

May 22, 1961

To the Graduate Council:

I am submitting herewith a thesis written by Joseph Wells Austin entitled "Comparison of Quality of Bull Semen Collected in the Artificial Vagina or by Electroejaculation". I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

R. L. Murphree
Major Professor

We have read this thesis
and recommend its acceptance:

Charles S. Hobbs

O. Glen Hall

Accepted for the council:

H. E. Spivey
Acting Dean of the Graduate School

COMPARISON OF QUALITY OF BULL SEMEN COLLECTED IN THE
ARTIFICIAL VAGINA OR BY ELECTROEJACULATION

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Joseph Wells Austin
June 1961

ACKNOWLEDGMENTS

I am indeed grateful to Dr. R. L. Murphree for his splendid guidance throughout my period of graduate study at the University of Tennessee and for his assistance in conducting this study and in preparing this thesis. I also appreciate the help of Dr. E. W. Hupp, Associate Scientist, UT-AEC Agricultural Research Laboratory, for his assistance throughout this study and Dr. M. A. Kastenbaum, Oak Ridge National Laboratory, for his assistance and advice in the statistical analyses of the data.

My heartfelt thanks go to Dr. Nathan S. Hall for his cooperation and kindness in awarding me an assistantship and making it possible for me to pursue an advanced degree. I greatly appreciate Dr. C. S. Hobbs, my graduate committee, and others who have reviewed this thesis and given their helpful suggestions. I wish to express my appreciation to the UT-AEC Agricultural Research Laboratory for providing the animals and facilities used in this investigation. I am deeply indebted to the UT-AEC staff personnel, laboratory technicians, and animal caretakers for their help and cooperation.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
III. PROCEDURE	7
IV. RESULTS	14
V. DISCUSSION	20
VI. SUMMARY	24
BIBLIOGRAPHY	25

CRANE & CREST

LIST OF TABLES

TABLE	PAGE
I. Characteristics of Semen Collected in an Artificial Vagina or by Electroejaculation	15
II. Effects of Collection Method Sequence on Total Number of Sperm per Ejaculate	17
III. Motility Ratings of Samples Collected in the Artificial Vagina and by Electroejaculation That Were Stored for a 4-day Period at 5° C	18
IV. Correlation Table	19

LIST OF FIGURES

FIGURE	PAGE
1. Schematic Wiring Diagram of the Electrical Unit	8
2. Relationship between Sperm Concentration and Optical Density of Electroejaculated Semen	11
3. Relationship Between Sperm Concentration and Optical Density of Semen Collected in the Artificial Vagina	12

CHAPTER I

INTRODUCTION

The artificial vagina has become the generally accepted method for collecting semen from bulls. It was invented by an Italian, Amantea (Willet 1956), in an attempt to increase the service of highly desirable sires. This was not a satisfactory method when using beef bulls to study the effects of radiation on sperm production at the UT-AEC Agricultural Research Laboratory. Two problems were encountered in attempting to use this method of collection. First, the percentage of untrained and unselected bulls that would serve the artificial vagina was relatively small; and second, it was difficult to obtain consistently timed collections. It was, therefore, necessary to have a large herd of bulls from which to select the desired number of experimental animals. The failure of selected bulls to ejaculate at scheduled intervals prompted the subsequent use of an electroejaculation technique in such investigations.

The ease and consistency of obtaining semen from bulls by electroejaculation is a great aid in conducting experiments and can be used to obtain semen for breeding purposes when the male is unable or unwilling to serve the artificial vagina.

Despite the fact that a number of papers have been published describing electroejaculation techniques, few intensive comparisons of the characteristics of semen collected in the artificial vagina with that collected by electroejaculation have been reported.

The purpose of this investigation was to examine and compare the quality of semen collected by the artificial vagina and electroejaculation. By so doing, the results of studies on semen obtained by electroejaculation could then be compared more accurately with the results of other methods found in the literature.

CHAPTER II

LITERATURE REVIEW

Various methods used to collect semen from bulls have been described by Winters (1938), Lambert and McKinzie (1940), Willett (1956), and Rice et al. (1957). These include (1) insertion of a sponge or breeding bag into the vagina of a cow prior to service, (2) aspiration of semen from the vagina of a non-estrous female after service, (3) messaging the ampulla, (4) electrical stimulation, and (5) use of an artificial vagina. With the exception of the electrical stimulus and artificial vagina, none of these procedures consistently yield semen of satisfactory quality.

Successful ejaculation of rams using an electrical stimulus was accomplished by Gunn (1936). One electrode was placed in the rectum and the other inserted between the fourth lumbar and last sacral vertebra. Even though this method was successful, the animal had to be securely restrained.

Brady and Gildow (1939) employed three methods for collecting ram semen: (1) from the vagina of a non-estrous ewe, (2) the artificial vagina, and (3) electrical stimulation. The largest volume of semen was obtained by electrical stimulation and the smallest from the vagina of a ewe. Sperm concentration was highest with the artificial vagina and lowest from electroejaculation. The greatest percent of motile sperm was in samples from the artificial vagina, and no difference between the other two methods was observed. From

these findings they concluded that the artificial vagina serves as the most useful method of collecting semen from rams, and electroejaculation has a very definite use especially when rams will not or cannot serve a ewe or the artificial vagina.

Terrill (1940) compared semen quality and fertility using semen collected by an artificial vagina, electrical stimulus, and from the vagina of a ewe. About half as many sperm were obtained with electrical stimulation as with the other two methods. Semen collected by electroejaculation exhibited slightly poorer motility, and the number of sperm per collection was more variable than in semen obtained by the other methods. The difference in sperm number and semen quality, however, did not appear to affect fertility following artificial insemination.

Dziuk et al. (1954) and Marden (1954) described equipment and techniques for collecting bull semen by electroejaculation. Dziuk et al. used a 30-volt 60-cycle alternating current which could be varied from zero to 30-volts by means of a variable transformer. An isolation step-down transformer was used to isolate the current from the power source and to allow a more gradual change in voltage. A bipolar rectal probe 1-1/2 inches in diameter with six rings spaced 1-3/4 inches apart was used. They obtained 111 collections from 18 bulls. The average volume was 12.3 ml. per collection with sperm concentration ranging from "very low to 1.27 billion sperm per ml." Even though the volume was larger and the concentration lower for the

electroejaculated semen, the total number of sperm was comparable to that obtained with an artificial vagina.

Marden (1954) reported that the most satisfactory results were obtained using frequencies between 20 and 30 cycles with a pure sine wave pulse. Zero to 130 volts were obtained by using a power source consisting of a transformer with a variable secondary winding. This source, in turn, actuated a 20-volt step-down transformer, thus enabling a gradual rising and falling potential to be applied to the electrodes. The cylindrical lucite probe, 1-7/8 inches in diameter and 13 inches long, consisted of four electrodes spaced 90 degrees apart and connected in pairs at 180 degrees.

Rowson and Murdoch (1954) found that the greatest response occurred when the probe was in close contact with the prostate gland. They used two ring electrodes which were inserted into the rectum on a rubber-gloved hand. This permitted more accurate placement of the electrodes than was possible with a probe. These three types of electrical units have given good results.

In breeding trials using electroejaculated semen from nine dairy stud bulls, which were collected the previous year with an artificial vagina, Dziuk *et al.* (1954) found essentially no difference in the 60- to 90-day nonreturn rates for the two years. A comparison of fertility with semen collected from two bulls by artificial vagina and electroejaculation was also reported by Manton (1956). The 90-day nonreturn rate of 1,438 first insemina-

tions with semen collected in the artificial vagina was 64%, while that for 3,712 first inseminations with electroejaculated semen was 68%.

Electroejaculated samples collected by Hill et al. (1956) had a slightly higher pH and lower volume than those from the artificial vagina. No cell type differences were found in morphological studies of semen collected by the two methods.

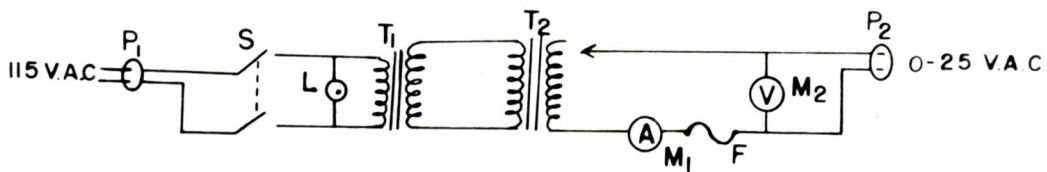
CHAPTER III

PROCEDURE

Twelve Hereford bulls, 2 to 6 years of age, were used in this study which extended from February 23 to May 15, 1959. They were selected from a group of 33 bulls on the basis of their willingness to consistently serve an artificial vagina during a preliminary training period. They were maintained as a single group on hay and corn silage with access to pasture during the latter part of the experimental period.

The finger electrode method described by Parish (1958) was used to collect the electroejaculated semen. The equipment consisted of: (1) an isolation transformer (to isolate the circuit from the standard 110 volt, 60 cycle, a-c source); (2) a variable transformer with a stop at 25 volts; (3) 0-1 ampere ammeter; (4) 0-25 volt voltmeter; (5) 0.5 ampere fuse; (6) pilot light; (7) off-on switch; and (8) two ring electrodes (schematic diagram, figure 1). The ring electrodes were made from number 10 copper wire by forming rings that fitted snugly over the first and second fingers of the operator. The rings were soldered to a suitable length of rubber insulated extension cord. A regular artificial vagina rubber cone and a graduated centrifuge tube were used to collect the semen.

Usually a variable amount of clear fluid precedes the sperm containing fraction during electroejaculation. This pre-sperm fraction was discarded during this experiment.



- P_1 = Standard male plug
 P_2 = Standard female plug
 S = DPST Switch
 L = Pilot light
 T_1 = 1:1 Isolation transformer
 T_2 = Variable transformer variac W-2
 M_1 = 0-1 AMP A.C. Ammeter
 M_2 = 0-25 V.A.C. Voltmeter
 F = 0.5 AMP Slo- Blo fuse

Figure 1. Schematic wiring diagram of the electrical unit. All components are standard items and are available from radio supply firms.

Figure 1. Relationship between a series of measurements and the density of carbonized material. The relationship between the density of a carbonized material and the density of a carbonized material is shown in Figure 1. The density of a carbonized material is shown in Figure 1. The density of a carbonized material is shown in Figure 1.

The generally accepted method and equipment were employed in collecting semen samples with the artificial vagina (Lambert and McKenzie, 1940). The collecting vials were warmed and insulated to prevent the sperm from being cold shocked. A non-estrous cow fastened in a stationary breeding stall was used as a mount. The bulls were not restrained nor allowed intentional false mounts before serving the artificial vagina.

At the beginning of the experiment, and periodically throughout, the preputial hair was clipped. The preputial area was brushed, prior to each collection by electroejaculation to remove any loose dirt.

An attempt was made to obtain two consecutive ejaculates from each bull on Monday and Friday each week. To compensate for seasonal and other uncontrolled environmental trends, a single crossover design was used. The bulls were divided into two comparable groups of six animals (A and B) on the basis of the average number of sperm obtained per ejaculate during a pre-experimental period.

The experimental period was divided into two unequal phases of 8 (first) and 4 (second) weeks duration. During the first phase both first and second ejaculates from each bull were obtained by the same method of collection. Semen was collected from the bulls in group A during the first 4 weeks in the artificial vagina and the next 4 weeks by electroejaculation. During the second phase the first ejaculate was obtained by one method and the second ejaculate by the alternate method. During the first 2 weeks of the second phase, the

first ejaculate from the bulls in group A was collected in the artificial vagina and the second by electrical stimulus. During the last 2 weeks, the first ejaculate was obtained by electroejaculation and the second by the artificial vagina. Throughout the experiment semen was collected from the bulls in group B in the same collection sequence but by the alternate method from that used with group A.

Immediately after each collection, the volume of semen was recorded and the initial sperm motility in undiluted semen was microscopically evaluated. Percent motile sperm was estimated from 0-100 in increments of 10 and the rate of progressive motility was evaluated through a range of 0-4. The pH of all samples of sufficient volume (1.5 ml.) was determined using a Beckman pH Meter (Model G).

Sperm concentration was estimated from optical density readings with a Klett-Summerson colorimeter. Optical density and hemocytometer counts were made on semen samples diluted with a 2.8% sodium citrate solution at a ratio of 1:39. The values of 52 samples collected in the artificial vagina from 16 bulls and 42 samples collected by electroejaculation from seven bulls were plotted (figures 2 and 3, respectively). Linear regression lines were fitted to these data by the method of least squares (Snedecor, 1956). From the equation $Y = a + bX$ (Y , is the predicted sperm number; a , is the intercept of the regression line with the ordinate; b , is the slope of the line; and X is the observed optical density) a table of predicted sperm numbers for each method of collection was constructed. Correla-

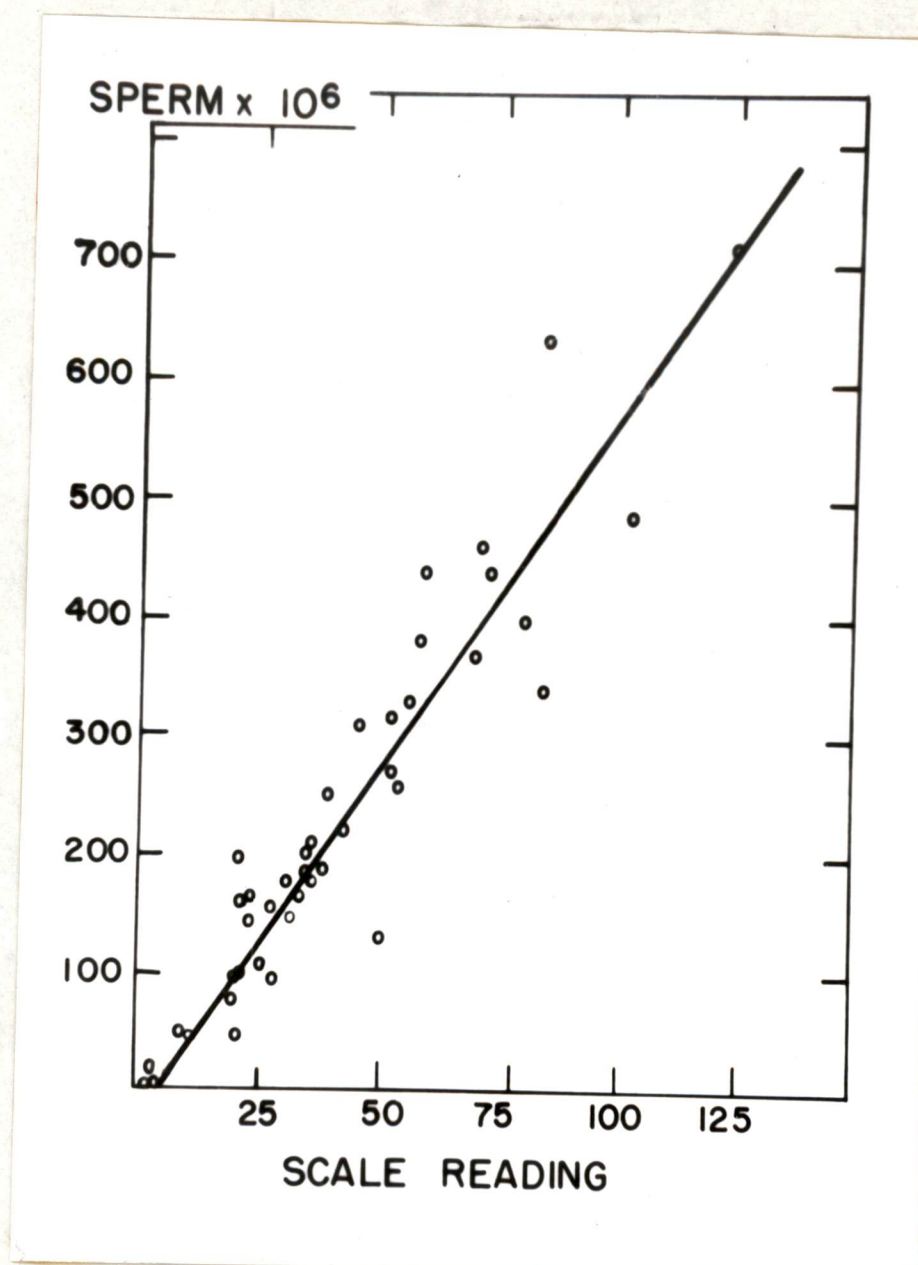


Figure 2. Relationship between sperm concentration and optical density of electroejaculated semen. The regression of hemocytometer counts of sperm numbers ($\times 10^6$ per ml.) on Klett-Summerson Colorimeter scale reading about 3 when semen was diluted 1:39 ($Y = -19.86 + 5.87 X$).

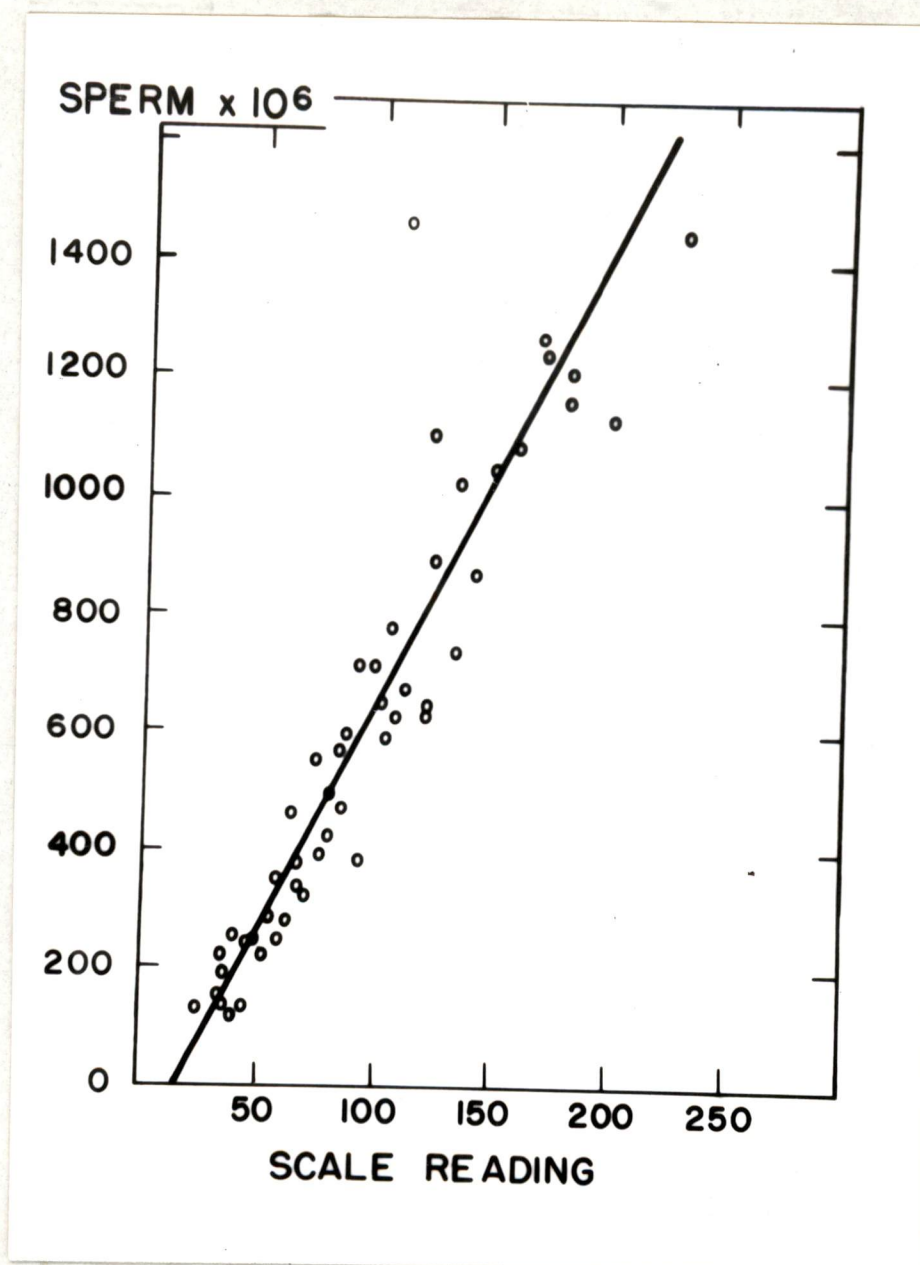


Figure 3. Relationship between sperm concentration and optical density of semen collected in the artificial vagina. The regression of hemocytometer counts of sperm numbers ($\times 10^6$ per ml.) on Klett-Summerson Colorimeter scale reading above 17 when semen was diluted 1:39 ($Y = -143.1 + 7.93 X$).

tion coefficients between optical density in the Klett-Summerson colorimeter and hemocytometer counts were + 0.951 and + 0.954 for the artificial vagina and electroejaculated samples, respectively.

Initial fructose concentration of all ejaculates obtained on Friday during weeks 3-7 and 9-12 was determined by the resorcinol reaction (Roe, 1934), as modified by Erb et al. (1956).

Maintenance of sperm motility during a 4-day storage period for all samples obtained on Monday during weeks 3 and 4, 7 and 8, and 9 through 12 were evaluated. Within 3 minutes after completing each collection, 0.5 ml. of semen was diluted in 4.5 ml. of diluent. The diluted semen was placed in a refrigerator and cooled to 5.0° C. Subsequently all samples were further diluted to 12 million total sperm per ml. by taking aliquots of the previously diluted samples and more of the same diluent. This diluent was composed of one part egg yolk to three parts of 3.2% sodium citrate containing 1000 units of penicillin per ml. Motility ratings in the diluted semen were made independently by two individuals immediately after the final dilution and after 2 and 4 days of storage at 5.0° C.

Statistical analyses of the data for the various criteria were by a weighted t-test or chi-square.

CHAPTER IV

RESULTS

The total number of semen samples collected during the 12-week period by each method would have been 288 if all bulls had ejaculated as scheduled. The number of samples obtained were 248 (86%) and 288 (100%), respectively, for the artificial vagina and electroejaculation methods of collection. Three bulls were responsible for 27 of the 40 failures to ejaculate in the artificial vagina and one of these bulls refused 15 times. When consecutive samples were obtained by the same method the interval between electroejaculated samples was about 3 minutes as compared to 5 to 20 minutes between ejaculates in the artificial vagina.

The data on volume, sperm concentration, total sperm, motility, and pH for the entire 12 week period are summarized in table 1. A larger volume of semen and lower sperm concentration was consistently obtained by electroejaculation ($P < 0.001$). The total number of sperm obtained by the two methods, however, was not statistically different ($P > 0.05$). The electroejaculated semen exhibited a higher degree of progressive motility ($P < 0.01$) and also had a higher pH ($P < 0.001$).

The major difference between the first and second phases was in the total number of sperm obtained by the two methods. During the first phase when consecutive ejaculates from each bull were

TABLE I

CHARACTERISTICS OF SEMEN COLLECTED IN AN ARTIFICIAL VAGINA
OR BY ELECTROEJACULATION

Criteria	Number of ejac. ^a		Method of collection	
	A.V.	E.E.	A.V.	E.E.
Volume (ml.)	247	287	2.8	6.3***
Sperm Conc. (x 10 ⁶ ml.)	247	287	625	293***
Sperm per Ejac. (x 10 ⁶)	247	287	1693	1826
Motility ^b	247	287	2.2	2.7**
pH	196	287	6.7	7.3***

^aOne sample from each method was lost before analyses were completed; A.V., artificial vagina; and E.E. electro-ejaculated samples.

^bMotility is expressed as the rate of progressive forward motion on a gradient of 0-4.

**Means differ significantly at the 0.01 level of probability

***Means differ significantly at the 0.001 level of probability.

collected by the same method there was not a statistically significant difference in the number of sperm obtained. During the second phase, however, when the first ejaculate was obtained by electroejaculation and the second by the artificial vagina, significantly less sperm ($P < 0.001$) were obtained in the artificial vagina (table 2).

The initial fructose concentration was 3.3 and 5.5 mg. per ml. in the semen collected by electroejaculation and in the artificial vagina, respectively. The electroejaculated samples, however, contained more total fructose (20.3 mg. compared to 15.4 mg.) and 2.1 mg. more fructose per billion sperm than did the samples obtained in the artificial vagina.

In comparing the motility ratings of all stored semen, the electroejaculated samples consistently had a higher motility rating during the 4-day storage period; however, these differences were not significant. At the beginning of storage, 94 and 68%, respectively, of the electroejaculated and artificial vagina samples were considered to be acceptable for storage to use in artificial insemination (i.e., rate and percent motility of at least 2 and 50, respectively). At the end of 4 days storage, 55% of the electroejaculated and 18% of the artificial vagina collected samples still had a motility rating acceptable for artificial insemination (table 3). This difference was significant ($P < 0.02$).

The correlation coefficients between the various criteria used to evaluate the semen in this study are shown in table 4.

TABLE II

EFFECT OF COLLECTION METHOD SEQUENCE OF TOTAL NUMBER
OF SPERM PER EJACULATE

Sequence	No ejaculates	Mean no. sperm ($\times 10^6$) ^a
<u>First phase</u>		
1st ejaculate (A.V.)	91 ^b	1818
2nd ejaculate (A.V.)	80	1593
1st ejaculate (E.E.)	96	1758
2nd ejaculate (E.E.)	96	1304
<u>Second phase</u>		
1st ejaculate (A.V.)	42	2092
2nd ejaculate (E.E.)	47 ^b	2078
1st ejaculate (E.E.)	48	2368
2nd ejaculate (A.V.)	34	1057 ^c

^aWeighted means of all observations based upon the number of observations for the artificial vagina method of collection for each bull.

^bOne sample of semen collected by each method was lost before being evaluated.

^cThe artificial vagina samples taken one hour after electroejaculation, had significantly less sperm ($P < 0.001$) than the electroejaculated samples taken after an artificial vagina collection.

TABLE III

MOTILITY RATINGS OF SAMPLES COLLECTED IN THE ARTIFICIAL VAGINA
AND BY ELECTROEJACULATION THAT WERE STORED FOR A 4-DAY
PERIOD AT 5° C

		Storage interval (days)					
		AV			EE		
		0	2	4	0	2	4
Mean motility rating for all samples stored	No. Samp.	79	79	79	96	96	96
	Degree ^a	2.5	2.0	1.3	2.8	2.3	1.9
	Percent ^b	52	47	34	65	56	53
Acceptable samples ^c (2-50)	No. Samp.	54	41	14	90	74	53 ^d
	Degree	2.9	2.6	2.1	2.9	2.4	2.3
	Percent	64	61	60	67	61	64

^a Degree of progressive motility (0-4).

^b Percent of motile sperm (0-100).

^c To be acceptable a sample had to have a degree of progressive motility rating of at least 2 and 50 percent or more motile sperm.

^d Significantly ($P < 0.02$) more electroejaculated than artificial vagina samples were still acceptable at the fourth day of storage.

TABLE IV

CORRELATION TABLE

Undiluted semen	Concen- tration	Degree motile	Percent motile	Volume	pH	Fructose	Rate of motility of stored semen	
							Initial	4-day
<u>Total Sperm</u>								
A.V.	.72**	.42**	.44**	.65**	-.05**	.01	.17**	.25**
E.E.	.97**	.48**	.32**	.10	-.34	.07	.32**	.43**
<u>Concentration</u>								
A.V.		.60**	.47**	-.43**	.18**	-.31**	.32**	.30**
E.E.		.46	.29	-.12	-.30	.06	.03	.46
<u>Motility (rate)</u>								
A.V.			.77**	.10	.10**	.05	.63**	.31**
E.E.			.72	.07	-.27	.04	.54	.62
<u>Motility (%)</u>								
A.V.				.26**	.06	.19	.62**	.41**
E.E.				.11	-.04	.09	.55**	.63**
<u>Volume</u>								
A.V.					.09**	.30*	-.09	.19
E.E.					-.22**	.09	-.06	.00
<u>pH</u>								
A.V.						-.46**	-.60**	-.03**
E.E.						-.58	-.36	-.48
<u>Fructose</u>								
A.V.							.06	.06
E.E.							.22	.07
<u>Motility (degree)</u>								
A.V.								.49**
E.E.								.57**

* $P < 0.05$ ** $P < 0.01$

CHAPTER V

DISCUSSION

Crombach et al (1956) reported that sperm concentration of electroejaculated samples could be greatly increased by teasing. This act of preparation, under normal conditions, removes considerable pre-sperm fluid which otherwise would be included in the semen. Since sperm concentration would have been reduced by this pre-sperm fluid, which might have been removed if teasing had been used, this fraction was discarded in this experiment.

Only two of the 12 bulls used in this study showed any indication of loss of libido following electroejaculation. These two bulls failed only one time to give a second ejaculate when collected with the artificial vagina during the first 8 weeks; however, during the second phase no second ejaculates were collected in the artificial vagina. With the exception of these two bulls there was no evidence of a detrimental effect on any bull's libido within one hour after being electroejaculated. Considering these facts and the consistency with which the other bulls worked, this experiment confirms other reports (Dziuk et al. 1954; Marden, 1954) that bulls do not form any unfavorable associations with electrical stimulation.

The marked reduction in total sperm collected in the artificial vagina samples, when preceded within an hour by electroejaculation, suggests temporary impairment of a bull's ability to ejaculate

naturally. The inhibiting effect is, however, of short duration since 3 and 4 days after being electroejaculated each bull produced a normal first ejaculate in the artificial vagina.

Rate of sperm motility and percent motile sperm in undiluted semen are greatly influenced by total sperm and concentration and are only slightly influenced by semen volume. Rate of sperm motility and percent motile sperm are highly correlated with each other, and each are about equally correlated with the other measurements performed. This indicates that one measure of motility is about as valid as the other, and that one gives almost as good an indication of sample quality as both.

Since the volume of semen obtained by electroejaculation was standardized to 6 ml. in this investigation, the variation in concentration was responsible for almost all of the variation in total sperm ($r = .97$). In samples collected in the artificial vagina, however, the variation in total sperm was about equally a result of the variation in volume and concentration. These two factors contribute independently to the increase in total sperm since they are negatively correlated with each other. The more concentrated samples and those with the greatest number of total sperm, when collected by either method, stored better on the average than samples that were less concentrated or contained less total sperm.

In the present study, semen from the artificial vagina had an average of 5.50 mg. of fructose per ml., while the electroejaculated semen contained an average of only 3.28 mg. per ml. The fructose con-

centration in the electroejaculated samples agree with the data of Freund and Murphree (1960) in which they reported a range of from 2.03 to 3.27 mg. of fructose per ml. of electroejaculated semen.

Lutwak-Mann and Rowson (1953) reported that fructose concentration in the pre-sperm fluid varied from 0.02 to 0.37 mg. per ml. and that it increased greatly in the sperm containing fraction (0.84 to 6.50 mg. per ml.); pH of the pre-sperm fluid varied from 7.8 to 8.0 as compared to 6.7 to 7.0 in the sperm containing fractions. Hess et al. (1960) reported that after removing the seminal vesicles, the mean semen fructose concentration of four bulls dropped from 2.56 to 0.10 mg. per ml. and the pH increased from 6.6 to 7.8. These data indicate that the seminal vesicles secrete the major portion of the fructose in bull semen. In the present investigation there was a negative correlation between pH and fructose concentration in the semen collected by both methods. These results and the results of others (Lutwak-Mann and Rowson, 1953; Hess et al., 1960) indicate that the high pH fluid comes from glands that do not secrete fructose.

The high pH fluids secreted by the bulls in this study reduced slightly the rate of sperm movement but did not affect the percent of sperm that were motile. In the electroejaculated semen pH was negatively correlated with all other measures of semen quality (table 4). These data support the findings of Herman and Swanson (1941) and Anderson (1942) that a high pH adversely affects semen quality. This is not true, however, when comparing the semen obtained by one method

of collection with the semen collected by another method. Within each method of collection the samples with the higher pH values were of lower quality; but even though the electroejaculated samples had a significantly higher pH (7.3 vs. 6.7), on the average they were not of inferior quality as compared to the artificial vagina samples. This is borne out by the fact that significantly more electroejaculated samples had an acceptable initial motility and maintained a higher rate of motility ($P < 0.02$) through 4 days of storage (table 3).

CHAPTER VI

SUMMARY

Electroejaculated semen is at least comparable in quality to semen collected in the artificial vagina. The volume of semen collected in the artificial vagina is less and the concentration higher than that obtained by electroejaculation; however, if a sufficient volume of semen is obtained by the electroejaculation method, essentially the same total number of sperm per ejaculate can be obtained by each method.

Samples with a higher pH are usually of inferior quality, when compared to other samples collected by the same method, but are not necessarily inferior to samples collected by a different method. The mean pH (7.3) for the electroejaculated semen samples was 0.6 of a unit higher than the samples collected in the artificial vagina but the electroejaculated samples maintained a higher degree of motility during 4 days of storage than those collected in the artificial vagina.

Since the electroejaculated samples had a higher pH and lower fructose concentration, it is postulated that the larger volume of fluid comes from the urethral glands with a slight increase from the seminal vesicles.

Semen was obtained more consistently by electroejaculation technique and it is adaptable to nearly all bulls with no selection on ability or desire to serve the artificial vagina.

BIBLIOGRAPHY

BIBLIOGRAPHY

- Anderson, James. 1942. The hydrogen-ion concentration of the semen of the bull. *J. Agri. Sci.* 32:298.
- Brady, D. E. and E. M. Gildow. 1939. Characteristics of ram semen as influenced by the method of collection. *Proc. Am. Soc. Animal Prod.* 250.
- Crombach, F. F. M. L., W. de Rover, and B. de Grott. 1956. The influence of preparation of dairy bulls on sperm production and fertility. *Int. Cong. Animal Repro. Sec.* Three p. 80.
- Dziuk, P. J., E. F. Graham, and W. E. Petersen. 1954. The technique of electroejaculation and its use in dairy bulls. *J. Dairy Sci.* 37:1035.
- Erb, R. E., F. H. Flerchinger, M. H. Ehlers, and F. X. Gassner. 1956. Metabolism of bull semen. II. Fructolysis relationships with sperm concentration and fertility. *J. Dairy Sci.* 39:326.
- Freund, M. and R. L. Murphree. 1960. Effect of whole-body gamma-irradiation on the characteristics and metabolism of bull semen during the early post-irradiation period. *J. Dairy Sci.* 8:1130.
- Gunn, R. M. C. 1936. Fertility in sheep; Artificial production of seminal ejaculation and the characters of the spermatozoa contained therein. *Coun. Sci. and Ind. Res. Aust. Bul.* 94.
- Herman, H. A. and Eric W. Swanson. 1941. Variations in dairy bull semen with respect to its use in artificial insemination. *Mo. Agri. Exp. Stat. Res. Bul.* 326.
- Hess, E. A., T. M. Ludwick, R. C. Martig, and F. Ely. 1960. Influence of seminal vesiculectomy on certain physical and biochemical properties of bovine semen. *J. Dairy Sci.* 43:256.
- Hill, H. J., F. S. Scott, N. Homan, and F. X. Gassner. 1956. Electroejaculation in the bull. *J. Am. Vet. Med. Assn.* 128:375.
- Lambert, W. V. and F. F. McKenzie. 1940. Artificial insemination in livestock breeding. *U. S. D. A. Cir.* 567.
- Lutwak-Mann, C. and L. E. A. Rowson. 1953. The chemical composition of the pre-sperm fraction of bull ejaculate obtained by electrical stimulation. *J. Agr. Sci.* 43:131.

- Manton, V. J. A. 1956. The fertility of bovine semen collected by electroejaculation. *Vet. Rec.* 68:1015.
- Marden, W. G. R. 1954. New advances in the electroejaculation of the bull. *J. Dairy Sci.* 37:556.
- Parish, N. R. 1958. An electroejaculation technique for the collection of semen from bulls. (Part II of M. S. Thesis. Department of Animal Husbandry) University of Tennessee, Knoxville, Tennessee.
- Rice, V. A., F. N. Andrews, E. J. Warwick, and J. E. Legates. 1957. *Breeding and Improvement of Farm Animals* (5th ed). McGraw-Hill Book Company, Inc., New York.
- Roe, J. R. 1934. A colorimetric method for the determination of fructose in blood and urine. *J. Biol. Chem.* 107:15.
- Rowson, L. E. and M. I. Murdoch. 1954. Electrical ejaculation in the bull. *Vet. Rec.* 66:326.
- Snedecor, G. W. 1956. *Statistical Methods*. Iowa State College Press, Ames. Iowa.
- Terrill, C. E. 1940. Comparison of ram semen collection obtained by three different methods for artificial insemination. *Proc. Am. Soc. Animal Prod.* p. 201.
- Winters, L. M., R. E. Comstock, C. L. Cole, W. W. Green, and J. J. Bulik. 1938. *Artificial insemination of farm animals*. Minn. Agr. Exp. Sta. Bul. 336.
- Willett, E. L. 1956. Development in the physiology of reproduction of dairy cattle and in artificial insemination. *J. Dairy Sci.* 39:695.