection with acute and chronic degeneration. They considered sperm with protoplasmic droplets as developmentally imperfect, and reduced fertility was associated therewith.

7. Longevity of Spermatozoa cf. N.R.%

No correlation has been found between N.R.% and longevity of the sperm as determined by motility in semen diluted with egg-yolk citrate buffer.

8. Percentage of Live Sperm in Fresh Semen and in Diluted Semen After Cold Shock cf. N.R.%.

Percentage of live sperm in fresh semen varied from 58 to 97 with an average of 82. Statistical treatments showed no correlation between this criterion and the breeding efficiency of the spermatozoa. However, the percentage of live sperm in egg-yolk citrate diluted semen (1:4) after cold shock (0°C., 10 minutes) showed very good correlation with fertility. This seemed to indicate that the percentage of live sperm in fresh semen was not related to fertility within the limits of these observations and was not an important criterion, at least when the dosage of sperm used per insemination was ashigh as in this experiment, while the percentage of live sperm after cold shock may be used as a reliable index in measuring semen quality.

SUMMARY

1. In cooperation with the Oregon Dairy Breeders Association present methods of semen evaluation have been applied to the semen of 15 bulls (6 Jerseys, 5 Guernseys, 4 Holsteins) owned or leased by the Association, with the hope of determining the better methods of semen evaluation so as to insure a higher and more economical conception rate. Semen in excess of current needs of the association has been made available for study from each use of these bulls from October 1947 to May 1948 (inclusive).

2. It is recognized that for most part this work has been with semen that has been of at least fair quality. The hunt has been for criteria that would distinguish between good semen and semen not quite good enough from the standpoint of getting cows to conceive and produce calves promptly and economically.

3. Semen was collected on every other morning and on the average collections were made from each bull every 6 days.

4. The non-return (N.R.) of estrus 50-80 days after insemination has been rated following the use of each semen specimen of the 432 sent out, and each of the various methods of evaluating semen was then checked against the N.R. (non-return of estrus) on 6419 first and second inseminations.

5. Certain tests of the evaluation of bull semen have been of little predictable value. These have been:

(1) Volume of semen per ejaculate

(2) Concentration of the semen

(3) Percentage of coiled tail sperm

(4) Percentage of tailless sperm

(5) Percentage of total morphologically abnormal sperm

(6) Percentage of live sperm in fresh semen

(7) Longevity of sperm as determined by motility has ranged from 24 to 49 days, (average 34.46 days), but there has been no correlation with the non-return of estrus rate evidenced.

6. Criteria that have proven helpful in predicting semen quality have been:

(1) Motility of sperm, expressed in swirls, observed immediately after collection on a warm stage at 37.75°C under low power magnification (100 X):

Excellent swirls ..... 60.38% N.R.

Good swirls ..... 56.93% N.R.

Fair swirls..... 50.63% N.R.

These differences are statistically significant.

(2) Motility of sperm in diluted semen after 6 days of storage at 5°C.: By grading the motility and noting the proportion of sperm actually motile a product was derived (Grade 10 to 0 x % motile). When this product was up to 450, the N.R. was 56.96%.

When above 450, the N.R. was 60.15%. This difference is statistically significant. This is one of the most dependable criteria found in this observation, but of course lacks the advantage of being available the day of collection and before the semen is shipped out.

(3) Morphology of the sperm: When the percentage of sperm showing middle piece enlargement and protoplasmic droplets was above 10%, the N.R. was 52.13%. When 1-9%, the N.R. was 60.07%. When none of these abnormalities was noticed, the N.R. was 60.32. These differences are statistically significant.

(4) The resistance of sperm to cold shock: When the percentage of sperm alive after the exposure of diluted semen (1:40) to 0°C. for 10 minutes, was up to 65%, the N.R. was 55.27%; when above 65%, the N.R. was 60.78%. This difference is statistically significant.

7. The N.R. averaged 59.52%. The group of bulls with fertility above the average had greater volume per ejaculate, higher percentage of live sperm after cold shock, lower percentage of tailless sperm and percentage sperm with middle-piece enlargements and protoplasmic droplets.

8. The motility of sperm after a storage period of 6 days is of value in predicting the general breeding performance of a bull but this method cannot be used to evaluate a particular semen sample prior to its use for insemination.

9. There were tendencies for: (1) more concentrated semen, (2) semen with a higher percentage live sperm or (3) lower percentage of tailless sperm to give better breeding results than when the semen qualities were the reverse. But the differences are not statistically significant.

10. When the records of individual bulls were considered there was noted a tendency for the 6-days' motility rating of the semen to be correlated with the N.R. percentage of the particular semen; and the incidence of a bull to produce semen of certain value (grade of 6-day motility x % motile sperm) of 6-day motility was found to be correlated, in some bulls, with the fertility level. The group of bulls with above average fertility had a higher percentage of samples showing better 6-day motility than that of the group below average fertility.

11. Occasionally there were indications that the different characters of semen may play various degrees of importance in fertility in different individuals. It seems, therefore, to be reasonable to recognize different "measuring sticks" for the evaluating of semen for different bulls.

12. It is emphasized that only the semen of at least fairly good quality - with fresh motility grading 9 or above and with at least fair swirls - was used in this artificial breeding program. Consequently it was impossible to have non-return of estrus records on poor quality semen. It seems reasonable to assume that if the

semen discarded for apparently low quality had been used for the insemination of cows, then a more complete picture might have been obtained and the tests evaluated on a scale of wider ranges. Nevertheless, it is true that the problem is to distinguish potentially good semen from semen not quite good enough to bring about an economically feasible conception rate; the problem is not to distinguish good semen from bad semen, that would be much simpler, but obviously of no practical help.

CONCLUSION

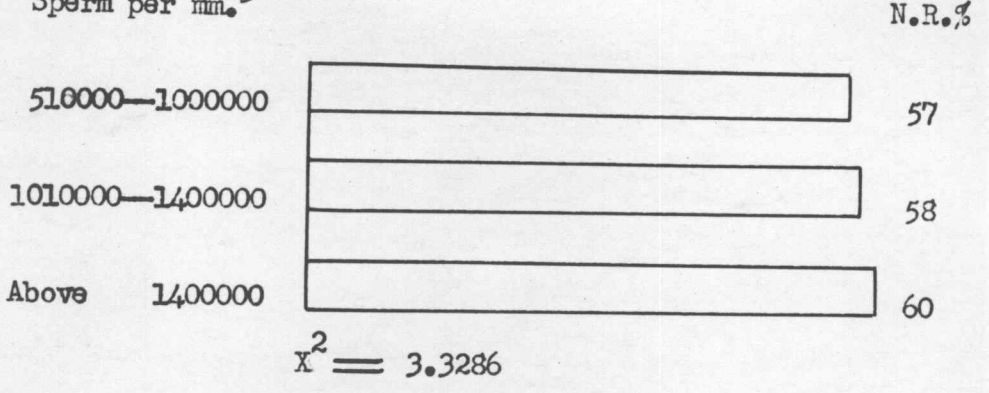
Since there are many factors involved in the problems of sperm physiology and fertility, it seems to be logical to use several characters of semen to predict the fertilizing capacity of the spermatozoa rather than only one criterion as a base.

From the results of this experiment the use of the following is suggested: Motility of sperm in fresh semen together with the examination on the percentage of live sperm in diluted semen after 10 minutes cold shock at 0°C., and in addition the percentage of sperm with protoplasmic droplets and middle-piece enlargements should be checked where impaired fertility is suspected and is not shown up decisively by other criteria.

It is recommended that in order to insure a high and economical conception rate, one should use the semens which have a fresh motility graded as good or excellent swirls, with more than 65% live sperm after cold shock and with sperm with protoplasmic droplets and middle-piece enlargement less than 10%.

Chart 1. Semen Characteristics and Fertility.

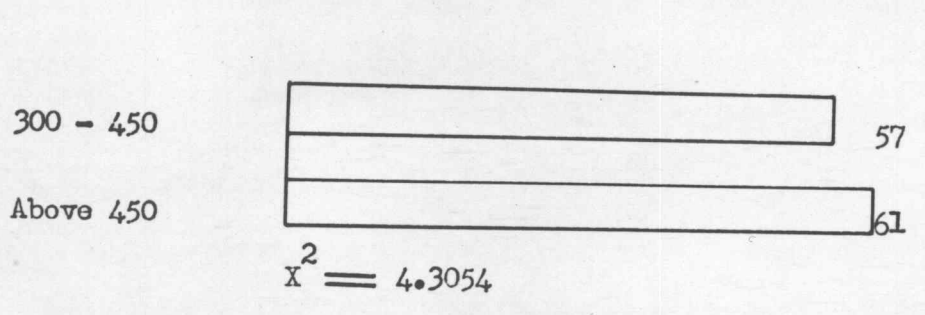
1. Concentration of Semen cf. % of Non-return.



$X^2 = 3.3286$

Difference is not significant.

2. Motility of Sperm after 6-days storage cf. % of Non-return.



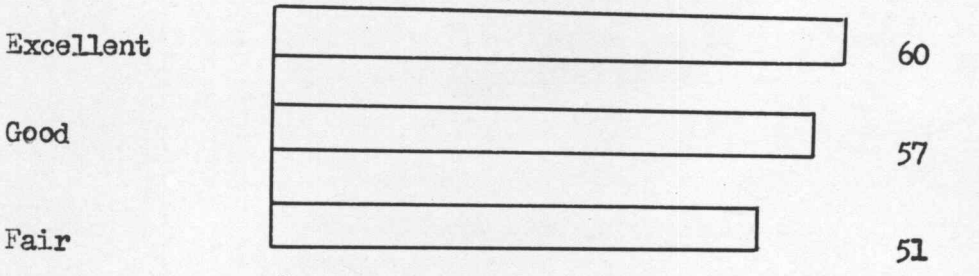
$X^2 = 4.3054$

Difference is significant.

Chart 1. (continued)

3. Motility of Sperm in Fresh Semen cf. % of Non-return.

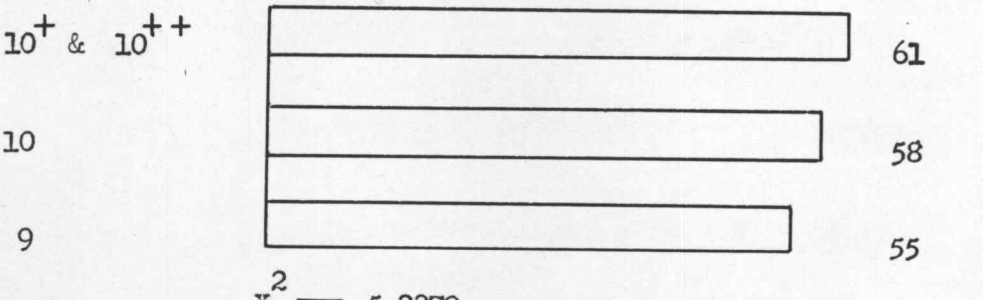
A. Swirls.



$X^2 = 8.1524$

Difference is significant.

B. Grades.

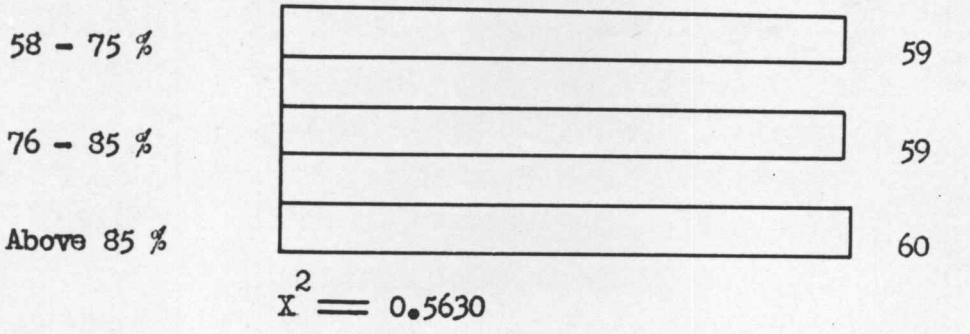


$X^2 = 5.2379$

Difference is just short of significance.

Chart 1. (continued)

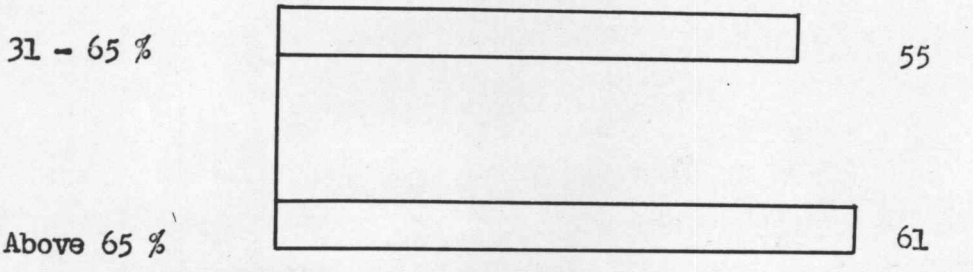
4. Percentage of Live Sperm in Fresh Semen cf. % of Non-return.



$X^2 = 0.5630$

Difference is not significant.

5. Percentage of Live Sperm after Cold Shock cf. % of Non-return.



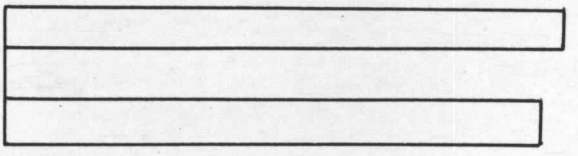
$X^2 = 6.8362$

Difference is significant.

Chart 1. (continued)

6. Percentage of Tailless Sperm cf. % of Non-return.

Tailless Sperm	N.R.%
None	62
Above 1 %	60

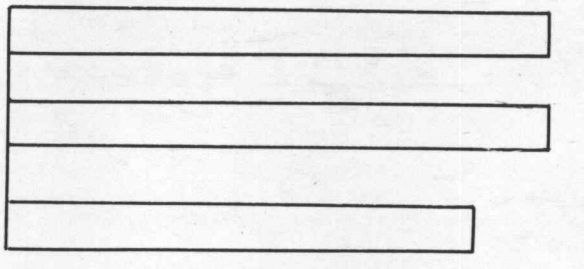


$X^2 = 3.0386$

Difference is not significant.

7. Percentage of Sperm with Middle-piece enlargement & Protoplasmic droplets cf. % of Non-return.

Abnormal Sperm	N.R.%
None	60
1 - 9 %	60
Above 9 %	52

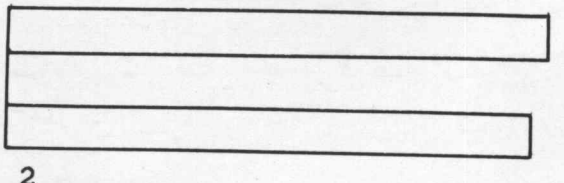


$X^2 = 17.3602$

Difference is significant.

8. Percentage of Coiled Tail Sperm cf. % of Non-return.

Coiled Tail Sperm	N.R.%
0 - 5 %	60
Above 5 %	59



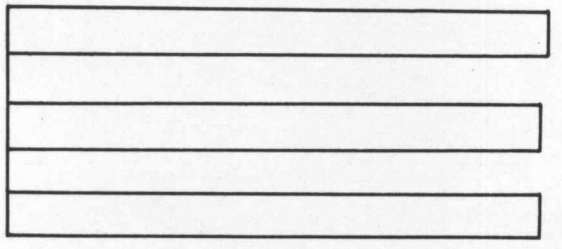
$X^2 = 0.0643$

Difference is not significant.

Chart 1. (continued)

9. Percentage of Total Morphologically Abnormal Sperm cf. % of Non-return

Total Abnormal Sperm	N.R.%
1 - 10 %	60
11 - 20 %	59
Above 20 %	59

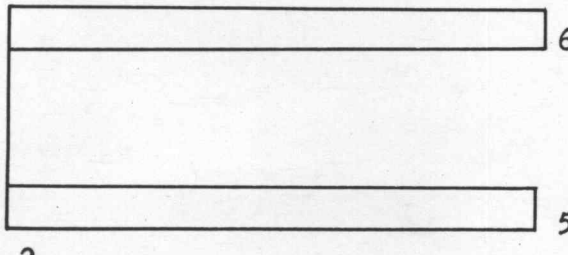


$X^2 = 0.7631$

Difference is not significant.

10. Longevity of the Spermatozoa cf. % of Non-return.

Longevity (days)	N.R.%
24 - 35	61
Above 35	59



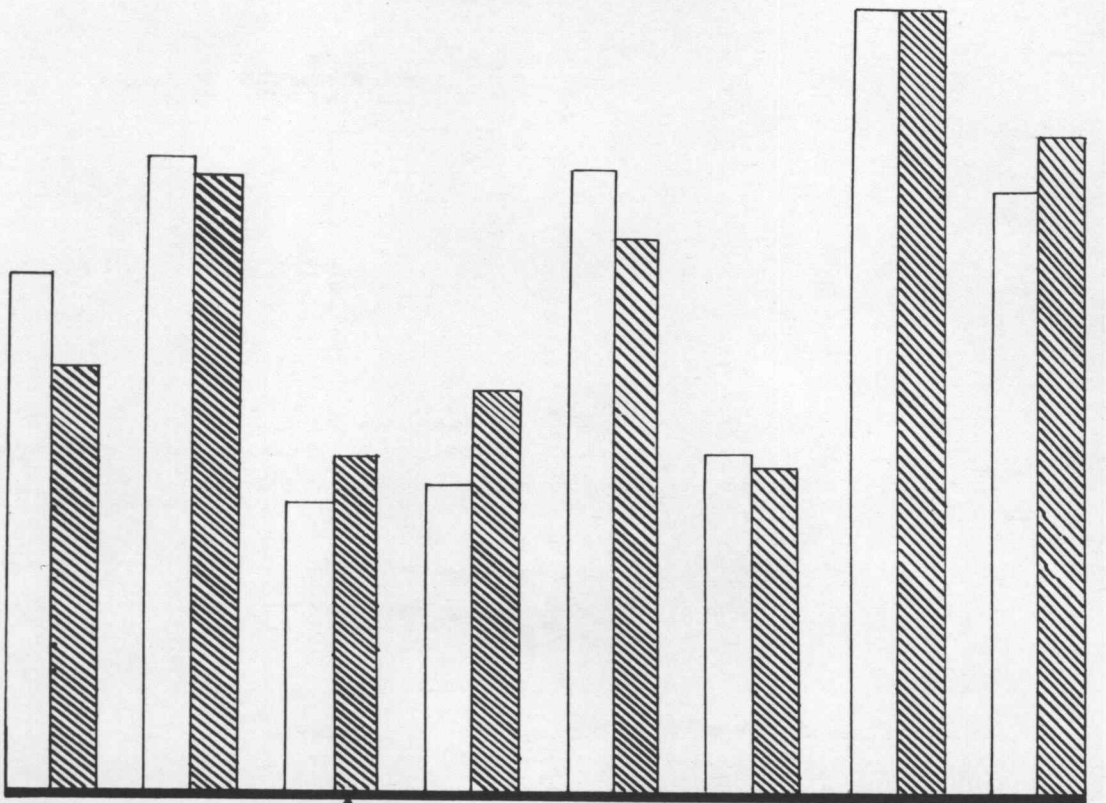
$X^2 = 1.7523$

Difference is not significant.

Chart 2. Average Semen Characters of Bulls above and below the Average Fertility Level:

Bulls with above average fertility

Bulls with below average fertility



Above av. Fert.	H 7	G 64	H 3	J 13	J 18	G 59	H 6	All
Non-return %	70	69	68	66	63	62	61	64.51
	59	58	56	56	54	41		53.47
Below av. Fert.	J 20	G66	H 1	G 65	J 8	J 16		All

Chart 3. Percentage of Semen Samples showing various Values of Motility after 6-days Storage.

1. Holstein Bulls:

Bull.	Month	N.R.%	Value of 6-days Motility			
			300 - 400	401 - 500	501 - 600	Above 600
H_1	Apr.	43			—	—
	Oct.	47		—	—	
	May	55		—	—	
	Jan.	57		—	—	
	Feb.	57			—	
	Mar.	62	—	—	—	—
	Dec.	64		—	—	
	Nov.	68	—		—	
H_3	Feb.	59		—		
	May	59	—	—		
	Oct.	67		—	—	
	Nov,	67	—	—	—	
	Jan.	67		—		
	Dec.	78	—		—	
H_6	Dec.	36		—	—	
	Oct.	52	—	—		
	Apr.	52		—	—	
	May	62		—	—	—
	Nov.	67	—		—	
	Mar.	67	—		—	
	Jan.	74		—	—	
H_7	May	60	—	—	—	
	Apr.	70		—	—	
	Mar.	72		—		
	Feb.	73		—	—	

Sequence for each bull is in increasing N.R.%.

Scale: 0 50 100

Chart 3. (continued)

2. Guernsey Bulls:

Bull	Month	N.R.%	Value of 6-days Motility			
			300 - 400	401 - 500	501 - 600	Above 600
G 59	Mar.	56				
	Feb.	58				
	Apr.	59				
	Nov.	62				
	Oct.	64				
	Dec.	64				
	Jan.	69				
	May	69				
G 64	Feb.	59				
	Jan.	63				
	Oct.	65				
	Apr.	69				
	Mar.	73				
	May	74				
	Dec.	76				
	Nov.	82				
G 65	May	40				
	Dec.	41				
	Oct.	56				
	Nov.	62				
	Jan.	62				
	Mar.	64				
	Apr.	64				
	Feb.	73				
G 66	Jan.	32				
	May	56				
	Mar.	58				
	Dec.	61				
	Apr.	62				
	Oct.	63				

Chart 3. (continued)

3. Jersey Bulls:

Bull	Month	N.R.%	Value of 6-days Motility			
			300 - 400	401 - 500	501 - 600	Above 600
J 8	Mar.	42				
	Feb.	49				
	Oct.	55				
	Jan.	55				
	Apr.	55				
	Nov.	57				
	Dec.	57				
	May	60				
J 16	May	33				
	Mar.	37				
	Jan.	40				
	Feb.	42				
	Apr.	45				
	Dec.	45				
	Oct.	46				
	Nov.	47				
J 17	Feb.	59				
	May	64				
	Oct.	66				
	Mar.	69				
J 18	Apr.	56				
	Mar.	59				
	Feb.	60				
	May	60				
	Dec.	65				
	Nov.	66				
	Jan.	69				
	Dec.	72				
J 20	Feb.	47				
	Jan.	51				
	Dec.	58				
	Mar.	62				
	Apr.	64				
	May	65				

Chart 4. Percentage of Semen Samples showing various grades of 6-days Motility between Bulls Above and Below Average Fertility Level.

	Bulls	N.R.%	Value of 6-days Motility (Grades of motility x % of motile sperm)			
			300 - 400	401 - 500	501 - 600	Above 600
Bulls with below av. Fertility	H 7	70	4	61	35	
	G 64	69	5	50	45	
	H 3	68	14	52	34	
	J 17	66		33	67	
	J 18	63		10	49	41
	G 59	62	6	47	44	3
	H 6	61	20	24	44	12
Weighted Means		Above av.	6.82	37.50	44.32	11.36
		Below av.	11.97	40.14	38.03	9.86
Bulls with above av. Fertility	J 20	59	12	35	35	18
	G 66	58	20	60	20	
	H 1	56	6	42	42	8
	G 65	56	3	35	41	21
	J 8	54		39	61	
	J 16	41	40	35	20	5

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