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# Studies of Respiration Rate of Dairy Bull Spermatozoa

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## ABSTRACT

Values for oxygen consumption of spermatozoa collected from 10 dairy bulls were determined by means of a modified Barcroft-Warburg respirometer. Oxygen consumption increased in case of sperm evidencing the longest survival time. Wide variations occurred in the oxygen consumption of different ejaculates of the same sire and are similar to variations occurring in other characteristics of different ejaculates collected from a given sire. Samples of semen containing large numbers of abnormal sperm were characterized by low oxygen consumption. Seminal fluid consumes oxygen at rates varying from 3.3 to 24.0 per cent of normal semen.

Spermatozoa of high initial motility have a higher average initial oxygen consumption, and on the average maintained a higher motility rate in storage.

The intracellular reserves are an important factor influencing spermatozoa metabolism and longevity.

## SUMMARY

1. Average values for oxygen consumption of bull spermatozoa increase with increases in survival time of the spermatozoa and a positive correlation between the two is observed. However, wide variations and overlapping of values occur in each group.

2. Variations in the intensity of oxygen consumption of different ejaculates of the same sire are similar to the wide variations occurring in other characteristics of the samples.

3. The presence of large numbers of abnormal spermatozoa in semen indicate a low quality of spermatozoa present, as judged by oxygen consumption per billion living spermatozoa.

4. Spermatozoa of high initial motility have a higher average initial oxygen consumption.

5. Seminal fluid consumes oxygen at rates varying from 3.3 to 24.0 per cent of whole semen.

6. Marked increases in oxygen consumption of spermatozoa were secured in a seminal fluid medium over control samples in phosphate buffer.

7. Centrifuging causes a depression of oxygen consumption of bull spermatozoa apparently due to a decrease in the number of living spermatozoa.

# Studies of Respiration Rate of Dairy Bull Spermatozoa

RAY E. ELY, H. A. HERMAN, AND C. F. WINCHESTER*

In recent years the energy metabolism of spermatozoa has been investigated with the view of obtaining a better understanding of their physiological requirements. Such information is of vital importance in the use and storage of semen used for artificial breeding.

Both biochemical methods and manometric techniques have contributed to our present knowledge of spermatozoa metabolism. In conducting investigations in this field it must be recognized that the sperm cell is a complex colloidal system of chemical constituents, similar to all biological material, whose actions and reactions are sensitive to minute changes in environmental conditions. Wide variations in the original characteristics of spermatozoa are undoubtedly an important factor in securing wide differences in response of various samples to the same environmental conditions.

These variations in sperm characteristics have been emphasized by the studies of Herman and Swanson (1941) who found ejaculates of the same sire varied widely in all properties studied including motility, numbers of abnormal spermatozoa, volume, and maintenance of motility on storage.

This investigation was undertaken to determine the relation between some of the characteristics of semen and the respiratory activity of spermatozoa. Studies are also included of some of the factors affecting respiration rate of bull spermatozoa, relative oxygen consumption of seminal fluid, and variations in the oxygen consumption of various bulls and different ejaculates of the same bull.

## REVIEW OF LITERATURE

Increased use of artificial breeding has recently stimulated an interest in the physiological requirements of spermatozoa and their source of energy. Many investigations have been directed toward a better understanding of the mechanism of energy metabolism

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of spermatozoa. Most studies, however, have not only been suggestive in explaining spermatozoa metabolism mechanisms but have added considerably to the practical applications of artificial breeding techniques and semen storage.

## Semen Characteristics and Their Relation to Fertility

Various characteristics of the semen have been studied in an effort to forecast the fertility of a given sire. Some of the more important measures and their relation to fertility are:

1. **Motility.**—The fact that motility is necessary for fertilization of the egg indicates that the degree of motility might be an important characteristic for determining the value of a given sample of semen.

A definite relation between motility and percentage of conceptions obtained was observed by Donham, Simms and Shaw (1931) in examinations of bull semen after natural matings. Davis (1938 found that motility or activity of the spermatozoan cell proved to be the best single indication of viability. Davis and Williams (1939) also found a correlation between percentage of motility and concentration of spermatozoa per cubic millimeter.

Herman and Swanson (1941) and Swanson and Herman (1941) found the length of survival with good motility was a good index of fertility of the semen. The initial motility, however, was related to fertility only to a limited extent.

2. **Glycolysis.**—Comstock (1939) and Comstock and Green (1939) found a definite correlation between rate of glycolysis of ram spermatozoa and duration of motility, which has been shown by others to be indicative of fertility.

3. **Oxygen Consumption.**—A comparison of the breeding record of thirteen bulls with the initial respiration rate of their semen showed a close relation between these two factors in a study by Walton and Edwards (1938). They gave the following summary of initial respiration rate and services per conception.

| No. Bulls | Services/conception | Ave. initial Resp. rate per billion Sperm. |
|---|---|---|
| 3 | 1-1.9 | 286 |
| 6 | 2-2.9 | 187 |
| 4 | 3-6.9 | 127 |

These figures are in agreement with an average value of 139 and 216 $mm^3$ oxygen per hour given by Shergin (1937) and Redenz (1933) respectively.

4. **Abnormal Spermatozoa.**—Williams (1920a) (1920b) found one of the most serious defects of the spermatozoa to be reduced size or underdevelopment of nuclear elements.

None of the bulls examined by Williams and Savage (1925) producing sperm having more than 16.6 per cent abnormal heads had a good breeding record, while 27 bulls with poor breeding records averaged 50.1 per cent abnormal heads. However, breeding efficiency was not always predictable from the examinations for abnormal spermatozoa. Three sterile bulls were reported by Addis (1937) as having a normal volume, concentration and motility of spermatozoa but had 40 to 50 per cent abnormal spermatozoa. Generales (1928) and Sciuchetti (1938) reported similar results.

Comstock and Green (1939) found large numbers of abnormal spermatozoa in a sample to affect the rate of glycolysis, however, the association was not always the same.

Swanson and Herman (1941) found the semen of bulls of good, questionable and poor fertility averaged respectively 142, 240 and 458 abnormal spermatozoa per 1000.

Studies of abnormal spermatozoa in human semen was believed to be one of the best methods of indicating fertility by Moench (1929) Moench and Holt (1931) and Mason (1929).

Studies with other species indicating that high fertility cannot be expected in samples with high percentages of abnormal spermatozoa have been reported by Savage, Williams and Fowler (1930) and McKenzie and Phillips (1934) for the ram, and McKenzie and Phillips (1933) and Phillips (1935) for boar semen. McKenzie and Berliner (1937) also found an increase in the number of abnormal spermatozoa in ram semen during their non-breeding season.

5. **Concentration of Spermatozoa.**—Comstock and Green (1939) found density or concentration of sperm to be an important factor in rate of glycolysis of spermatozoa. A slight correlation was also found between the percentage of motility and concentration of spermatozoa by Davis and Williams (1939). Herman and Swanson (1941) report that dairy bulls producing semen of greatest sperm concentration were generally among the most fertile.

## Spermatozoa Metabolism

More studies have been made of the relation between the physical and chemical characteristics of semen and the corresponding fertility than of the relation between metabolic activity of spermatozoa and fertility. It seems probable that further studies of spermatozoa metabolism may give a better explanation for the

relation between semen characteristics and fertility and a better understanding of spermatozoa requirements from the standpoint of storage and diluting materials used in artificial breeding practices.

1. **Glycolysis.**—Bernstein (1933a) reported the glucose content of the semen of one bull, one dog and one stallion as 300, 116 and 82 mg. per cent respectively. The concentration was relatively constant in the spermatozoa but varied in the seminal fluid. When semen was stored outside the body, the glucose content of the fluid fell but that of the spermatozoa remained relatively constant. The decrease in glucose was very small in seminal fluid freed of spermatozoa by centrifuging. From these studies he suggested that spermatozoa can metabolize the glucose of the seminal fluid.

Fresh ejaculates of bull and human semen were found by Bernstein and Slovochotov (1933) to contain 40 to 50 mg. per cent of lactic acid, which increased on storage. The motility of spermatozoa gradually declined, but ceased at different lactic acid levels. According to Bernstein and Shergin (1933) the hexose phosphoric acid content of semen varies in different animals of the same species, but variations in surviving semen could not be correlated with the disappearance of glucose or the formation of lactic acid.

Shergin (1937) determined glucose by a fermentation technique rather than the reduction method and found the true glucose content of the semen of the stallion and boar 3.5 and 7.25 mg. per cent respectively. The bull and ram contained 334 and 179 mg. per cent respectively.

Moore and Mayer (1941) reported the average sugar concentration in 26 analyses of semen from 7 rams as 516 mg. per cent with a range of 217 to 744 mg. per cent.

Redenz (1933) found that glycolysis was responsible for motility of bull spermatozoa under anaerobic conditions and under aerobic conditions glycolysis could be stimulated by addition of sugar.

On the other hand, Ivanov (1935) (1936) believed that motility of bull and boar spermatozoa did not depend on glycolysis because motility was retained several hours in the presence of monohaloacetic acids.

A close relation was found between glycolysis and duration of motility in studies by Comstock (1939). Very little glycolysis took place in seminal fluid freed of spermatozoa.

MacLeod (1940) found oxygen is detrimental to the motility of spermatozoa. He also found (1941a) (1941b) that monoiodo-

acetate and fluoride inhibited glycolysis and depressed motility of human spermatozoa. Glycolysis was relatively constant from specimen to specimen but large differences in individuals occurred.

Moore, Mayer and McKenzie (1940) and Moore and Mayer (1941) found a pronounced effect of temperature on the glycolysis rate of ram spermatozoa. Glycolysis did not take place at 0 degrees C. but rapid increases occurred with increases in temperature above 0 degrees. Moore and Mayer (1941) also found that sugar in ram semen is metabolized to lactic acid which affects the motility of the spermatozoa by changes in pH. In some experiments the motility ceased when the sugar concentration was still quite high and in others mobility continued after complete disappearance of the sugar.

2. **Aerobic Respiration.**—In addition to studies regarding the oxidative processes in spermatozoa metabolism, the interrelations of oxidative and glycolytic mechanisms as energy yielding reactions for spermatozoa have been extensively investigated.

Using dog spermatozoa, Ivanov (1931) reported that motility was retained under anaerobic conditions and also when respiration was inhibited with cyanide.

Ivanov (1936) found the respiratory quotient of sheep spermatozoa to be about 0.78, indicating that substances other than carbohydrates are oxidated. Shergin (1937) reported that spermatozoa consumed 29 $cm^3$ oxygen per gm. in one-half hour which is much lower than most other animal tissues. Ram spermatozoa consumed more oxygen than the spermatozoa of the boar or stallion but if diluted to the same concentration the oxygen consumption was essentially the same.

Walton and Edwards (1938) reported a range in initial oxygen consumption of bull spermatozoa from 13 sires as 74 to 356 $mm^3$ oxygen per billion spermatozoa per hour, which was closely related to the breeding efficiency of the sire.

MacLeod (1939) believed that nearly all the energy required for normal function of human spermatozoa is derived from glycolysis and not from respiration. Ross et al (1941) reported similar results with human spermatozoa. The respiration of human spermatozoa, according to Shettles (1940a) varies inversely with the age of the specimen and directly with the number of cells per unit volume. The R.Q. also varied inversely with the age of the sample and directly with the rate of oxygen consumption, indicating a shift in the metabolites being oxidized. No oxygen was consumed by semen devoid of spermatozoa.

Lardy and Phillips (1941a) indicated that oxygen was required for the utilization of intracellular reserves of the spermatozoa because in a Ringer-phosphate medium containing no sugar they remain motile only in the presence of air.

From studies of the use of phospho-lipids as a source of energy for bull spermatozoa Lardy and Phillips (1941b) found that in the presence of phospholipids the rate and duration of oxygen consumption is greatly increased. The oxygen consumption in a medium containing glucose was not appreciably increased by the addition of lecithin.

Henle and Zittle (1942) confirmed the observation of Redenz (1933) of a wide range of oxygen consumption by bovine epididymal spermatozoa which they attribute to varying degrees of maturity of the spermatozoan cells. Optimal pH for oxygen uptake of bovine epididymal spermatozoa was found to be pH 7.5 to 8.0. Seminal spermatozoa had a decidedly lower oxygen consumption than epididymal spermatozoa.

### Factors Affecting the Oxygen Consumption of Spermatozoa

A knowledge of the factors affecting the respiration rate of spermatozoa is important not only in analyzing the results of spermatozoa metabolism studies but also in a comparison of the results of various investigators.

1. **Effect of Dilution.**—Gray (1928) found that dilution of sea urchin spermatozoa increased the activity of the cells. Windstosser (1935) however, did not find this true of epidymal spermatozoa of the guinea pig, rat or bull, although oxygen consumption was increased. Shergin (1937) also found that concentration of spermatozoa in the medium was a factor affecting oxygen consumption. Winberg (1940) reported increases in respiration intensity on dilution of avian spermatozoa accompanied by a decrease in the length of life.

MacLeod (1914a) reported that human spermatozoa were activated by a substance in seminal fluid, possibly glucose, rather than by the dilution effect. This had previously been indicated by Nesmejanova (1937) who found that boar epididymal spermatozoa were in surroundings poor in electrolytes but during ejaculation the medium is rich in electrolytes, particularly sodium.

Winchester and McKenzie (1941a) found that at concentrations of spermatozoa from one to six billion cells per cc. an increase in concentration was accompanied by a decrease in respiration rate. At lower concentrations the effect was sometimes ob-

served but frequently there appeared to be no effect. Respiration of yeast cells remained constant at concentrations of one to eight billion cells per cc.

2. **Hydrogen Ion Concentration.**—Dilutions of epididymal spermatozoa of the guinea pig, rat and bull with non-isotonic buffer solutions at pH 6.1 to 8.0 showed no definite relation between respiratory rate and motility or pH, according to the studies of Windstosser (1935).

Winchester and McKenzie (1941b) reported the optimum pH for respiration of boar spermatozoa as 7.2 to 7.3 and for ram spermatozoa as 7.0 to 7.2. Both decline progressively on either size of the optimum pH, however, a unit change in pH has less influence on respiration rate of ram spermatozoa than on boar spermatozoa.

Henle and Zittle (1942) report the optimal pH for oxygen uptake of bovine epididymal spermatozoa as pH 7.5 to 8.0.

3. **Effect of Temperature.**—Shergin (1937) found temperature an important factor affecting respiration rate of spermatozoa. Chang and Walton (1940) found sudden changes in temperature and prolonged low temperatures to have a detrimental effect on the subsequent respiratory activity of ram spermatozoa.

4. **Effect of Various Gases.**—Shettles (1939) (1940a) (1940b) showed that spermatozoa were inactivated when exposed to carbon dioxide even when buffered and no measurable change in pH occurred. The concentration of carbon dioxide found in the testis, epididymis and vas deferens was lower than that required to immobilize the spermatozoa in vitro. Shettles (1940a) also found that spermatozoa showed motility for 17 to 36 hours in nitrogen, nitrous oxide, helium or air at very low pressure. In helium or oxygen there was a marked increase in activity. This is in contrast with MacLeod's observation that motility is markedly impaired in the presence of oxygen or air.

5. **Effect of Certain Chemicals.**—Lardy and Phillips (1941a) blocked both glycolysis and oxidative mechanisms of spermatozoa and found they still retained motility considerable periods of time. It seems possible that enzymatic stimulation of unknown intermediate energy-yielding reactions are an important source of energy to the spermatozoa under these conditions. Low concentrations of iodoacetic acid completely inhibited the breakdown of glucose to lactic acid but did not directly affect motility. Cyanide in concentrations of 0.001 M inhibited the motility of spermatozoa in egg-yolk buffer but had little effect on motility or lactic acid production in a Ringer-phosphate glucose medium.

Henle and Zittle (1941) found that gramicidin initially stimulated and then completely inhibited the oxygen consumption of bovine spermatozoa in Ringer phosphate. If sufficiently small amounts were used, only the stimulation was observed during the period of the experiment. In alkaline phosphate buffer only the stimulation of oxygen consumption is observed, while in Ringer bicarbonate of the same pH the respiration is greatly inhibited.

6. **Effect of Composition of Medium.**—The importance of composition of the medium in studies of spermatozoa metabolism is illustrated by the studies of Fenn (1931) with muscle metabolism, Shaffer, Chang and Gerard (1935) and Chang, Shaffer and Gerard (1935) with nerve metabolism and Canzanelli et al (1942a) (1942b) with brain, liver and kidney tissue who found ionic concentration of the medium; particularly sodium, potassium and calcium; to be highly important in controlling normal metabolism.

Windstosser (1935) found that motility is affected largely by the nature of the ions in the diluting medium.

Bernstein (1933) has reported that spermatozoa apparently had a very high resistance to pure solutions containing a single neutral salt or sugar. The studies of Nesmejanova (1937) indicate that the concentration of electrolytes, particularly sodium, may be important in the initiation of motility at the time of ejaculate.

MacLeod (1939) found that the respiration of human spermatozoa was almost entirely glycolytic in the presence of glucose.

Lardy and Phillips (1941b) found oxygen consumption of spermatozoa in the presence of glucose less than the "endogenous" respiration in a glucose-free medium. This indicates a preferential utilization of glycolytic mechanisms rather than oxidative mechanisms by spermatozoa. Similar evidence of a competition for substrates by the liver has been presented by Mirsky et al (1937).

Redenz (1933) showed that added lactate increased the respiration rate but did not increase the motility of spermatozoa suspensions containing no sugar, which indicates the oxygen uptake of spermatozoa may be for oxidative removal of the metabolic products.

MacLeod (1940) also found spermatozoa produced approximately twice the amount of lactic acid (aerobically and anaerobically) in seminal fluid as in Ringer's glucose. Since motility was about the same in both cases, he believed the increased lactic acid production may be related to the relatively high viscosity of seminal fluid and not to any specific stimulating substance in the seminal fluid itself. Winchester and McKenzie (1941c) found oxy-

gen consumption of the seminal plasma of the boar ranged from 5 to 22 per cent of that of the whole semen. Respiration of the seminal plasma continued at reduced intensity after heating at 100 degrees C. for five minutes, but the fraction of the seminal fluid passing through a porcelain filter did not consume oxygen.

Henle and Zittle (1942) reported a stimulation of oxygen consumption by additions of epididymal secretion to epididymal spermatozoa but not by addition of seminal fluid. The factor present was heat stable and not dialyzable. Dilute suspensions had the greater increases, while in preparations containing more than 500 million cells per milliliter no stimulation was noticed or even a slight depression took place. In a comparison of oxygen uptake in Ringer-glucose and Ringer-bicarbonate glucose, the oxygen uptake was markedly increased in the latter medium and the motility also appeared greater than in the bicarbonate-free medium.

A summary of the literature reveals wide variations in the characteristics of semen samples. The relation of various semen characteristics to the activity and longevity of the spermatozoa and their value in forecasting the fertility of a sire have been quite thoroughly investigated.

Investigations of the energy requirement of spermatozoa, however, have given less conclusive results. There is considerable evidence that the longevity of spermatozoa is an inherent quality of the cell affected largely by subsequent extracellular environment. On this basis the storage of spermatozoa is a problem of conservation of intracellular reserves, and providing an environment for the greatest efficiency in the utilization of these reserves would probably result in the most nearly ideal storage conditions.

## MATERIALS AND METHODS

**Bulls Used.**—Ten bulls in use at the University of Missouri dairy herd furnished semen for these studies. Examination of certain characteristics of the ejaculates indicate that they represent rather wide ranges in chemical and physical characteristics of the ejaculate and also in breeding efficiency.

**Experimental Procedure.**—Semen collections were made with the artificial vagina described by Herman and Ragsdale (1939). Precautions were taken to avoid rapid changes in temperature at the time of collection by collecting in a protected centrifuge tube. An endeavor was made to maintain uniform collection periods with the bulls used although all the samples collected were not used for respiration measurements. Immediately after collection

a portion of the sample (3 to 6cc.) was placed in a small sterile vial, wrapped in a small piece of dry paper towel, placed in a rubber finger stall and kept in a thermos bottle at 70° F. until delivered to the laboratory, and the respiration measurements begun. Another portion of the sample was held for examination of motility, percentage of abnormal spermatozoa, concentration of spermatozoa and whenever possible, determination of length of survival on storage at 40° F.

Motility ratings and determination of percentage of abnormal spermatozoa were made by the methods described by Herman and Swanson (1941).

In most cases the spermatozoa count was determined by two individuals with a hemocytometer diluting pipette and Levy counting chamber. In some instances a 1:200 dilution for the counting chamber was secured by diluting 0.05 ml. of semen to 10 ml. volume and checking with a hemocytometer dilution.

The samples were prepared for the respiration measurements by buffering the seminal fluid with M/8 phosphate buffer at pH 6.87. In most cases the samples were centrifuged, one-half the seminal fluid removed and replaced with a measured volume of phosphate buffer and the sample remixed. In this way the concentration of spermatozoa in the medium was adjusted to the desired count and a medium of seminal fluid buffered with an equal volume of phosphate buffer was secured. In some samples of a high spermatozoa count fluid-phosphate buffer medium was secured by diluting the semen with an equal volume of phosphate buffer.

A modification of the staining technique reported by Lasley, Easley and McKenzie (1942) for differentiating the living and dead spermatozoa was used to determine the percentage of living spermatozoa. A total of 500 spermatozoa were counted for each sample and the percentage of living spermatozoa calculated. Stains were made of the original sample, at the beginning and end of the respiration measurements, to detect any effect of treatment in preparing the samples and also to check the survival of spermatozoa during the determination of respiration rate. Determination of the pH at the end of the trials were made to avoid any marked changes in pH of the trials used for comparison.

**Respiration Measurements.**—Aerobic respiration measurements were made in air with a modified Barcroft-Warburg respirometer. The principles of this method have been described by Dixon (1934). The accuracy of the apparatus was determined by simultaneous respiration measurements of yeast suspensions in each of the

manometers. The error (mean deviation x 100/mean) was 2 per cent.

For the studies of oxygen consumption 2.0 ml. of the spermatozoa suspension was used and 0.1 ml. of 20 per cent KOH placed on a coil of Whatman No. 40 starch-free filter paper in the center well for absorption of carbon dioxide. Two and one-tenth ml. of distilled water was placed in the compensating flask.

A 10 minute shaking period was allowed for the flasks to come to an equilibrium before closing the manometer tubes and the first readings taken five minutes later. Subsequent readings were taken at ten minute intervals over a period of one hour for routine determinations. In all cases the first readings were secured between 90 minutes and 120 minutes after ejaculation, which makes our figures for "initial" oxygen consumption represent those for approximately the third hour after ejaculation. Oxygen uptake of buffered seminal fluid was determined at the same time in separate flasks.

## EXPERIMENTAL RESULTS

**1. Relation of Survival on Storage to Oxygen Consumption of Bull Spermatozoa.**—All samples with which it was possible to determine the length of survival on storage are grouped in Table 1 in three groups according to length of survival with a 2 motility rating on storage at 40° F. and compared with their respiration rate per billion spermatozoa and per billion living spermatozoa. The range of motility ratings given are from 0 to 5 according to the degree of motility described by Herman and Swanson (1941). The results in Table 1 show a definite trend to higher oxygen consumption in the groups having greater length of survival on storage. With the same intracellular reserves one would expect an increased metabolic rate to shorten the length of life of the spermatozoön cell, however. as increased oxygen consumption is associated with greater length of survival on storage it probably indi-

TABLE 1.—RELATION OF SURVIVAL ON STORAGE TO INITIAL OXYGEN CONSUMPTION OF BULL SPERMATOZOA.

| Sire | Survival* | $mm^3$ $O_2$/hour/billion sperm | $mm^3$ $O_2$/hour/billion living sperm |
|---|---|---|---|
| 6 | 24 | 187.67 | 228.59 |
| 3 | 24 | 52.70 | 155.45 |
| 4 | 24 | 123.03 | 149.11 |
| 3 | 24 | 95.57 | 114.05 |
| 7 | 24 | 60.52 | 109.05 |
| 7 | 24 | 47.12 | 84.29 |
| Average | 24 | 94.43 | 140.09 |
| | (48-96 hrs. survival) | | |
| 6 | 48 | 71.85 | 104.59 |
| 1 | 48 | 95.97 | 290.82 |
| 2 | 72 | 51.59 | 73.70 |
| 1 | 72 | 197.69 | 339.67 |
| 6 | 96 | 57.51 | 77.48 |
| 6 | 96 | 72.44 | 99.10 |
| 6 | 96 | 97.05 | 185.56 |
| Average | 75 | 92.01 | 168.84 |
| | (over 96 hrs. survival) | | |
| 1 | 120 | 89.22 | 132.96 |
| 1 | 120 | 162.30 | 228.91 |
| 6 | 168 | 294.39 | 362.55 |
| 4 | 192 | 308.59 | 405.50 |
| Average | 150 | 213.63 | 282.48 |

*Hours 2 motility was maintained.

cates a difference in the intracellular reserves of the spermatozoa or possibly a difference in the available metabolizable substances in the medium, assuming that spermatozoa can utilize constituents of the medium.

2. **Relation Between Characteristics of the Ejaculate and Oxygen Consumption.**—While the data in Table 1 represent comparisons with determinations made on the same samples, the oxygen consumption measurements and survival on storage figures in Table 2 are average figures for the number of samples indicated, which were collected and studied over a period of six months, but both determinations were not made on the same samples in all cases.

These data show the same wide variation in length of survival on storage as previously reported by Herman and Swanson (1941). Equally as great a range of values are also found in the oxygen consumption values which makes it difficult to accurately predict the breeding ability of a sire from a single determination of any one characteristic of a sample. All sires had some samples with a relatively short survival time on storage, but sires having a longer average survival time had some samples maintaining 2 motility longer than the best samples of the poorer sires. The same is true of values for initial oxygen consumption.

TABLE 2.—RELATION BETWEEN CHARACTERISTICS OF THE EJACULATE AND OXYGEN CONSUMPTION.

| | Survival on Storage | | | Respiration Rate | | | |
|---|---|---|---|---|---|---|---|
| Sire | No. Samples | Ave.* Survival | *Range | Samples | $O_2$/hr. per billion sperm | $O_2$/billion Living sperm | Range |
| 1 | 15 | 110 | 24-192 | 6 | 137.56 | 210.01 | 94-459 |
| 2 | 6 | 76 | 24-144 | 4 | 63.49 | 95.99 | 52-167 |
| 3 | 20 | 68 | 24-168 | 6 | 71.32 | 108.43 | 73-155 |
| 4 | 4 | 48 | 24-120 | 6 | 74.44 | 201.83 | 88-149 |
| 5 | 10 | 48 | 24-96 | 4 | 59.00 | 103.62 | 73-130 |
| 6 | 10 | 46 | 24-96 | 9 | 88.37 | 161.95 | 70-290 |
| 7 | 6 | 24 | All>24 | 4 | 44.81 | 79.88 | 60-109 |

*Hours 2 motility was maintained.

3. **Relation of Abnormal Spermatozoa to Oxygen Consumption of Bull Spermatozoa.**—The results in Table 3 show a decrease in intensity of initial oxygen consumption with increasing numbers of abnormal spermatozoa. However, the number of abnormal spermatozoa may not be the only contributing factor as the samples representing the high percentage of abnormal spermatozoa were also largely samples having a short survival of maintenance on

storage. Also, all samples having a low number of abnormal spermatozoa do not have high initial oxygen consumption.

TABLE 3.—RELATION OF ABNORMAL SPERMATOZOA TO OXYGEN CONSUMPTION OF BULL SPERMATOZOA.

| Sire | Abnormal Sperm per 1,000 | $mm^3$ Oxygen per billion sperm | $mm^3$ Oxygen per billion Living Sperm |
|---|---|---|---|
| 1 | 45 | 72.44 | 99.10 |
| 1 | 57 | 49.15 | 94.52 |
| 1 | 27 | 197.69 | 339.67 |
| 1 | 81 | 97.17 | 125.38 |
| 1 | 45 | 308.59 | 459.89 |
| 4 | 69 | 63.28 | 103.40 |
| 4 | 66 | 123.02 | 149.11 |
| 4 | 84 | 89.22 | 139.49 |
| 3 | 66 | 83.63 | 137.10 |
| 3 | 54 | 74.01 | 86.97 |
| 3 | 48 | 97.57 | 114.05 |
| 6 | 72 | 71.85 | 104.59 |
| Average | 59.5 | 110.47 | 162.77 |
| 1 | 111 | 100.34 | 141.52 |
| 5 | 155 | 42.58 | 107.52 |
| 5 | 102 | 50.27 | 73.28 |
| 4 | 183 | 33.30 | 88.10 |
| 3 | 114 | 52.70 | 155.45 |
| 2 | 111 | 28.12 | 52.46 |
| 2 | 198 | 42.91 | 75.81 |
| 6 | 123 | 26.67 | 71.31 |
| 6 | 117 | 95.97 | 290.82 |
| 6 | 116 | 108.08 | 281.46 |
| 6 | 180 | 187.67 | 228.59 |
| Average | 137.3 | 69.87 | 142.39 |
| 5 | 274 | 84.16 | 130.08 |
| 7 | 552 | 47.12 | 84.29 |
| 7 | 645 | 30.01 | 60.02 |
| 7 | 645 | 60.52 | 109.05 |
| 7 | 624 | 41.60 | 66.14 |
| Average | 548 | 52.68 | 89.92 |

4. **Relation of Initial Motility to Oxygen Consumption of Bull Spermatozoa.**—Samples having the higher initial motility included those samples having higher initial oxygen consumption, however, some samples having an initial motility rating of 5 also had low initial oxygen consumption. While the average figures show a lower oxygen consumption for samples of poor initial motility, such wide ranges occur in the respiration values of any one group that there is apparently not the close association one might expect.

TABLE 4.—RELATION OF INITIAL MOTILITY TO OXYGEN CONSUMPTION OF BULL SPERMATOZOA.

| Sire | Initial Motility | $mm^3$ oxygen per billion sperm | $mm^3$ oxygen per billion living sperm |
|---|---|---|---|
| 1 | 5 | 308.59 | 459.89 |
| 1 | 5 | 197.69 | 339.67 |
| 6 | 5 | 187.67 | 228.59 |
| 6 | 5 | 97.05 | 185.56 |
| 3 | 5 | 83.63 | 137.10 |
| 1 | 5 | 97.17 | 125.38 |
| 1 | 5 | 72.44 | 99.10 |
| 1 | 5 | 49.15 | 94.52 |
| 2 | 5 | 42.91 | 75.81 |
| 5 | 5 | 50.27 | 73.28 |
| 2 | 5 | 28.12 | 52.46 |
| Average | | 110.43 | 170.12 |
| 6 | 4 | 95.97 | 290.82 |
| 3 | 4 | 52.70 | 155.45 |
| 4 | 4 | 89.22 | 139.49 |
| 6 | 4 | 71.85 | 104.59 |
| 3 | 4 | 74.01 | 86.97 |
| Average | | 76.75 | 155.46 |
| 6 | 3 | 108.08 | 154.40 |
| 4 | 3 | 63.36 | 168.96 |
| 4 | 3 | 123.02 | 149.11 |
| 1 | 3 | 100.34 | 141.52 |
| 5 | 3 | 42.58 | 107.52 |
| 4 | 3 | 63.28 | 103.40 |
| 4 | 3 | 33.30 | 88.10 |
| Average | | 76.28 | 130.43 |
| 5 | 2 | 84.16 | 130.08 |
| 3 | 2 | 95.57 | 114.05 |
| 7 | 2 | 60.52 | 109.05 |
| 7 | 2 | 47.12 | 84.29 |
| 6 | 2 | 26.67 | 71.31 |
| 7 | 1 | 41.60 | 66.14 |
| 7 | 1 | 30.01 | 60.02 |
| Average | | 55.09 | 90.71 |

5. **Relative Metabolic Rates of Bull Semen and Seminal Fluid.**—Oxygen consumption of 23 samples of seminal fluid varied from 4.52 to 67.33 $mm^3$ oxygen per hour per 2 cc., which represented a range of 3.3 to 24.0 per cent of the oxygen consumption of the semen from which it was centrifuged. Table 5 shows there was no relation between the intensity of oxygen consumption of whole semen and the oxygen uptake of its seminal fluid.

TABLE 5.—RELATIVE METABOLIC RATES OF BULL SEMEN AND SEMINAL FLUID.

| Semen | Seminal fluid | Percentage of semen |
|---|---|---|
| 201.33 | 6.60 | 3.3 |
| 143.66 | 5.24 | 3.6 |
| 97.36 | 4.52 | 4.6 |
| 229.03 | 11.78 | 5.1 |
| 247.03 | 16.06 | 6.5 |
| 123.40 | 9.16 | 7.4 |
| 89.67 | 7.29 | 8.1 |
| 79.24 | 7.02 | 8.9 |
| 111.59 | 10.29 | 9.2 |
| 98.72 | 10.10 | 10.2 |
| 56.10 | 6.17 | 11.0 |
| 79.54 | 9.72 | 12.2 |
| 101.15 | 12.90 | 12.8 |
| 162.55 | 21.07 | 13.0 |
| 76.24 | 10.54 | 13.8 |
| 159.12 | 22.62 | 14.2 |
| 94.85 | 15.50 | 16.3 |
| 145.74 | 26.83 | 18.4 |
| 340.05 | 67.33 | 19.8 |
| 136.13 | 29.98 | 22.0 |
| 44.87 | 10.24 | 22.8 |
| 50.03 | 12.00 | 24.0 |

Examination of the seminal fluid for spermatozoa after centrifuging was negative in all of the trials used for comparison.

6. **Effect of Seminal Fluid in Medium on Oxygen Consumption of Bull Spermatozoa.**—In this experiment control samples were suspended in a phosphate buffer medium by diluting the semen with an equal volume of phosphate buffer, centrifuging out the spermatozoa, washing the spermatozoa once in phosphate buffer before making the final dilution with phosphate buffer. Samples in the seminal fluid-phosphate buffer medium received the same treatment of centrifuging and remixing as the controls except half the seminal fluid was retained and the sample brought up to volume with phosphate buffer. Oxygen consumption measurements of buffered seminal fluid samples were made at the same time. Table 6 shows a stimulation of oxygen consumption by spermatozoa in the presence of seminal fluid after correction for the oxygen uptake of the seminal fluid used. While increases in oxygen consumption were secured in all trials conducted, quite wide difference in the percentage increase was secured.

TABLE 6.—EFFECT OF SEMINAL FLUID ON OXYGEN CONSUMPTION OF BULL SPERMATOZOA.

| | $mm^3$ $O_2$/hr./billion living spermatozoa | | |
|---|---|---|---|
| | Phos. buffer pH 6.87 | Phos. buffer +Seminal Fluid | Percentage Increase |
| 3-25-42 | 75.67 | 198.31* | 162.1 |
| 4-11-42 | 96.24 | 141.11* | 46.6 |
| 4-18-42 | 33.50 | 56.17* | 67.7 |
| Average | 68.47 | 131.86 | 92.6 |

*Corrected for oxygen uptake of seminal fluid.

**7. Effect of Centrifugation on the Subsequent Oxygen Consumption of Bull Spermatozoa.**—A comparison of the oxygen consumption of centrifuged samples with control samples shows a decrease in oxygen consumption after centrifuging. Differential staining of live and dead spermatozoa also showed a decrease in the percentage of living spermatozoa in the centrifuged samples. Calculation of the results on the basis of oxygen consumption per billion living spermatozoa show essentially the same values for both centrifuged and control samples, which indicates that the metabolic rate of the living spermatozoa was the same in both cases, although some spermatozoa were killed by centrifuging. Table 7 presents the results of these comparisons of centrifuged and control samples. Motility ratings of control and centrifuged samples were the same at the beginning of the experiment.

TABLE 7.—EFFECT OF CENTRIFUGATION ON THE SUBSEQUENT OXYGEN CONSUMPTION OF BULL SPERMATOZOA.

| Cmm. $O_2$/hr. | | $mm^3$ $O_2$/billion | | $mm^3$ $O_2$/billion | | Per Cent Living | |
|---|---|---|---|---|---|---|---|
| Control | Centrifuged | Control | Centrifuged | Control | Centrifuged | Control | Centrifuged |
| 130.78 | 77.39 | 162.18 | 95.97 | 289.60 | 290.82 | 56.0 | 33.0 |
| 63.65 | 54.67 | 95.57 | 82.09 | 114.05 | 114.05 | 83.8 | 72.0 |
| 92.61 | 63.30 | 187.67 | 137.60 | 228.59 | 196.50 | 82.1 | 70.0 |
| Average: | | | | | | | |
| 95.68 | 65.12 | 148.47 | 105.22 | 210.75 | 200.46 | 74.0 | 58.3 |

## DISCUSSION

From these investigations it would appear that spermatozoa exhibiting greater activity may live longer than those of lower activity. For instance, it is well recognized that spermatozoa of good initial motility have the best opportunity to survive and fertilize the egg in the female genital tract. The same spermatozoa

would also be expected to retain good motility longer on storage. Walton and Edwards (1938) indicated that spermatozoa with a high initial respiration rate retained this quality longer than spermatozoa with a low initial respiration rate. This would indicate that spermatozoa vary widely in (1) their inherent intracellular composition (2) metabolizable nutrients of their environment (2) physico-chemical characteristics of their environment or (4) their efficiency of utilization of metabolizable substances.

Gray (1931) was probably the first to discuss the reasons for the decline in respiration of semen of different individuals declining at different rates, presumably due to intrinsic properties of the spermatozoa determining the rate and duration of metabolic activity. The fact that Bernstein (1933b) did not increase the survival time of spermatozoa by addition of nutrient substances to the medium further indicates their dependence on intracellular reserves for energy metabolism. Moore and Mayer (1941) also found that if sugar is a source of energy for ram spermatozoa, it is not the only source. In an experiment at 22.5 degrees C. ram spermatozoa retained motility for 30 hours in the absence of sugar.

There is considerable evidence of the importance of the consumption and physico-chemical characteristics of the medium on spermatozoa motility and metabolism but no evidence that these factors are not affecting the inherent intracellular reserves of the spermatozoa.

Swanson and Herman (1941) report that changing the seminal fluid of good and poor semen samples did not affect the characteristics of the spermatozoa probably indicates that the physcial characteristics of a natural environment or the presence of metabolizable nutrients are probably of minor importance in altering spermatozoa metabolism.

This study of the relation between spermatozoa survival and initial oxygen consumption further indicates the importance of the intracellular reserves in spermatozoa metabolism and longevity. Samples maintaining good motility longer on storage also had higher initial oxygen consumption.

The relation of percentage of abnormal spermatozoa and initial motility to metabolic rate suggest a relation between the original quality of spermatozoa and their activity or longevity. Semen samples having large numbers of abnormal spermatozoa also had lower oxygen consumption per billion living spermatozoa.

The oxygen uptake of seminal fluid ranged from 4.52 to 67.33 $mm^3$ oxygen per hour per 2.0 cc., representing from 3.3 to

24.0 per cent of the oxygen consumption of the semen from which it was obtained. These figures are comparable to those obtained by Winchester anl McKenzie (1941c) for boar seminal fluid. No relation was observed between the intensity of respiration of spermatozoa and their surrounding fluid, necessitating the measurement of oxygen consumption of seminal fluid in determinations of the true metabolism of spermatozoan cells.

The stimulation of oxygen consumption of spermatozoa in the presence of seminal fluid or their natural medium is not unique with spermatozoa cells alone. Similar results have been secured with nerve tissue by Shaffer, Chang and Gerard (1935), with leucocytes by MacLeod and Rhoads (1939), bone marrow by Warren (1940), and by Canzanelli et al (1939 (1942a) (1942b) with guinea pig kidney, liver and heart tissue. Brain tissue, however, showed a response opposite that of other tissues studied.

The mechanism of this increased oxygen consumption of spermatozoa in the presence of seminal fluid over that in a phosphate buffer medium is as yet unexplained. There is no indication that it is beneficial to spermatozoa, as the motility was not improved nor was the length of survival at room temperature increased. This work emphasizes the importance of the knowledge of the composition of the medium in respiration studies, especially in a comparison of the results of various workers.

Increases of 60 to 120 per cent in oxygen consumption of centrifuged egg suspensions over that of unstratified controls has been reported by Velick (1941). He believed damage of the membrane, also observed with shaking, was responsible. Fleischmann (1939) reported that injury causes a change in cell permeability usually resulting in an increased respiration. Other studies indicate that complex cellular changes are responsible.

Our studies of the effect of centrifugation on the subsequent oxygen consumption of spermatozoa show a decrease in oxygen consumption apparently due to a decrease in the number of living spermatozoa as determined by the differential staining technique described previously.

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