A COMPARISON OF CENTRIFUGED AND NONCENTRIFUGED STALLION SEMEN

A Thesis

by

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Submitted to the Graduate College of
Texas A&M University in
partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

August 1969

Major Subject: Physiology of Reproduction

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ABSTRACT

A Comparison of Centrifuged and Noncentrifuged Stallion Semen. (August 1969)

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Four ejaculates were taken from each of four stallions during the Spring of 1969. Two samples were taken from each ejaculate. One sample of semen was extended 1:1 with buttermilk-glucose (BMG). The other semen sample was first centrifuged and the seminal plasma decanted before BMG extender was used to bring it to twice its original volume. Both centrifuged and noncentrifuged samples of extended semen were stored at 4° C. and evaluated daily for percent motility and rate of forward movement.

From the four stallions tested there was an indication of a difference among stallions in the ability of their spermatozoa to withstand storage without the seminal plasma.

The seminal plasma from each of the sixteen collections was also evaluated, using pH, freezing point depression and millivolts conductivity as criteria for the evaluation. It was determined that only osmotic pressure varied significantly between stallions and this variation was due to a single stallion that had the lowest freezing point depression.

ACKNOWLEDGEMENTS

To Dr. A.M. Sorensen, my committee chairman, I would like to extend my deepest gratitude. Had it not been for his guidance both academically and personally the writing of this thesis might not have been possible. I wish to thank also the members of my committee, Professor J.K. Riggs and Dr. H.R. Crookshank, for their consideration both as being one of their students and for helping to guide the writing of this thesis.

I am also grateful to Dr. L.H. Breuer for his willingness to help program the data used in this thesis.

I would like to thank Mr. and Mrs. J.P. McCall, Sr., my parents, for the sacrifices they have made for my education.

Last, I would like to dedicate this to Karen McCall, my wife.

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CHAPTER I

INTRODUCTION

The purpose of this study was to determine if the seminal plasma produced by the stallion is detrimental to the life of the spermatozoa, and if so, is it possible to remove this plasma and replace it with a more suitable medium? In this way superior males of the species may be utilized.

In order to determine the effect of the seminal plasma on storage time of extended semen, percent survival of centrifuged and noncentrifuged sperm was checked after storage at 4° C.

To establish what properties of the seminal plasma might be detrimental or advantageous to the semen, pH, freezing point depression and milli-volts conductivity were determined for each sample. A pH reading was made to establish changes in acidity. Freezing point depressions were run as a means of establishing osmotic pressure, and milli-volts conductivity were determined as an estimate of ionization in the seminal plasma. This was done in such a manner that differences between stallions and ejaculates could be determined.

"The citations on the following pages follow the style of the Journal of Animal Science."

CHAPTER II

REVIEW OF LITERATURE

It was reported by Gazder (1956) that solutions containing various levels of buttermilk ranging from 3 to 21 percent were used as extenders for stallion semen. Later he tried the addition of glucose and obtained better results. The most effective level was 5 gm. powdered buttermilk to 5 gm. glucose in 100 ml. of distilled water.

Buttermilk-glucose extender was significantly better than mare's milk, cow's milk or egg-yolk-glucose. Survival was best when the ratio of stallion semen to extender was 1:4. The addition of 400 I.U. of penicillin and 1 mg. of streptomycin per milliliter of diluted semen effectively controlled bacterial growth without adversely affecting the viability of the sperm.

Roberts (1956) stated that stallion spermatozoa varied greatly in vigor and resistance when extended. Generally the greater the concentration, the better the sperm withstood extension. Extender added in a 1:1 ratio was well tolerated for insemination in volumes up to 100 milliliters.

Kotjagina et al. (1965) reported that in Rombe's trials horse semen was centrifuged for one minute at 1,000 rpm, then at 1,500 rpm for nine to ten minutes. After centrifugation, 70 to 80 percent of the supernatant fluid was poured off and the semen brought up to the original volume with an extender of

lactose-glycerol-yolk and antibiotics. Semen extended in this manner gave a conception rate of eighty percent in mares. Kotjagina et al. (1966) reported that motility and survival rate were increased by ratio of 1:4 of sperm to extender over the method used by Kotjagina et al. in 1965.

Polge and Minotakis (1964) stated that in experiments designed to reduce the ampule space required to freeze and store a whole ejaculate of semen, the sperm were concentrated by centrifugation at 1,000 to 1,200 rpm for twenty to thirty minutes in a refrigerated centrifuge at 4° C. Seventy-five percent of the supernatant fluid was then removed and the packed sperm resuspended in the remaining 25 percent before ampuling and freezing. In all experiments the concentrated semen samples from stallions and jacks showed approximately the same recovery and survival rates as the uncentrifuged frozen semen, especially when they were re-extended with freshly prepared extender. This was further substantiated by Nagase (1966) who reported using centrifuged stallion semen in his freeze pelleting method of storage.

Hendrikse (1966) found the average pH of stallion semen to be 7.4 with high pH being associated with large ejaculates. He further found a significant negative correlation between the volume of ejaculate and the concentration. Evidence was also found that nonmotile spermatozoa were not necessarily dead.

Bogart and Mayer (1950) concluded that even at low temperatures, metabolic by-products accumulated at a relatively rapid rate. Their work showed that egg yolk provided a factor which enabled the spermatozoa to withstand some of the adverse conditions encountered during storage, especially cold shock. Presence of egg yolk not only protected spermatozoa from drastic temperature changes, but also protected them from many types of adverse environmental conditions such as changes in pH and osmotic pressure.

They also reported an association between the degree of ionization of the salts in an extender and the harmful effects of these salts on the spermatozoa. Favorable results were obtained with isotonic glucose and sucrose solutions and could be attributed to the low degree of ionization of these substances in solutions rather than the role they played in spermatozoan metabolism.

They recommended that egg yolk combined with an isotonic solution of either sucrose or glucose be used in the extension of stallion semen. Optimum survival of spermatozoa was obtained in a buffer composed of equal parts egg yolk and 5 percent glucose solution at an extension ratio of 1:4.

CHAPTER III

MATERIALS AND METHODS

Four ejaculates were taken from each of four stallions during a period ranging from February 3, 1969 through June 3, 1969. Stallion A was a fifteen year old Thoroughbred, Jedgar Ruler who was in heavy use, being mated approximately twice daily except for those days when collections were taken for this test. Stallion B, Leo Star Adams, was an eight year old Quarter Horse under light use, being mated only twice a week. Stallion C, Stick, was a twelve year old Quarter Horse not in use for breeding, and the same was true for Stallion D, Merry Legs, Jr., a twelve year old Quarter Horse. These stallions were collected by means of an artificial vagina with temperatures of the A.V. ranging from 45° C. to 49° C. The semen was then evaluated. Volume was measured in the collection bottle, a 250 cc. plastic baby bottle, and included the preseminal fluids, sperm fraction and plug. However, in most instances there was no measurable amount of plug, and when a measurable amount was present it never exceeded 10 cc. Next the semen was filtered through two layers of cheese cloth to remove as much gelatinous material and debris as possible. Concentration was determined by the use of a hemacytometer. The semen was first diluted to a ratio of 1:200 using disposable dilution bottles. Then the diluted semen was placed on the hemacytometer where the number of sperm cells in

five large squares was counted. The five large squares contained a volume of 1/50 cu. mm., therefore, 50X200X1000 equalled the number of spermatozoa in one cc., which is how concentration was reported. Percent motility and percent abnormal spermatozoa were both reported as visual estimates. Rate of forward movement (RFM) was also reported as a visual estimate with a range of 1+ to 4, 1+ being the best possible RFM and a 4 denoting lack of movement.

The ejaculate was next divided into three samples of 5 cc. each with each sample being placed in a 20 cc. test tube at room temperature. One sample was extended at a ratio of 1:1 with pre-warmed (38° C.) extender containing 5 gm. dehydrated buttermilk and 5 gm. glucose in 100 cc. of distilled water (BMG). The other 5 cc. portions of the ejaculate were centrifuged at room temperature in a slant type medical centrifuge for four minutes at 1,500 rpm in order to remove the majority of the seminal plasma. Seventy-five to eighty percent of the seminal plasma was then decanted from each of the test tubes and combined for later use. Then the BMG extender was used to bring the remaining sperm rich fraction of one of the centrifuged samples to twice the original volume, therefore making a total of 10 cc. The sperm rich fraction of the other centrifuged sample was discarded. This left two samples, each containing 10 cc. of extended spermatozoa. The main difference was that one sample contained seminal plasma and the other sample had been centrifuged and the

seminal plasma decanted and replaced by BMG.

The two samples were then placed in refrigeration at 4° C. and checked every 24 hours for percent motility and RFM until the percent motility for each sample became less than 5 percent.

The seminal plasma which was decanted from the two centrifuged samples was placed in a 150 ml. beaker and evaluated. A Corning Scientific Instrument Model 10 pH Meter was used to determine the pH. A Cryoscope was used to determine freezing point depression as a measure of osmotic pressure. In order to obtain the freezing point, 2 cc. samples of seminal plasma were placed in 10 cc. test tubes and stored in a freezer until all sixteen samples had been collected. Each sample was placed in the Cryoscope individually and the freezing point depression was read. Reruns were made on six of the samples to determine repeatability. The repeatability was found to be within 2/1000 of a degree centigrade. As an indicator of ionization in the seminal fluids, conductivity of the sample was measured. This was done immediately after pH was determined by switching the Corning Model 10 pH Meter to read milli-volts of current instead of pH. The range possible was from -1400 to +1400 milli-volts.

An analysis of variance after H.O. Hartley (1962) was used to evaluate the stored centrifuged and noncentrifuged sperm, and the seminal plasma.

CHAPTER IV

RESULTS AND DISCUSSION

Listed in Appendix tables 1, 2, 3 and 4 are the comparisons by stallions of centrifuged and noncentrifuged sperm. Also recorded in these tables are the date, volume, concentration and percent abnormalities for each of the sixteen separate collections. The comparisons were made using percent motility, rate of forward movement and days stored at 4° C. to evaluate.

Figures 1 through 4 graphically compare the percent and motility of centrifuged and noncentrifuged sperm after fixed periods of storage at 4° C. for Stallions A, B, C and D respectively. In these figures the percent motility for each day is a mean value for all four collections on that day.

For Stallion A the centrifuged sperm were 35.00 percent motile on day zero. This was 6.25 percent higher than the motility for the noncentrifuged sperm which was 28.75. The centrifuged sperm were 8.75 percent motile on day one, 1.25 percent motile on day two and zero percent motile on day three, while the noncentrifuged sperm had a motility of zero on day one. The centrifuged sperm, therefore, were motile two days longer than the noncentrifuged.

The noncentrifuged sperm for Stallion B had a motility of 55.00 percent on day zero, 5 percent higher than the centrifuged sperm at that time. On day one the noncentrifuged sperm were 26.25 percent motile and the centrifuged sperm were 20.00 percent

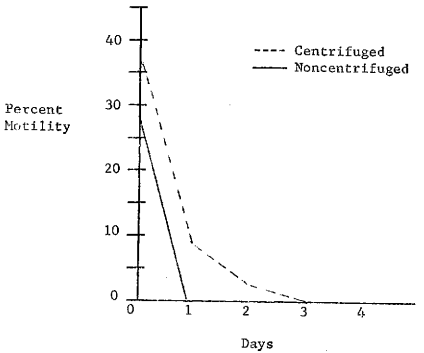

Figure 1. Comparison of centrifuged and noncentrifuged sperm using percent motility and days stored at 4° C. to evaluate Stallion A.

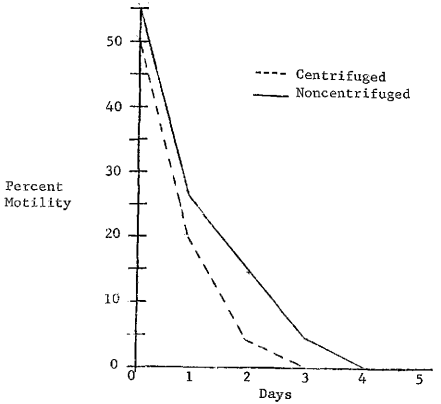

Figure 2. Comparison of centrifuged and non-centrifuged sperm using percent motility and days stored at 4° C. to evaluate Stallion B.

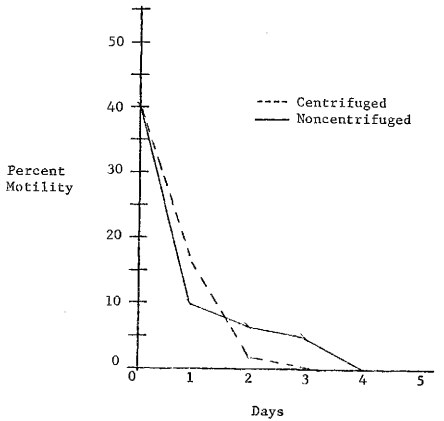

Figure 3. Comparison of centrifuged and non-centrifuged sperm using percent motility and days stored at 4° C. to evaluate Stallion C.

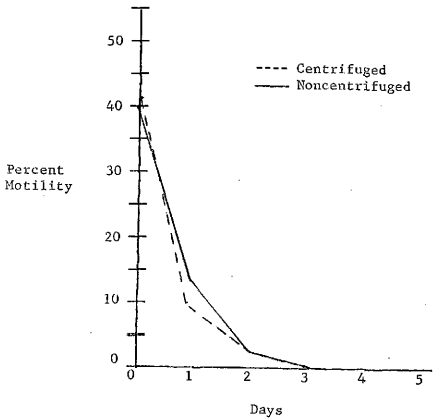

Figure 4. Comparison of centrifuged and non-centrifuged sperm using percent motility and days stored at 4° C. to evaluate Stallion D.

motile. On day two the noncentrifuged sperm had a motility of 13.25 percent and the centrifuged sperm had a motility of 5.00 percent. By the third day the noncentrifuged wperm had a motility of 5.00 percent and the centrifuged sperm showed no motility. On the fourth day the noncentrifuged sperm showed no motility.

Both the centrifuged and noncentrifuged sperm from Stallion C had 40.00 percent motility on day zero. On day one the centrifuged sperm were 16.25 percent motile, 5 percent better than the noncentrifuged sperm which were 11.25 percent motile. On day two, however, the noncentrifuged sperm were 6.25 percent motile or 5 percent better than the centrifuged. By day three all the centrifuged sperm were nonmotile, yet the noncentrifuged sperm were 5.00 percent motile and did not become completely nonmotile until day four.

The motility of sperm from Stallion D for day zero was 36.25 percent for the centrifuged and 35.00 percent for the noncentrifuged. The motility on day one was 13.75 percent for the noncentrifuged and 10.00 percent for the centrifuged. On day two both the centrifuged and noncentrifuged sperm had a motility of 1.25 percent and on day three both were nonmotile.

In composite (figure 5), the centrifuged sperm were 40.31 percent motile on day zero, 0.63 percent higher than the noncentrifuged sperm on that day. On day one the centrifuged sperm showed 13.75 percent motility and the noncentrifuged showed 12.81 percent, a difference of .94 percent. On day two the noncentrifuged sperm were 3.13 percent more motile with the

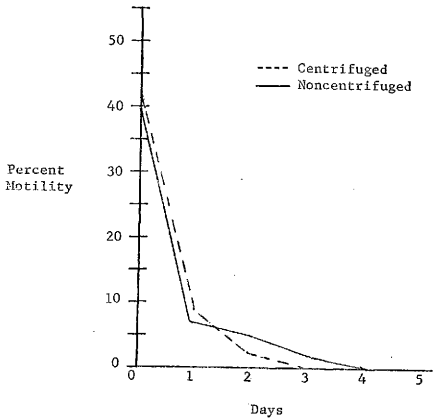

Figure 5. Comparison of centrifuged and non-centrifuged sperm using percent motility and days stored at 4° C. to evaluate a composite of Stallions A, B, C and D.

noncentrifuged sperm showing 5.31 percent and the centrifuged 2.18 percent. By day three there were no motile centrifuged sperm, but 2.81 percent of the noncentrifuged sperm remained motile. On day four all sperm were nonmotile.

An analysis of variance with factorial design using the three factors; stallions, percent motility and days, was used to analyze the survival data of centrifuged and noncentrifuged sperm following extension and storage at 4° C. Table 1 shows the source of variation, sum of squares, degrees freedom, means squares, F values and significance levels obtained from this analysis. The difference between animals for their ability to produce motile sperm was significant. The differences between the motility of centrifuged and noncentrifuged sperm were not significant. This was the same result observed by Polge et al. (1964) and Nagase (1966). However, when animal interaction was included there was a highly significant difference between centrifuged and noncentrifuged sperm. This indicated that centrifugation caused a variation in storageability of sperm from certain stallions. There was a highly significant variation from day zero to day five in percent survival of spermatozoa. There was also a highly significant variation among animals for their ability to withstand storage for different lengths of time. The F value determined for centrifuged and noncentrifuged sperm with day interaction was nonsignificant. Also, animal, centrifuged, noncentrifuged and day

TABLE 1. THE COMPARISON OF THE PERCENT SURVIVAL OF CENTRIFUGED AND NONCENTRIFUGED SPERM FOLLOWING EXTENSION AND STORAGE AT 4° C.

Source of Variation	Sum of Squares	Degrees Freedom	Means Squares	F
Animal	2249.21800	3	749.73950	16.94814**
Cent. & Noncent.	29.40625	1	26.40625	.59692
Animal X Cent. & Noncent.	341.71870	3	118.90620	2.57489*
Days	35605.61000	4	8901.40200	201.21951**
Animal X Days	1540.62500	12	128.38540	2.90219**
Cent. & Noncent. X Days	111.87500	4	27.96875	.63224
Animal X Cent. & Noncent. X Days	266.87500	12	22.23958	.50273
Collections	1067.96800	3	355.98950	8.04621**
Error	5175.76059	117	44.23727	

*Significant at the 0.05 level
**Significant at the 0.01 level

interaction were nonsignificant. There was a highly significant variation between collections within stallions.

Figures 6 through 9 compare centrifuged and noncentrifuged sperm using rate of forward movement (RFM) and days stored at 4° C. to evaluate each stallion. In these figures the rate of forward movement for each day is a mean value for all four collections on that day. The trends are the same by stallions as those shown in figures 1 through 4 for motility.

Figure 6 is a graph showing the RFM values for sperm from Stallion A on days zero through three. On day zero both the centrifuged and noncentrifuged sperm had a RFM of 1.00. On day one the centrifuged sperm had a RFM of 2.00 while the noncentrifuged sperm RFM was 4.00. On day two the RFM for the centrifuged sperm was 3.75 and by day three it was 4.00. The centrifuged sperm lived two days longer than the noncentrifuged sperm.

Figure 7 is a graph of the RFM values for sperm from Stallion B on days zero through four. On day zero the RFM for noncentrifuged sperm was 1.00 and for centrifuged was 1.25. On day one the RFM for both centrifuged and noncentrifuged sperm was 2.75. On day two the noncentrifuged sperm had a RFM of 3.00 and the centrifuged sperm had a RFM of 3.50. It was not until day three that the noncentrifuged sperm had a RFM of 3.50 and by this time the centrifuged sperm had a reading of 4.00. On day four the noncentrifuged sperm read 4.00.

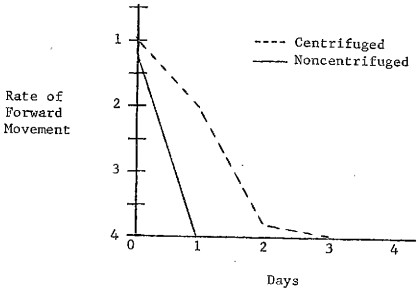

Figure 6. Comparison of centrifuged and non-centrifuged sperm using rate of forward movement and days stored at 4° C. to evaluate Stallion A

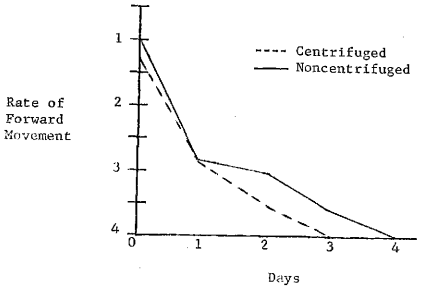

Figure 7. Comparison of centrifuged and non-centrifuged sperm using rate of forward movement and days stored at 4° C. to evaluate Stallion B.

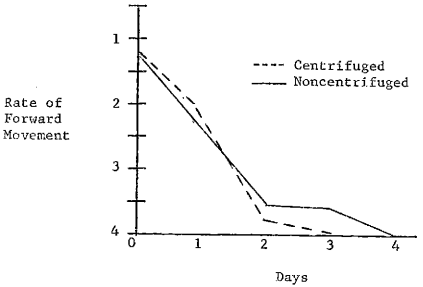

Figure 8. Comparison of centrifuged and non-centrifuged sperm using rate of forward movement and days stored at 4° C. to evaluate Stallion C.

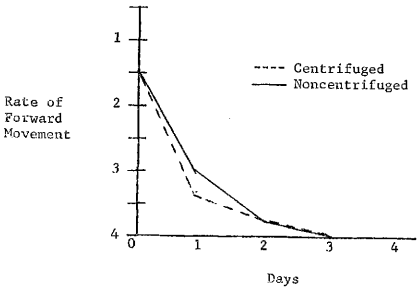

Figure 9. Comparison of centrifuged and non-centrifuged sperm using rate of forward movement and days stored at 4° C. to evaluate Stallion D.

Figure 8 is a graph of the RFM by days for Stallion C. The day zero reading of RFM was 1.25 for both centrifuged and non-centrifuged sperm. On day one the centrifuged sperm had a RFM of 2.00 and the noncentrifuged sperm a RFM of 2.25, however, by day two the RFM for the noncentrifuged sperm was better, having a value of 3.50 while the centrifuged sperm had a value of 3.75. On day three the noncentrifuged sperm still had a RFM of 3.50 but the centrifuged sperm were already at the 4.00 level. On day four the noncentrifuged sperm had a RFM value of 4.00.

Figure 9 is a graph of RFM by days for Stallion D. On day zero both the centrifuged and noncentrifuged sperm had a RFM of 1.50. On day one the noncentrifuged sperm had a RFM of 3.00 which was slightly better than the centrifuged which was 3.25. Days two and three showed no difference in RFM for centrifuged and noncentrifuged sperm. On day two both had a RFM of 3.75 and on day three both had a RFM of 4.00.

Figure 10 is a composite of figures 6, 7, 8 and 9. Here also the trend is the same as the composite (figure 5) for motility. The day zero values for RFM are the same for both centrifuged and noncentrifuged sperm each having a value of 1.25. On day one the RFM for the centrifuged sperm was 2.50, 0.5 better than the 3.00 RFM for the noncentrifuged sperm. On day two centrifuged and noncentrifuged sperm had almost identical RFM, the centrifuged having a reading of 3.68 and the noncentrifuged a reading of 3.56. However, on day three the centrifuged sperm had a RFM of 4.00

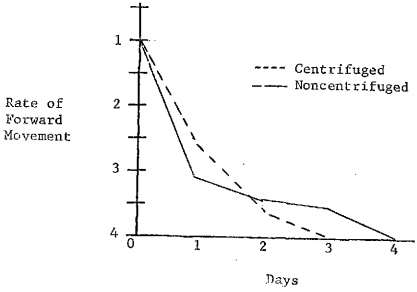

Figure 10. Comparison of centrifuged and non-centrifuged sperm using rate of forward movement and days stored at 4° C. to evaluate Stallions A, B, C and D collectively.

while the noncentrifuged sperm had a RFM of 3.68. It was day four before the noncentrifuged sperm were given a RFM of 4.00.

In tables 2 through 4, evaluation of the physical properties of the seminal plasma are given by stallion and by collection. An analysis of variance with factorial design and just one factor, stallions, was used to analyze for differences in the physical properties of seminal plasma. In tables 5, 6 and 7 are given the source of variation, sum of squares, degrees freedom, means squares, F values and significance levels for the properties measured.

Table 2 contains the pH values obtained for each sample of seminal plasma. Average values for each of the four stallions and the overall average of 7.62 pH units are also given. There was no significant variation in pH of seminal plasma between stallions as shown by the statistical analysis in table 5. The overall average of 7.62 was .22 pH units higher than reported for semen by Hendrikse (1966) and .64 units lower than that determined for the BMG extender.

The freezing point depression (FPD) is expressed in degrees centigrade at which the sample became solid. Table 3 gives the data on all sixteen samples and the statistical analysis is shown in table 6. There was a 95 percent significant variation between stallions for freezing point depression of the seminal plasma. The average FPD of the entire group of samples was $-.702^{\circ}$ C. The FPD of the BMG was -.760. In order to determine

TABLE 2. pH VALUES OF SEMINAL PLASMA BETWEEN COLLECTION AND STALLIONS

Stallion	COLLECTION #1	#2	#3	#4	Avg.
A	7.62	7.65	7.58	7.72	7.64
B	7.31	7.55	7.55	7.88	7.57
C	7.55	7.49	7.65	7.80	7.62
D	7.20	7.60	7.80	7.92	7.63
					7.62

TABLE 3. FREEZING POINT DEPRESSION VALUES OF SEMINAL PLASMA BETWEEN COLLECTIONS AND STALLIONS

Stallion	COLLECTIONS #1	#2	#3	#4	Avg.
A	-1.200	-.962	-.769	-.626	-.889
B	-.676	-.599	-.702	-.728	-.676
C	-.637	-.589	-.720	-.591	-.634
D	-.716	-.558	-.603	-.570	-.611
					-.702

TABLE 4. MILLI-VOLT VALUES OF SEMINAL PLASMA BETWEEN COLLECTIONS AND STALLIONS

Stallion	COLLECTION #1	#2	#3	#4	Avg.
A	-32	-35	-27	-37	-32
B	0	-7	-35	-49	-22
C	-26	-19	-20	-25	-27
D	-8	-34	-28	-30	-25
					-27

TABLE 5. A MEASURE OF pH OF SEMINAL PLASMA TO DETERMINE DIFFERENCES BETWEEN STALLIONS

Source of Variation	Sum of Squares	Degrees Freedom	Means Squares	F
Animal	0.13207	3	0.04402	1.22926
Error	0.42983	12	0.03581	---

TABLE 6. A COMPARISON OF THE FREEZING POINT DEPRESSION OF SEMINAL PLASMA TO DETERMINE THE DIFFERENCES BETWEEN STALLIONS

Source of Variation	Sum of Squares	Degrees Freedom	Means Squares	F
Animal	0.19432	3	0.06477	3.4488*
Error	0.22541	12	0.01878	---

*Significant at the 0.05 level

TABLE 7. A TEST OF THE DIFFERENCES IN THE DEGREE OF IONIZATION TAKING PLACE IN THE SEMINAL PLASMA OF DIFFERENT STALLIONS

Animal	221.50000	3	73.83333	1.61875
Error	2542.50000	12	136.83333	---

the sample causing the greatest variation, a multiple-range test was run. The test used was Duncan's new multiple-range test from Steel and Torrie (1960) illustrated in table 8. Average FPD of seminal plasma for Stallion A was -.889 and this was significantly different at the 95 percent level from the other three stallions.

This would indicate that the same factor causing a decrease in the freezing point of the seminal plasma was detrimental to the storage life of spermatozoa, since Stallion A had improved storage life of the spermatozoa when the seminal plasma was removed from the semen. This was also indicated by Bogart and Mayer (1950) in their discussion of various physical and chemical factors detrimental to spermatozoan viability.

Table 4 lists the milli-volts of conductivity for each sample with averages for each stallion also listed. The overall average was -27. The statistical analysis in table 7 indicated no significant variation between stallions for milli-volts conductivity of their seminal plasma. The BMG extender had a milli-volt reading of +22, which was 49 more milli-volts conductivity than the average for seminal plasma.

The results obtained in this study indicated that stallion semen does vary in its ability to withstand storage. It is also indicated that freezing point depression of the seminal plasma is one of the factors causing this variation. Therefore in those stallions that have an impaired length of storage due to a low FPD of the seminal plasma a more suitable medium

TABLE 8. DUNCAN'S NEW MULTIPLE RANGE TEST FOR THE FREEZING POINT DEPRESSION OF SEMINAL PLASMA OF 4 STALLIONS WITH 4 COLLECTIONS EACH.

$S\bar{x}$ = .0685

Value of P		2	3	4
SSR	5%	3.08	3.23	3.33
	1%	4.32	4.55	4.68
LSR	5%	.211	.221	.228
	1%	.296	.312	.321

STALLION	A	B	C	D
Average Freezing Point Depression for 4 Collections	-.889	-.676	-.634	-.611*

*Any two means not underscored by the same line are significantly different.

could be substituted for the seminal plasma to increase storage time. In this way superior males of the species might be better utilized.

This study was beneficial too, in that it provided information about pH, FPD and milli-volts conductivity of the seminal plasma not previously known.

CHAPTER V

SUMMARY

Four ejaculates were taken from each of four stallions during the Spring of 1969. Two samples were taken from each ejaculate. One sample of semen was extended 1:1 with buttermilk glucose extender. The other semen sample was first centrifuged and the seminal plasma decanted before buttermilk glucose extender was used to bring it to twice its original volume. Both the centrifuged and noncentrifuged samples were than stored at 4° C. and evaluated daily for percent motility and rate of forward movement until the percent motility was less than five percent.

Of the four stallions tested, two showed a difference in the storage life of the spermatozoa due to removal of the seminal plasma. Storage life was improved when the seminal plasma was removed from the semen of Stallion A. However, the reverse was true for Stallion B, since the storage life of the spermatozoa from this stallion was impaired by the removal of the seminal plasma.

The decanted seminal plasma for each of the sixteen collections was also evaluated to determine the variation among stallions for the physical properties of their seminal plasma. Freezing point depression, pH, and milli-volts conductivity were the physical properties evaluated. Only freezing point depression

indicated a significant variation among stallions and this variation was due to Stallion A, who had the lowest freezing point depression. This would indicate that the same factor causing a decrease in freezing point depression was detrimental to the storage life of the spermatozoa.

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APPENDIX

TABLE 1. COMPARISON OF CENTRIFUGED AND NONCENTRIFUGED SEMEN, USING PERCENT MOTILITY, RATE OF FORWARD MOVEMENT AND DAYS STORED TO EVALUATE STALLION A.

	COLLECTION															
	#1				#2				#3				#4			
	Date 5/20/69				Date 5/20/69				Date 6/3/69				Date 6/3/69			
	Vol. 25 cc.				Vol. 25 cc.				Vol. 25 cc.				Vol. 20 cc.			
	Abn. <10				Abn. <10				Abn. <5				Abn. <5			
	Conc. $400X10^6$				Conc. $60X10^6$				Conc. $280X10^6$				Conc. $120X10^6$			
	Cent.		Noncent.		Cent.		Noncent.		Cent.		Noncent.		Cent.		Noncent.	
Day	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM
0	30	1	15	2+	35	1	35	1-	35	1	35	1-	40	1+	35	1
1	5	3	<5	4	10	3	<5	4	10	1	<5	4	10	1	<5	4
2	<5	4			5	3-			<5	4			<5	4		
3					<5	4										

TABLE 2. COMPARISON OF CENTRIFUGED AND NONCENTRIFUGED SEMEN, USING PERCENT MOTILITY, RATE OF FORWARD MOVEMENT AND DAYS STORED TO EVALUATE STALLION B.

	COLLECTION															
	#1				#2				#3				#4			
	Date 2/25/69				Date 3/4/69				Date 5/13/69				Date 5/13/69			
	Vol. 70 cc.				Vol. 75 cc.				Vol. 50 cc.				Vol. 35 cc.			
	Abn. <5				Abn. <5				Abn. <10				Abn. <10			
	Conc. $100X10^6$				Conc. $100X10^6$				Conc. $360X10^6$				Conc. $100X10^6$			
	Cent.		Noncent.		Cent.		Noncent.		Cent.		Noncent.		Cent.		Noncent.	
Day	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM
0	60	1+	55	1-	70	1+	65	1	25	2-	45	1-	45	1-	50	1+
1	10	2-	5	3-	10	3	50	2-	25	3	35	3	35	3	30	3-
2	<5	4	5	3-	<5	4	20	3	10	3	10	3-	10	3-	15	3-
3			<5	4			10	3	<5	4	<5	4	<5	4	10	3-
4							<5	4							<5	4

TABLE 3. COMPARISON OF CENTRIFUGED AND NONCENTRIFUGED SEMEN, USING PERCENT MOTILITY, RATE OF FORWARD MOVEMENT AND DAYS STORED TO EVALUATE STALLION C.

	COLLECTION															
	#1				#2				#3				#4			
	Date 2/3/69				Date 2/5/69				Date 2/11/69				Date 2/13/69			
	Vol. 20 cc.				Vol. 100 cc.				Vol. 25 cc.				Vol. 30 cc.			
	Abn. <5				Abn. <5				Abn. <5				Abn. <5			
	Conc. $40X10^7$				Conc. $260X10^6$				Conc. $480X10^6$				Conc. $140X10^6$			
	Cent.		Noncent.		Cent.		Noncent.		Cent.		Noncent.		Cent.		Noncent.	
Day	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM
0	30	2	30	2	50	1+	50	1+	50	1	30	1	30	1	50	1
1	15	2	10	3	20	2	20	3+	10	2	<5	4	20	2-	15	1-
2	5	3	<5	4	<5	4	15	3+	<5	4			<5	4	10	3-
3	<5	4					10	3+							10	3-
4							<5	4							<5	4

TABLE 4. COMPARISON OF CENTRIFUGED AND NONCENTRIFUGED SEMEN, USING PERCENT MOTILITY, RATE OF FORWARD MOVEMENT AND DAYS STORED TO EVALUATE STALLION D.

	COLLECTION															
	#1 Date 2/3/69 Vol. 20 cc. Abn. <5 Conc. 240X10^6				#2 Date 2/5/69 Vol. 125 cc. Abn. <5 Conc. 120X10^6				#3 Date 2/11/69 Vol. 25 cc. Abn. <5 Conc. 420X10^6				#4 Date 2/13/69 Vol. 25 cc Abn. <5 Conc. 420X10^6			
	Cent.		Noncent.		Cent.		Noncent.		Cent.		Noncent.		Cent.		Noncent.	
Day	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM	% Mot.	RFM
0	30	2	30	2	50	1-	50	1-	30	2-	20	2-	35	1+	40	1+
1	10	3	10	3	<5	4	20	2	10	3	<5	4	20	3+	25	3
2	<5	4	<5	4			<5	4	5	3			<5	4	5	3
3									<5	4					<5	4

VITA

James P. McCall, Jr. was born in Magnolia, Arkansas, on June 17, 1943, and was the only child of James P. and Francis McCall. He started his education by attending the schools of the El Dorado School System at El Dorado, Arkansas. After graduating from El Dorado High in May 1961, he started college by attending the Agricultural and Mechanical College of Texas at College Station, Texas, and graduated from Texas A&M University with a Bachelor of Science in January 1966.

He immediately started graduate study, having as his goal a Master of Science in Animal Science. After completing the course work for this degree in the Spring of 1967, he was employed by the Texas Agricultural Extension Service as an Assistant County Agricultural Agent for eight months. Next he was employed as Director of Research for Horse Breeders Service of Sonoma, California. In September of 1968 he returned to Texas A&M University to complete the requirements for the Master of Science Degree for which he is now a candidate. At present he resides with his wife and two sons at Route 2, Box 274, Bryan, Texas.

Typist for this thesis was Mrs. Karen McCall.

Xeroxing was done by the Engineering Department of Texas A&M University.