A STUDY OF THE RELATIONSHIP OF THE MORPHOLOGY AND THE PROGRESSIVE MOTILITY OF BOVINE SPERMATOZOA

by

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B. S., Kansas State University, 1959
D.V.M., Kansas State University, 1959

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

Approved by:

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INTRODUCTION

The technique of diluting and storing of bovine semen has been developed to the extent that artificial insemination of cattle is an economical practice. Laboratory techniques that permit rapid evaluation of semen quality have aided in the development of the artificial insemination program. Concentration of spermatozoa and the estimation of the gross motility of the spermatozoa are two techniques that are used to determine the acceptability of bovine semen. Determination of per cent live spermatozoa and morphology of the spermatozoa are included with concentration and motility when bull semen is evaluated for potential future use of a bull, whether for natural service or for artificial insemination programs. However, even with the use of the laboratory techniques to determine the potential value of a bull for semen production, the final factor that must be considered is the ability of the semen to produce conception in the female irregardless of the method of use.

It has been observed that semen from some individual bulls will not survive the semen freezing techniques commonly used by artificial insemination programs. The inability of the spermatozoa to survive freezing is not detectable with the use of the usual laboratory techniques. At present, the freezability of bovine semen is based on the determination of sperm concentration, gross motility, and trial freezing procedures. A survey of the literature failed to reveal work in which the morphology of the spermatozoa had been studied in relation to the degree of motility of the surviving spermatozoa following the semen freezing technique.

This study was undertaken to investigate the relationship of the morphology of the spermatozoa in the raw semen sample and the degree of progressive motility of the spermatozoa which survive the semen freezing technique.

It was postulated that such a relationship, if present, might lead to the use of spermatozoa morphology studies as indicators as to when the semen of an individual bull may be subjected to semen freezing techniques and the spermatozoa be expected to survive.

Such a relationship would enable the laboratory personnel performing semen evaluation another criterion for predicting the value of a given sample of raw semen for use in an artificial insemination unit.

REVIEW OF LITERATURE

Semen Dilution

Artificial insemination aroused only scientific interest when an Italian physiologist, Lazzaro Spallanzani, first utilized the procedure in dogs in 1780. Hafez (1962) suggests that the technique of artificial insemination was not practically used until the Russian scientist, Ivanhoff, used the technique for the breeding and improvement of horses, sheep, and cattle about 1900. Ivanhoff improved the process to the extent that by 1938 artificial insemination was of practical use in domestic animals. Hafez did not indicate what techniques Ivanhoff employed, however, it is assumed the semen was diluted with either normal saline or a phosphate buffer for use within a short period of time since

diluents providing protection and nutrients to the spermatozoa were not developed until a later date.

Maule (1962) credits the widespread development of artificial insemination to the work of Lardy and Phillips, in 1939, which demonstrated the protective action of egg yolk against cold shock. Several workers have studied the composition of egg yolk, presumably with a view to developing synthetic diluents, but the results of isolation of what were considered to be the essential fraction varied considerably. Mayer and Lasley claimed, in 1945, that the fraction which had this protective action was insoluble in acetone, alcohol and ether. Walton in 1947 reported that complete protection could be obtained with the ether soluble fraction. Blackshaw, in 1954, found that the alcohol soluble, acetone insoluble fraction was protective and that it contained lecithin. It is now generally agreed that the protective action of egg yolk is lecithin or a similar phospholipid.

The original yolk diluent described by Lardy and Phillips consisted of equal volumes of egg yolk and a phosphate buffer. A distinct disadvantage of the egg yolk-phosphate diluent was the presence of large fat globules which made it impossible to observe individual spermatozoan motility under the microscope. Salisbury et al. (1941) described a diluent consisting of equal parts of egg yolk and either 3.6 or a 2.9% sodium citrate solution. This diluent provided a clearer field in which spermatozoa motility could be assessed under the microscope.

Modifications of the egg yolk-citrate diluent were studied by Almquist (1951), Holt (1952), and Stewart (1950). All reported

no adverse effect when the yolk concentration was reduced to 20 to 25% egg yolk.

Maule (1962), discussing the ultra-low temperature storage of bovine semen, recognized that the first significant work on this technique was done by Smith and Polge in 1950. These workers reported the best survival rates of sperm when 15% glycerol was added to the 3.9% sodium citrate buffered diluent. Successful conceptions in 79% of 38 cows occurred when the semen was diluted with an equal volume of yolk-citrate buffer, cooled to 5 C. and then further diluted with an equal volume of glycerol-citrate to give a final 10% glycerol concentration by Polge and Rawson (1952).

It is noted in the texts of Maule (1962), Hafez (1962), and Salisbury and Van Denmark (1961) that there is a variation of the ability of spermatozoa to survive freezing in ejaculates between bulls but not within the ejaculates of an individual bull. Willett and Ohms (1958) indicated that this variation may be within the spermatozoal cell itself and not to the surrounding seminal plasma. This observation was based on studies involving centrifugation and separation of the spermatozoa and the seminal fluid, exchanging the fractions of semen and then freezing the spermatozoa.

Morphology of Spermatozoa

The classification of abnormal spermatozoa, as used by Carroll et al. (1963), is that of primary and secondary abnormalities. Primary abnormalities are those deviations from normal that result from abnormal spermatogenisis within the seminiferous tubules of the testicle. These include abnormal heads, midpieces

and abaxial attachment of the midpieces. The secondary abnormalities are assumed to be due to some nonphysiologic condition affecting the spermatozoal cell after passage through the efferent ducts of the testicle. The most common deviations associated with secondary abnormalities include detached heads, coiled tails, and distal protoplasmic bodies.

Maule's (1962) review of the published work on spermatozoa abnormalities quoted work by Williams and Savage in 1925 and by Lagerlof in 1934 indicated that there was decreased fertility of bulls when there was more than 17% spermatozoa abnormalities. Both groups of workers attached most importance to the morphology of the spermatozoal head. Their observations were based on studies on semen samples from 208 bulls by Williams and Savage, and semen samples from 50 bulls by Lagerlof.

Blom, as quoted by Maule, studied 100 normal fertile bulls and found that from 5 to 10% of the spermatozoa possessed a primary abnormality, and that the incidence of secondary abnormalities was variable. It was his opinion that some of the secondary abnormalities were artefacts due to staining techniques.

Roberts (1956) also classified the morphologic abnormalities of the spermatozoa as either primary abnormalities or secondary abnormalities. The primary abnormalities were microcephalic and macrocephalic sperm cells, short broad heads, elongated narrow heads, pyriform heads, double heads, double middle pieces and tails, swelling of the middle piece, kinked or coiled middle pieces and tails, and abaxial attachment of the middle pieces. The secondary abnormalities included free heads, free tails, and

proximal or distal protoplasmic droplets on the middle pieces.

Mann (1954) suggested there could be a high degree of sperm abnormality within a normal semen sample. He described abnormalities as any form of degenerate, abnormal, or immature forms consisting of every conceivable deviation from the normal cell structure.

Motility of Spermatozoa

Currently used methods for the assessment of sperm motility are primarily visual, and the results are usually expressed in comparative rather than absolute terms. No means are readily available for characterizing the distribution of motility of the individual sperm cells in a semen sample. Thus an attempt is made to characterize the motility of spermatozoa in a sample as a whole or as the mean of the population. Such visual estimates are apt to be influenced by the concentration of the cells in a semen sample (Salisbury and VanDemark, 1961).

Lagerlof (1936) observed that bulls whose spermatozoa exhibited poor motility were usually of poor fertility. Motility estimation in this study was based on the number of motile spermatozoa in a given sample of raw semen.

The percentage of spermatozoa exhibiting motility has been the basis for determining the acceptability of semen extenders by many workers including Bishop (1955) and Albright et al. (1960).

Brady and Gildow (1939) described a method of determining the per cent motile sperm in raw semen by utilizing a hemacytometer. They filled both sides of the counting chamber, one with semen

diluted 1:100 with normal saline and the other with semen diluted 1:100 with alcohol. The dead sperm were counted in both sides and the difference in the counts was considered the per cent of motile spermatozoa. This technique has been used by others with slight modifications in the method of killing the spermatozoa.

Mercier (1944), in the course of a study of the relationship between the proportion of morphologically abnormal spermatozoa and other criteria of bull semen quality, evaluated motility of the spermatozoa as the per cent motile spermatozoa in a serum sample. His results indicated that a high motility of the sperm cells was associated with a low proportion of abnormal sperm cells.

Zemjanis (1962) described the examination of fresh, unstained bovine semen for motility as a dual procedure. The first examination was observation of the wave pattern or swirl motion which reflected the combined effect of sperm cell concentration and the viability of the sperm cell. The second procedure for the motility test was to estimate the percentage of motile cells in the semen ejaculate. The estimation was performed by first diluting the fresh semen in warm saline and then examining the diluted semen under a cover slip. He categorizes the motility in the following classes;

(1) Progressive, rectilinear motility in which the sperm cells move rapidly in straight forward direction.

(2) Circling motility where cells move in circles because of midpiece or tail defects.

(3) Reverse circling motility characterized by sperm cells

which circle in a backward direction.

(4) Pendulation motility where in the cells exhibit jerky, serpentine motility without showing marked forward progress.

Zemjanis expressed the opinion that at least 70% of the motile cells should exhibit the progressive type of motility. In an earlier publication, Diseases of Cattle (1956), Zemjanis had expressed the opinion that, in order to classify semen as good, at least 40% of the sperm cells should exhibit progressive motility.

Trimberger, G. W. (1962) rated the motility of sperm as gross motility in terms of percentage at intervals of 10 ranging from 0-100. He recognized the three main types of sperm motility as progressive motion, rotary or circular motion, and ocillatory motion without change of position. He recorded the rates of motility on the following basis:

0 - No progressive motility

1 - Sluggish motility

2 - Motility somewhat sluggish

3 - Intermediate motility

4 - Maximum progressive motility

This classification was used on diluted semen and after semen storage to indicate the semen quality.

MATERIALS AND METHODS

Experimental Animals

Seventeen bulls were used in this study of the morphology and progressive motility of spermatozoa in extended and frozen

semen. Ten of the 17 bulls were of the Holstein breed. The remaining 8 bulls consisted of one Guernsey, two Angus, two Hereford, one Shorthorn, and one Charolais. The ages of the bulls ranged from one year to 14 years of age.

Code No.	Breed	Age*
H-50	Holstein	1
H-49	Holstein	2
H-48	Holstein	2
809	Hereford	2
M-17	Shorthorn	3
AA-2	Angus	4
904	Charolais	6
H-41	Holstein	6
G-30	Guernsey	6
H-35	Holstein	8
H-47	Holstein	9
H-38	Holstein	9
H-32	Holstein	9
807	Hereford	9
H-34	Holstein	10
H-39	Holstein	11
701	Angus	14

*Age is given to the nearest year.

Collection of Samples

The bulls were selected and the semen specimens collected at random. The samples were obtained at the time of routing collections by K.A.B.S.U.[1] personnel. The demand for the semen of any individual bull in commercial artificial breeding programs supplied by K.A.B.S.U. determined the frequency of collection in bulls.

Samples were taken at three stages in the process of extending, freezing, and storing bull semen. Sample A was taken as soon as possible after the fresh semen sample entered the laboratory. This sample was used for the morphological study of the spermatozoa. Sample B was taken at the stage when the fresh semen was extended to the calculated concentration of approximately 30 million spermatozoa per milliliter of extended semen. This sample was used to ascertain the degree of progressive motility of the spermatozoa prior to freezing the semen. Sample C was obtained one week later and consisted of a portion of a vial of frozen semen after it had been in storage for six days. It was used to determine the degree of progressive motility of the spermatozoa following storage.

Morphology examinations were made on 49 separate specimens of Sample A. Progressive motility was determined on 38 different specimens of Sample B and on 26 different specimens of Sample C.

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[1]Kansas Artificial Breeding Service Unit, Department of Dairy Science, Kansas State University.

Procedure for Determining Morphology

One drop of fresh undiluted semen was obtained in the laboratory following the measurement of the quantity of semen. The drop of semen was placed on a clean microscope slide, previously warmed to an estimated 100 F. One drop of live-dead stain[1] was placed adjacent to the drop of semen and the two drops were gently mixed. Mixing was accomplished by using a second slide as a stirring rod for a period of not less than fifteen seconds nor more than thirty seconds. The mixture of stain and semen was then spread, as a thin film, on the warmed slide and allowed to air dry. If the slide appeared to be excessively stained on the first attempt, additional slides were immediately prepared with lesser quantities of the stain-semen mixture. This was to insure that there would be a slide so stained that individual spermatozoa could be observed without superimposition of another spermatozoa.

The stained spermatozoa were studied with the microscope using the high power lens (430 times magnification). A minimum of 200 spermatozoa were observed and the sperm abnormalities were classified and recorded as being either primary or secondary abnormalities. Those classed as primary abnormalities were spermatozoa with malformations of the head and the midpiece. Those classed as secondary abnormalities included abnormalities of the

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[1]The live-dead stain used was of the formula
4.8 Gm. sodium citrate dihydrate
0.8 Gm. eosin bluish
0.5 Gm. Nigrosin
100.0 ml. Distilled water
This mixture was heated to 180 F., cooled and filtered 3 times. The live-dead stain used was obtained from Colorado State University.

tail, proximal or distal protoplasmic bodies, and free or detached heads. Only the spermatozoal cell that included a head was used in the counts. Spermatozoa having both a primary and secondary abnormality were classed as primary abnormalities and not as secondary abnormalities.

Procedure for Determining Progressive Motility

Sample B for progressive motility was taken at the stage when the semen had been extended with egg yolk-citrate extender. One milliliter of the extended semen was withdrawn with a sterile 1 milliliter glass pipette and transferred to a 5 milliliter plastic semen vial that had been prewarmed in a water bath to 80 F. To this sample, one additional milliliter of egg yolk-citrate diluent was added so that the concentration of the spermatozoa would be the same as the concentration of the frozen semen.

A "bright-line" hemacytometer was utilized in the process of observing and studying the progressive motility. The hemacytometer was used in preference to the usual microscope slide and cover slips because of the constant depth of the counting chamber and the total thickness of the hemacytometer. The total thickness of the hemacytometer aided in maintaining a constant temperature, thus preventing a slow down in motility of the spermatozoa due to the decrease in environmental temperature.

The hemacytometer and cover slip were preheated to 105 F. prior to positioning on the stage of the microscope. The time interval required to position the hemacytometer on the microscope stage and to focus the microscope allowed the hemacytometer to

cool to an estimated 100 F. The microscope was prefocused in order to allow more time for determining sperm motility.

A capillary hematocrit tube was filled by capillary action from the specially extended semen sample. The filled capillary tube was then touched to the margin of the hemacytometer and the cover slip and the counting chamber was allowed to fill by capillary action.

Only the spermatozoa that exhibited motility were classified as to the degree of progressive motility.

The progressive motility of the spermatozoa was observed in the center square of the calibrated area on the platform of the hemacytometer. This was indexed as Class 1 progressive motility or Class 2 progressive motility. The criteria for Class 1 progressive motility was the progression of the spermatozoa in a forward path across the .05 millimeter calibrated square. Class 2 progressively motile spermatozoa included those that traveled in a circular or rotary fashion, those that showed an oscillatory motion with very little progression, and those with reverse or backward motion. One hundred motile spermatozoa were observed and classified. The 100 spermatozoa were counted in groups of 25 in order to assure representation of the total field of the hemacytometer and to have a check system on any possible decrease in the sperm motility due to cooling of the counting chamber. The results consisted of an average of 25 of the four group counts and were recorded as either Class 1 or Class 2 progressive motility.

Samples C, for the study of progressive motility, were obtained from a vial of frozen semen which had been in storage for

6 days following the completion of the semen freezing process. The vial was removed from liquid nitrogen storage and placed in ice water until the semen became liquified. Upon completion of thawing, the vial was rotated slowly to insure an even dispersion of the spermatozoa within the vial. The vial was opened and a capillary tube was used to transfer Sample C to the hemacytometer.

Preparation of the hemacytometer and determining the classification of the progressive motility of the spermatozoa was performed according to the procedures and standards described for Sample B. The essential differences between Samples B and C were that Sample C contained glycerol and had been frozen and stored for six days at ultra-low temperature (-196 C.). Sample C was placed in the heated counting chamber for approximately 20 seconds before the evaluation was undertaken. The 20 second interval was sufficient to warm the semen sample to assure good spermatozoa motility.

RESULTS

The semen of 17 bulls was examined in the course of this investigation. The number of separate semen samples per bull varied from a low of 1 to a high of 13, with at least a 6 day interval between any 2 semen studies from a given bull. The number of examinations performed with the semen of each bull is shown in Table 1.

As indicated in Table 1, there were 49 examinations for morphology of the spermatozoa, 38 progressive motility examinations of Sample B, and 26 progressive motility examinations of

TABLE 1 - Number of examinations performed.

	Code #	Morph.[1]	Progressive Motility Prefreezing[2]	Progressive Motility Post freezing[3]
	H-50	1	1	1
	H-49	1	1	0
	H-48	2	2	1
	809	2	2	1
	M-17	6	1	0
	AA-2	3	3	1
	904	1	1	1
	H-41	1	0	0
	G-30	1	1	1
	H-35	2	1	1
	H-47	13	13	10
	H-38	5	5	4
	H-32	1	1	1
	807	1	0	0
	H-34	5	3	1
	H-39	1	1	0
	701	3	3	3
Total	17	49	38	26

[1]Morphology examination - Sample A

[2]Sample B

[3]Sample C

Sample C. Of the 49 original semen samples, 11 samples were discarded prior to dilution with egg yolk-citrate extender, thus preventing examinations of Samples B and C for progressive motility. The semen was discarded because of insufficient quantity, concentration, or unfavorable gross semen quality as determined by K.A.B.S.U. personnel.

Pre-freezing progressive motility of Sample B was determined on the 38 samples which were diluted in preparation for processing frozen semen. Of the 38 samples that were frozen, post-freezing progressive motility, Sample C, was determined on 26 frozen semen samples following storage at the ultra-low temperature for 6 days.

Morphology of Spermatozoa

Sample A, obtained soon after the raw semen entered the K.A.B.S.U. laboratory, was prepared and examined as rapidly as the technique would allow. Morphologic abnormalities of the spermatozoa was determined and recorded as the per cent of primary abnormalities of the head and the midpiece, and secondary abnormalities including the free or detached head, coiled tails, and the presence of a protoplasmic body on the midpiece of the sperm cell.

The recorded observations of 39 spermatozoa morphology examinations are shown in Tables 2 through 5. The semen samples in these tables were grouped according to the bull's age.

Morphological examinations made on the spermatozoa obtained in Sample A, Tables 2 through 5, revealed the presence of abnormalities in all the semen samples. The incidence varied between

TABLE 2

H-50, H-48, H-49, 809, M-17, - 1-3 years of age

Bull	Age in Years	Date	Primary Abnormalities			Secondary Abnormalities			
			Head	Mid-piece	Total	Tail	Prot. Bodies	Free Head	Total
H-50	1	12-17-63	1	3	4	2	6	1	9
H-49	2	11-5-63	1	3	4	1	0	2	3
H-48	2	9-4-63	1	3	4	3	6	1	10
		12-17-63	2	1	3	4	0	2	6
809	2	12-17-63	6	8	14	2	15	0	17
		12-31-63	3	4	7	1	14	2	17
M-17	3	11-26-63	2	4	6	1	1	2	4

TABLE 3

AA-2, 904, H-41, G-30, H-35 - 4-8 years of age

			Primary Abnormalities			Secondary Abnormalities			
Bull	Age in Years	Date	Head	Mid-piece	Total	Tail	Prot. Bodies	Free Head	Total
AA-2	4	9-4-63	1	5	6	4	4	0	8
		9-11-63	10	3	13	4	3	8	15
		9-18-63	5	4	9	3	0	2	5
904	5	10-15-63	4	14	18	4	0	5	9
H-41	6	12-23-63	2	2	4	1	2	2	5
G-30	6	10-29-63	1	2	3	1	3	4	8
H-35	8	9-24-63	1	12	13	1	0	6	7

TABLE 4

H-47, H-38, H-32, 807 - 9 years of age

			Primary Abnormalities			Secondary Abnormalities			
Bull	Age in Years	Date	Head	Mid-piece	Total	Tail	Prot. Bodies	Free Head	Total
H-47	9	9-4-63	0	3	3	2	31	0	33
		9-11-63	2	10	12	4	0	2	6
		9-18-63	3	5	8	4	0	3	7
		9-24-63	1	9	10	2	10	1	13
		10-2-63	4	6	10	3	2	0	5
		10-15-63	7	12	19	4	28	0	32
		10-22-63	0	6	6	3	2	4	9
		11-5-63	1	4	5	3	4	7	14
		11-26-63	1	3	4	1	1	2	4
		12-3-63	3	5	8	0	2	5	7
		12-10-63	4	0	4	0	3	2	5
		12-17-63	4	2	6	2	1	1	4
		12-23-63	2	2	4	2	0	6	8
H-38	9	9-11-63	2	10	12	2	4	6	12
		9-18-63	1	4	5	1	3	5	9

		10-2-63	2	11	13	0	6	6	12
		10-15-63	1	4	5	1	2	0	3
H-32	9	10-15-63	2	3	5	0	9	1	10
807	9	12-23-63	4	4	8	2	1	6	9

TABLE 5

H-34, H-39, 701 - 10-14 years of age

			Primary Abnormalities			Secondary Abnormalities			
Bull	Age in Years	Date	Head	Mid-piece	Total	Tail	Prot. Bodies	Free Head	Total
H-34	10	10-29-63	3	7	10	6	4	1	11
		11-5-63	3	5	8	0	4	3	7
H-39	11	11-5-63	2	8	10	3	5	0	8
701	14	10-22-63	2	6	8	8	2	0	10
		10-29-63	2	3	5	0	3	5	8
		12-3-63	2	8	10	3	5	0	8

samples from an individual bull as well as between samples of different bulls.

The total number of spermatozoal morphologic abnormalities in the 39 samples concerned with both primary and secondary abnormalities is 683 out of 3900 spermatozoa counted and recorded. Of the 683 abnormal spermatozoa, 306 showed primary morphologic abnormalities and 377 showed secondary morphologic abnormalities.

The 39 samples had an average percentage of 7.85% primary abnormalities, 9.66% secondary and 17.5% of the combined abnormalities.

Primary Abnormalities of Spermatozoa

Primary abnormalities include those spermatozoal morphologic abnormalities that occur during the process of spermatogenesis within the seminiferous tubules of the testicles (Carroll, 1963). Such abnormalities are observed in conjunction with the head and midpiece of the spermatozoal cell (Carroll, 1963). The primary abnormalities of the spermatozoa observed in this study were giant heads, small heads, pyriform heads, and deviations of the contour of the head. Midpiece abnormalities observed in this study were abaxial attachment of the midpiece to the head, double midpiece, coiled midpiece which in nearly all instances involved the distal one-fourth of the midpiece, and granular or beaded midpiece.

The primary abnormalities in this study were classed as abnormalities of the head of the spermatozoal cell and as abnormalities of the midpiece. The average incidence of

abnormalities of the head was 2.51%. Abnormalities of the midpiece occurred with the average incidence of 5.33%.

The occurrence of primary abnormalities at the rate of 7.84% is within normal limits associated with semen produced by fertile bulls (Lagerlof, 1936). However, it was noted that there was a variation in the percentage of primary abnormalities in separate semen collections from a given individual. There was no indication of faulty spermatogenesis as a result of the morphology studies within the scope of Tables 2 and 5.

Primary abnormalities are associated with faulty spermatogenesis. It would require further investigation on this subject in order to correlate sperm morphology and degenerative changes within the seminiferous tubules.

Secondary Abnormalities of Spermatozoa

Secondary morphologic abnormalities are considered to be those abnormalities of the spermatozoa that occur after the spermatozoal cell has left the seminiferous tubules. Secondary abnormalities include such abnormalities as loose or detached head of a spermatozoa, coiled tails, and presence of protoplasmic bodies along the midpiece. Both the free heads and the coiled tails may be caused by faulty techniques of semen handling, such as severe mechanical irritation, cold shock due to rapid temperature change, or the inclusion of some abnormal material such as contamination with as little as a drop of water. The protoplasmic bodies occur as a result of inadequate aging of the spermatozoal cell within the tubular genitalia of the bull

(Roberts, 1956).

The greatest variation in secondary abnormalities in this study occurred among the protoplasmic bodies in the samples from H-47. The presence of the protoplasmic bodies did not appear to affect the progressive motility of the semen samples.

The over-all average of secondary abnormalities in the 39 samples that were recorded was 9.66%. The average number of protoplasmic bodies was 4.77%. Free heads occurred at the average rate of 2.64%. Tail abnormalities had the average rate of 2.23%. This incidence of secondary abnormalities was considered to be low as compared to accepted methods of semen evaluation in which a tolerance of up to 20% secondary abnormalities is allowed in the morphologic grade of very good.

It is theorized that the low incidence of secondary abnormalities noted in this study were due to the excellent technique employed by the personnel at K.A.B.S.U. in the collection and handling of semen. Another factor which may have contributed to the low incidence of secondary abnormalities was the short interval between the collection of the semen and the staining of the A sample. The time lapse approximated two minutes in most instances. Proof of these factors would necessitate further study involving the relationship of staining technique and the secondary morphologic abnormalities.

As was indicated in Materials and Methods, spermatozoa having both a primary and secondary abnormality were classed according to the primary abnormality. Morphologic examinations of 10 samples of semen from 4 bulls revealed that the morphologic

grade for each sample would have been either fair or poor. The standards for grading the morphology was that standard used at the Dykstra Veterinary Clinic, Kansas State University, where in semen with 20% to 30% primary abnormalities is graded as fair, and semen with more than 30% is graded as poor. The location of the primary abnormalities as observed in Sample A from bulls M-17, H-38, H-34, and H-35 are listed in Table 6. These represent the 10 samples in which more than 20% primary abnormalities were observed.

TABLE 6

M-17, H-35, H-38, H-34

			Primary Abnormalities		
Bull	Age in Years	Date	Head	Midpiece	Total
M-17	3	9-18-63	26	30	56
		9-24-63	20	37	57
		10-2-63	5	30	35
		10-15-63	10	16	36
		10-29-63	12	10	22
H-35	8	10-2-63	7	23	30
H-38	9	9-24-63	1	21	22
H-34	10	10-2-63	2	26	28
		10-15-63	4	26	30
		10-22-63	20	4	24

The average primary abnormality for the samples in Table 6 was 10.7% for the head and 22.3% for the midpiece, with a combined total of 33%. Secondary abnormalities were not recorded on the semen samples with a incidence of more than 20% primary abnormalities because of the occurrence of both classifications present on the same sperm cell.

Variation of the per cent primary abnormalities occurred between samples from M-17, H-38, H-34, and H-35, as was observed in all bulls, and the morphologic data for the above mentioned bulls was shown in Tables 2 to 5 with the date indicating at which time the morphologic quality was improved to not more than 20% primary abnormalities. Secondary abnormalities were then counted and recorded.

Relationship of the Bull's Age to Morphology of Spermatozoa

The rate of incidence of spermatozoal morphologic abnormalities did not indicate an apparent variation between the different ages of the bulls used. Carroll (1963) observed that the morphologic character of the sperm cells had a greater correlation to the final semen quality classification than did the concentration, motility, and per cent live spermatozoa. In his study of age incidence and semen quality, he found that yearling bulls and those 10 years and over had the highest incidence of unsatisfactory semen quality.

To accurately define the effect that age would have on spermatozoa morphology, further work would be required.

Progressive Motility of Spermatozoa

The degree of progressive motility of the motile spermatozoa was determined from samples of diluted semen both before and after freezing and was classified as Class 1 or Class 2 progressive motility. Sample B was obtained from the diluted semen before the addition of glycerol, equilibration, freezing, and storage of the semen. Sample C was obtained from a vial of frozen semen after it had been stored for six days. The relative number of Class 1 and Class 2 progressive motility in Samples B and C of paired samples are indicated in Table 7.

The average ratio of Class 1 to Class 2 progressive motility in Sample B was 20.4 to 4.6 whereas in Sample C it was 16.5 to 8.5 respectively. The decrease in the Class 1 progression of the motile sperm revealed an average drop of 3.9 progressively motile sperm. A corresponding increase in the Class 2 progressive motility between Samples B and C was present. Converting the decrease of Class 1 progressive motility reveals a relative decrease of 15.6% between Sample B and C.

Explanation of the decrease of Class 1 progressive motility between the paired samples would be speculative at this time. Some of the factors that may have had an effect are the aging of the spermatozoa, the addition of glycerol to the diluent, and/or the ultra-low temperatures necessary for the freezing and storage of frozen semen.

TABLE 7 - Degree of progressive motility in paired samples

	Sample B		Sample C	
	Class 1	Class 2	Class 1	Class 2
H-50	22	3	21	4
H-48	23	2	17	8
809	14	11	3	22
AA-2	21	4	12	13
904	20	5	17	8
G-30	22	3	20	5
H-35	21	3	17	8
H-47*	20.8*	4.2*	17.6*	7.4*
H-38**	20.25**	4.75**	17.5**	7.5**
H-32	19	6	14	11
H-34	17	8	15	10
701***	20.6	4.4***	16.0***	9.0***
Average[1]	20.4	4.6	16.5	8.5

*Average of 10 separate samples.
**Average of 4 separate samples.
***Average of 3 separate samples.

[1]Recorded at the nearest tenth.

The Relationship of Sperm Morphology to Progressive Motility

The relationship of spermatozoal primary morphologic abnormalities of Sample A and the respective class of motility in Samples B and C is shown in Tables 8 and 9.

Table 8 contains results of the examination of samples in which primary abnormalities did not exceed 10% whereas the results

TABLE 8 - Relationship of the spermatozoal primary morphologic abnormalities of Sample A with 10% or less primary abnormalities to the progressive motility of Samples B and C

Code #	Date	% Prim. Ab.[1]	Progressive Motility Sample B Class[1]	Class 2[2]	Sample C Class 1[3]	Class 2[3]
H-47	9-18-63	8	19	6	17	8
	9-24-63	10	19	6	14	11
	10-2-63	10	19	6	15	10
	10-22-63	9	21	4	17	8
	11-5-63	3	22	3	18	7
	11-26-63	4	23	2	19	6
	12-3-63	8	22	3	20	5
	12-10-63	5	22	3	19	6
	12-17-63	6	22	3	19	6
AA-2	9-18-63	9	21	4	12	13
H-48	12-17-63	3	23	2	17	8
H-32	10-15-63	5	19	6	14	11
701	10-22-63	6	21	4	13	12
	10-29-63	5	21	4	17	8
	12-3-63	10	20	5	18	7
G-30	10-29-63	3	22	3	20	5
H-50	12-17-63	6	22	3	21	4
H-38	9-18-63	5	22	3	21	4
	10-15-63	5	22	3	21	4
H-34	10-22-63	4	19	6	15	10
Average[4]		6.2	21.1	3.9	17.4	7.6

[1]Primary abnormalities as recorded from Sample A.
[2]Progressive motility from Sample B.
[3]Progressive motility from Sample C.
[4]To the nearest tenth.

of the examination of samples in which more than 10% primary abnormalities are noted in Table 9.

The basis for separation of the relationship by the per cent of primary abnormalities present was the classification of spermatozoal morphology by Carroll et al. (1963) in which they classified the morphology as very good (V.G.) when 1-10% primary abnormalities occurred in the sample, good (G.) when 11-20% primary abnormalities occurred in the sample and fair (F.) when 21-30% primary abnormalities were noted. These semen samples with more than 30% primary abnormalities were considered poor (P.).

The averages given in Table 8 indicate that 6.2% of the spermatozoa had a primary morphologic abnormality. In these samples an average of 21.1 of 25 motile spermatozoa had a Class 1 progressive motility following the initial extension of semen in egg yolk-citrate diluent. Converting this ratio to percentage, it may be stated that an average of 84.4% of the motile spermatozoa in Sample B had a Class 1 progressive motility. Following glycerolation, freezing, and storage in liquid nitrogen for six days the ratio of motile spermatozoa that exhibited Class 1 progressive motility decreased to an average of 17.4 per 25 cells or 69.4%.

The average occurrence of primary abnormalities in the semen samples represented in Table 9 was 16.5%. The ratio of Class 1 progressive motility was 18.5 per 25 motile spermatozoa in Sample B, the diluted semen prior to the addition of the

TABLE 9 - Relationship of the spermatozoal primary morphologic abnormalities of Sample A with more than 10% primary abnormalities to the progressive motility of Samples B and C.

Code #	Date	% Prim. Ab.[1]	Sample B[2] Class 1	Sample B[2] Class 2	Sample C[3] Class 1	Sample C[3] Class 2
809	12-17-63	14	14	11	3	22
904	10-15-63	18	20	5	17	8
H-35	9-24-63	13	21	4	17	8
H-47	10-15-63	19	19	6	18	7
H-38	9-24-63	22	17	8	11	14
H-38	10-2-63	13	20	5	17	8
Average[4]		16.5	18.5	6.5	13.8	11.2

[1]Per cent of spermatozoa with primary abnormalities.
[2]Class of progressive motility of the extended semen sample.
[3]Class of progressive motility of the semen sample after freezing and storage for six days in liquid nitrogen.
[4]To the nearest tenth.

glycerol and the freezing and storage of the semen. After the semen had been processed and stored for six days, the examination of Sample C revealed that an average of 13.8 per 25 motile spermatozoa exhibited Class 1 progressive motility. This represents a decrease from 73% of the motile spermatozoa with Class 1 progressive motility in Sample B to 55.2% progressive motility in Sample C following processing and storage.

The results, as indicated in Tables 8 and 9, show a decrease from 69.6% to 55.2% motile spermatozoa showing Class 1 progressive motility in the final frozen semen product when the rate of primary abnormalities increased approximately 10%, from 6.2% to 16.5%.

The presence of spermatozoal primary morphologic abnormalities within a semen sample is an indication of faulty spermatogensis that occurred during the development of the spermatozoa in the seminiferous tubules of the testicle. It is logical to assume that faulty spermatogensis, which results in morphologic abnormalities of the spermatozoa, may influence some spermatozoa that are normal insofar as can be detected by the usual microscopic methods to be abnormal in metabolic functions of the spermatozoa cell. This study indicates a trend in this direction as there is a reasonable inverse relationship between the increasing per cent of morphologic abnormalities in raw semen and the decreasing per cent of motile spermatozoa that exhibit Class 1 progressive motility from a vial of frozen semen. If this indication is substantiated by future studies, morphology examinations could be utilized to indicate the approximate per cent of motile spermatozoa that will retain a Class 1 progressive motility of the sperm cells that survive the techniques used in the production of frozen semen.

Secondary morphologic abnormalities were not correlated to the classification of progressive motility in this study due to the relatively low incidence of these abnormalities in semen used in the course of this work.

SUMMARY

Semen samples from 17 bulls, owned by K.A.B.S.U., were studied for the relationship of spermatozoal primary abnormalities and the degree of progressive motility of the motile sperm

in extended and frozen bovine semen.

Twenty-six samples were utilized for the studies in which primary abnormalities were recorded and in which motility was evaluated on both the pre-freezing and post-freezing samples. The 26 were divided into groups, according to the incidence of spermatozoal primary morphologic abnormalities. On group included those samples with less than 10% primary abnormalities and the other group included those samples with more than 10% primary abnormalities.

Progressive motility of the motile spermatozoa was classified as Class 1 and Class 2 progression. The 26 samples in which paired progressive motility was studied the average Class 1 progressive motility was 81.6% of the motile spermatozoa present in diluted, unfrozen semen sample as compared to 66% in the determination of the frozen semen sample.

In the group of 20 samples in which the occurrence of primary abnormalities was 10% or less, 84.4% of the motile spermatozoa had Class 1 progressive motility in the prefreezing sample and an average of 69.6% in the post freezing sample.

In the six samples with more than 10% primary abnormalities, the per cent of Class 1 progressively motile spermatozoa for the prefreezing and post freezing classification was 73% and 55.2% respectively.

A relationship of spermatozoal primary abnormalities to the ability of a semen sample to withstand the freezing process was not demonstrated when the average number of primary abnormalities in fresh diluted semen was compared with the average number

of Class 1 progressively motile spermatozoa present in sixth day post freezing sample. Samples with less than 10% primary abnormalities had an average Class 1 progressive motility of 69.6%, whereas 55.2% of the motile spermatozoa had Class 1 progressive motility in the samples with more than 10% primary abnormalities. It would appear that there was a close inverse relationship between the increasing per cent of primary morphologic abnormalities of the spermatozoa and the per cent of the surviving motile spermatozoa exhibiting Class 1 progressive motility.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. J. L. Noordsy for his guidance and advice, to Mr. C. Ancel Armstrong, Mr. Paul Rempel, Mr. Jesse Estes and other personnel of the Kansas Artificial Breeding Service Unit for their many considerations in the course of this work, and to Dr. J. E. Mosier of the Department of Surgery and Medicine for many hours of consultation and advice throughout the course of study and preparation of this thesis.

Appreciation also is extended to the Kansas Artificial Breeding Service Unit, Department of Dairy Science, Kansas State University for making available the semen from bulls present within their unit.

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A STUDY OF THE RELATIONSHIP OF THE MORPHOLOGY AND THE PROGRESSIVE MOTILITY OF BOVINE SPERMATOZOA

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

Commercial artificial insemination of cattle became an economic reality when semen diluents containing egg yolk proved to have a protective action on bovine spermatozoa.

Techniques for the freezing and storing of bovine semen were developed following the discovery by Smith and Polge that glycerol, added to the semen diluent, would protect the sperm cell at ultra-low temperatures necessary for the preservation of bovine semen.

Semen evaluation of bulls has developed in recent years to the extent that many cattle breeders are now using this service as an aid in selecting potentially satisfactory sires for increased efficiency of reproductive pgorams. This evaluation is generally based on concentration, gross motility, morphology, and the per cent live spermatozoa in a sample of raw semen.

A literature survey did not reveal reports of attempts to correlate the morphology of spermatozoa in raw semen with the degree of progressive motility of the spermatozoa in extended and frozen bovine semen.

This study was performed to determine if a relationship did exist and if present would the morphology examination be of any value in predicting the potential use of semen from an individual bull in an artifical insemination program that required the freezing and storing of semen.

A technique to classify the degree of progressive motility in diluted semen is described which entails the use of a hemacytometer in observing and classifying the motile spermatozoa according to the degree of progression within a given sample.

Semen from 17 bulls representing six breeds of cattle was used in this study. Forty nine sperm morphology examinations were made on raw unprocessed semen. Classification of the motile spermatozoa as to the degree of progressive motility was determined on 38 samples of diluted semen prior to freezing. The same classification was repeated on 26 samples of frozen semen that had been in storage in liquid nitrogen for a period of six days.

Results of the examinations at the three stages of semen production were correlated in 26 samples for the relationship of spermatozoa morphology and the ability of the spermatozoa to survive ultra-low temperatures as determined by classification the degree of progressive motility of the spermatozoa prior to and after freezing.

Twenty of the samples were grouped as having 10% or less spermatozoal primary abnormalities. The average incidence of primary abnormalities in this group was 6.2%. Class 1 progressive motility was exhibited by 21.1 of 25 motile spermatozoa or in 84.4% of the motile spermatozoa in the diluted semen sample prior to freezing. Class 1 progression of the motile spermatozoa decreased to an average of 17.4 per 25 cells that survived freezing in the sample of frozen semen that had been in storage for six days. This represents a decrease to 69.6% Class 1 progressively motile spermatozoa.

Six samples were grouped as having more than 10% primary abnormalities of the spermatozoa. In this group primary abnormalities accured at the average rate of 16.5%. Class 1 progression was

exhibited by 74% of the motile spermatozoa in the diluted, unfrozen sample of semen and at the rate of 55.2% in the sample taken from the frozen semen.

Although it is known that there are many factors involved which influence the ability of spermatozoa to survive the semen freezing procedures, the results of this study would indicate that the sperm morphology and the degree of progression of the motile spermatozoa that survive freezing have an inverse relationship. Results of this investigation indicate that the use of morphology studies of raw semen may be of value in predicting the per cent of Class 1 progressive motility of the motile spermatozoa that would survive the freezing and storage processes currently used in artificial breeding units.