May 12, 1965

To the Graduate Council:

I am submitting herewith a thesis written by Pin Kwen Chung entitled "Effects of Management and Nutrition on Semen Quality of Beef Bulls." 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.

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Major Professor

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EFFECTS OF MANAGEMENT AND NUTRITION ON SEMEN QUALITY OF BEEF BULLS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Pin Kwen Chung

June 1965

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CHAPTER I

INTRODUCTION

The calf crop represents the only source of animal income from the beef cow herd. Infertility or sterility in the bull can cause either reduction or complete loss of income from the herd for a given year. Thus two factors become important:

1. A study of those semen qualities which may predict the probability of a bull's fertility.
2. The avoidance of nutritional and management factors which might decrease bull fertility.

Semen quality and characteristics have, in a number of studies, shown varying degrees of correlation to fertility. Since these characteristics will vary even between ejaculates from the same bull, they cannot be expected to give more than an estimation of the animal's fertility. The actual fertility can be proved only by breeding trials. Nevertheless, the study of these semen qualities and characteristics currently form the only basis for rapid prediction of the potential fertility of the bull.

It also follows that a study of the influences which different types of management or nutrition may have on bull semen quality allow a rapid method of estimating the effect that management or nutrition might have upon the fertility of the bull.

At the Tennessee Experiment Station, several management-nutrition regimes have been developed for beef bulls. It was the purpose of this study to determine the effects of these nutrition regimes on the semen collected by electroejaculation from Hereford, Polled Hereford, and Angus bulls. Conception rate studies were not made.

CHAPTER II

REVIEW OF LITERATURE

The major authors who first reported on studies in the field of fertility of the bovine male appear to be Williams in 1920 and Williams and Savage, as cited by Salisbury and Van Demark, in 1925. A large number of papers have followed. Although each of these were reviewed only the pertinent ones will be recorded herein. It is the purpose of this literature review to examine (1) the pioneering work of Williams and of Savage; (2) studies of the relationship, singly or together, of volume of semen ejaculated, concentration of spermatozoa, total number of spermatozoa, spermatozoa motility, percentage of live spermatozoa, total number of live spermatozoa, and morphological abnormalities of spermatozoa upon either fertility estimates or actual breeding performance of bulls; and (3) research dealing with the effects of various levels of nutrition upon the development and breeding soundness of bulls.

Williams (1920) concluded that semen fertility depends not only upon physical properties of semen but upon the number of spermatozoa which are motile, the degree of spermatozoa motility, degree of oligospermia, and the percentage of imperfect spermatozoa, either deformed or immature. Williams and Savage (1925), as cited by Salisbury and Van Demark (1961), found that when the percentage of abnormal spermatozoa reached 18 per cent or more, the fertility of the bull was impaired.

Laing (1945) found that on the basis of the density of the semen, the total number of spermatozoa ejaculated, the motility, and change in pH of the semen upon incubation at 37°C. the animals could be divided into a "higher fertility group" with services per conception of slightly more than 1.0 to about 3.5, and a "lower fertility group" with services per conception from above 3.5 to infinity. Totally sterile animals could also be detected.

Leuchtenberger et al. (1956) show clearly that not all morphologically normal spermatozoa are physiologically normal. The size of the nucleus of morphologically normal spermatozoa from either fertile or infertile bulls does not differ significantly. However, the deoxyribonucleic acid (DNA) content of spermatozoa from infertile bulls is significantly less and more variable than is the DNA content of spermatozoa from fertile bulls.

The relationship of the methylene-blue test to other criteria of measuring semen quality was shown by Van Demark, Mercier and Salisbury (1945). The methylene-blue test was not significantly correlated with the proportion of morphologically abnormal spermatozoa, but highly significant correlations were shown between the methylene-blue reduction time and volume of the ejaculate, spermatozoa concentration, initial spermatozoa motility, and initial pH of fresh semen.

Interclass correlation coefficients between certain semen characteristics were obtained by Lasley (1944). A highly significant correlation was found to exist between live and motile spermatozoa ($r = +0.615$ for 167 semen samples) and between live and progressively-

motile spermatozoa ($r = +0.696$ for 158 semen samples). Percentage of motile spermatozoa was also correlated with fertility by actual breeding tests. In those semen samples which contained fewer than 40 per cent motile spermatozoa, the average fertility was 54 per cent as compared to 69.4 per cent in those which contained 61 to 70 per cent motile spermatozoa.

Correlations of semen characteristics and fertility were also shown by Bratton et al. (1956) by actual breeding studies. Statistically significant linear correlations were obtained between the fertility of bovine ejaculates extended 1:100 and 1:300 and the concentration of spermatozoa in the ejaculates, the per cent of motile spermatozoa, the methylene-blue reduction time, the pH, the per cent of unstained live spermatozoa, the per cent of morphologically abnormal spermatozoa, the liveability of spermatozoa at 5°C., and the oxygen uptake of spermatozoa at 37.5°C. The average 60 to 90 day non-returns to 7,168 first services with semen extended 1:100 was 56.6 per cent and the per cent non-returns for 5,785 first services with semen extended 1:300 was 52.8 per cent. A 3.6 per cent citrate-sulfanilamide-yolk extender was used.

Bishop (1955) reported that the fertility of tested samples of semen measured by conception rate was significantly correlated ($P < 0.05$) to methylene-blue reduction time and resistance of spermatozoa to cold shock and was inversely related to the proportion of dead spermatozoa, ejaculate volume, and age of bull. Fertility was not significantly related to total sperm concentration, the concentration of living spermatozoa, the proportion of morphologically abnormal spermatozoa,

motility rating, number of living spermatozoa inseminated, or the dilution ratio of samples used for insemination. Seven breeds--Friesian (26), Shorthorn (23), Ayrshire (6), Guernsey (5), Jersey (6), Hereford (6), Red poll (4)--were used in Bishop's studies. The significant differences ($P < 0.001$) in fertility among breeds were not related to differences in the semen characteristics examined.

Much higher associations between semen characteristics and fertility have been published. Lasley and Bogart (1943) reported correlations of 0.909 between ejaculate volume and fertility, of 0.708 between concentration and fertility, and of 0.831 between resistance to cold shock in egg-yolk buffer and fertility.

Branton et al. (1951) reported that concentration of spermatozoa in individual ejaculates was highly significantly correlated with fertility ($r = +0.2834$) as shown by breeding tests. When the means for the individual bulls were used for the analysis, a more highly significant correlation coefficient ($r = +0.5678$) was obtained. The numbers of motile spermatozoa per milliliter of extended semen or per insemination were highly significantly correlated with fertility ($r = +0.4912$), the within-bull correlation coefficient was highly significant ($r = +0.3637$), but the between-bull correlation coefficient was not significant ($r = +0.0990$). Highly significant negative correlation coefficients of -0.6675, -0.3147, -0.3790, and -0.3415 were obtained between methylene-blue reduction time and concentration, initial motility, motility after cold shock, and motility at the end of 3 days of storage at 40°F., respectively. The partial correlation coefficient between methylene-blue

reduction time and initial motility, independent of concentration was -0.5003, as compared to that of -0.7339 between the same methylene-blue reduction time and concentration independent of initial motility. Branton and his coworkers (1951) concluded that no single rapid test had been devised that would accurately and reliably measure or predict semen quality and fertility. They recommended the following combination of tests and minimum standards for evaluating bull semen: (a) an initial progressive motility of at least 50 per cent; (b) a concentration, as determined by hemocytometer, photoelectric colorimeter, or opacity standard method, of at least 500 million spermatozoa per milliliter; (c) the modified methylene-blue reduction time using 300 million spermatozoa per milliliter preferably should not exceed seven minutes, and definitely should not exceed nine minutes.

An enlightening work in predicting fertility by measuring semen quality was published by Buchner, Willett and Bayley in 1954. Three trials comparing correlations between non-return rates and tests or combinations of tests for measuring quality of semen were completed and the results studied by simple correlation and by multiple-correlation techniques. The linear correlation coefficients were reported on the basis of the total correlation among ejaculates, the among-bull correlations, and those among ejaculates within bulls. The best single estimates for predicting differences in fertility of bulls, using the average semen quality of a number of ejaculates from each bull, were progressive motility after incubation for 16 hours at 38°C. in yolk citrate plus antibacterial agents ($r = 0.90$); total motility after the

same treatment (r = 0.86); and methylene-blue reduction time (r = 0.72). Such results indicated that from 50 to 81 per cent of the fertility variance among bulls was associated with measurable variances in these semen-quality tests. Throughout these studies the among-bull correlations were, in most instances, higher than the total or within-bull correlations.

Linear correlation coefficients on an among-bull basis and a multiple correlation between fertility and concentration, motility, and methylene-blue reduction time were calculated by Salisbury (1955) for two periods of time (1943, 1945). The single correlation coefficients between fertility and concentration (0.65, 0.40), fertility and motility (0.67, 0.34), and fertility and methylene-blue reduction time (-0.72, -0.51) were significant. The multiple correlation between fertility and volume, concentration, motility (per cent), and methylene-blue reduction time were also significant.

The fact that fertility appears to decrease as the number of morphologically abnormal spermatozoa increases has been shown by several workers. Rollinson (1951) divided bulls into five groups--fertile bulls, infertile bulls, sterile bulls, congenitally sterile bulls, and impotent bulls--on the basis of semen characteristics. Actual fertility rates of the infertile bulls ranged from 0 to 65 per cent as compared with 50 to 92 per cent for the fertile bulls. Fertile semen contained the least number of abnormal spermatozoa. Increasing numbers of abnormal forms were found through two groups of infertile bulls (21 to 41 per cent and over 41 per cent). Bulls in the 4 per cent to 20 per cent groups and acquired sterility groups did not continue this trend, although the

percentages of abnormals in those groups were above fertile bulls. Congenitally sterile bulls showed the highest percentage of abnormal spermatozoa.

Wiltbank (1963) found that bulls having 5 to 9 per cent abnormal spermatozoa had a 70 per cent conception rate, bulls with 10 to 18 per cent had a 50 per cent conception rate, and those with 20 to 28 per cent had a 52 per cent conception rate. Bulls with more than 50 per cent abnormal spermatozoa had a conception rate of 36 per cent.

Maddox et al. (1958) have attempted to predict bull fertility by examining density of semen, motility, rate of movement, and morphology of spermatozoa. They reported that in a state-wide study in Texas approximately 7 per cent of the 1,369 beef bulls evaluated were infertile, 9 per cent were questionable, and 84 per cent were satisfactory as measured on the basis of semen evaluation but without actual breeding trials.

A total of 10,940 beef bulls were examined by Hill, Faulkner and Carroll (1958) and by Carroll, Ball and Scott (1963) for breeding soundness by physical examination and semen quality evaluation only. Semen collections were obtained with the electroejaculator. Motility, concentration, morphologic characteristics, and percentage of live cells were graded separately. Morphologic character of sperm cells had the greatest correlation to the final semen quality classification. The results indicated that 82.9 per cent of the bulls were satisfactory, 11.8 per cent questionable, 4.9 per cent unsatisfactory, and 0.3 per cent could not be classified.

The effect of nutrient intake, especially total digestible nutrients (TDN), on reproductive performance of dairy bulls has been under rather intensive investigation. Asdell (1949) indicated that on very low planes of nutrition sexual desire or libido may diminish, whereas, on a high plane, excessive weight may cause the male to become sluggish and cause development of fat above the testes in quantities sufficient to interfere with spermatogenesis. Olsen et al. (1950) stated that bulls fed a subnormal ration produce semen of poorer quality based upon density, total number of spermatozoa per ejaculate, motility, methylene-blue reduction tests, and per cent of abnormal spermatozoa and a smaller volume of ejaculate than bulls fed normal or above normal rations on the basis of TDN.

The relationship between plane of nutrition and onset of semen production was studied by Flipse and Almquist (1961). The average age of bulls at the onset of semen production was 61, 45, 41, and 44 weeks, respectively, for rations delivering 70, 100, 115, and 130 per cent of the National Research Council's recommended TDN requirements. Comparable figures for body weight at the onset of semen production were 523, 643, 675, and 784 pounds. The average motile spermatozoa output per ejaculate at 57 to 80 weeks of age was 1.01, 1.10, 2.21, and 2.30 billion for the bulls receiving 70, 100, 115, and 130 per cent rations, respectively. After 112 weeks of age, there was no difference in output of motile spermatozoa per ejaculate associated with level of energy intake. Between 150 and 208 weeks of age, the bulls in the group of 130 per cent rations became much slower in sexual reaction time than bulls in other groups.

Van Demark, Mauger and Fritz (1964a and 1964b) have extensively studied causes and prevention of reproductive failures in dairy bulls. Specifically, their experiments dealt with the growth, sexual development, and semen production of young bulls. Two groups of Holstein bulls were fed from 8 weeks to 46 months of age on 60 per cent (underfed) or 100 per cent (control) of the TDN intake recommended in Morrison's feeding standards. From 46 to 58 months of age the feeding regime of the two groups was reversed. Underfeeding greatly impaired body, endocrine gland, and reproductive tract growth, especially during the early period of rapid growth. Puberty was delayed in the underfed group. Body weight and testes size in the underfed bulls closely followed the percentage difference in TDN intake until the rations were switched. Following a change in rations the bulls originally underfed gained in body weight to near normal, but no appreciable recovery occurred in bone, endocrine, or testes growth. Reducing the rations of the normally fed bulls to 60 per cent of the recommended TDN intake caused slight reductions in body weight, but little or no change was observed in the other characteristics measured. The production of semen in appreciable quantities following puberty was delayed 8 months or more in the underfed group. After 20 to 24 months of age the percentage reduction between the groups for body size, testes size, and semen production were quite similar. Underfeeding during the period of 8 weeks to 46 months of age caused the bulls to be slower in replenishing depleted supplies of sperm reserves. Although from 20 to 44 months of age the control bulls produced approximately 43, 73, 98,

and 125 per cent as many spermatozoa at invervals of 1, 4, 7, and 13 days after a previous partial-depletive collection, the underfed bulls produced only 27, 56, 75, and 86 per cent as many spermatozoa at the respective intervals.

CHAPTER III

PROCEDURES

Animals

The 48 weanling bull calves were obtained from various branch experiment stations in the state and two private breeders and were brought to the Main Experiment Station at Knoxville in November of 1963. There were 27 Angus, 11 Herefords, and 10 Polled Herefords. The bulls were divided into groups of 4 to 5 bulls and placed on three different levels of feed for wintering. In March of 1964 the bulls were assigned to three different feeding and management regimes. Following the pasture period, the bulls were again reassigned to two different feeding regimes, most of them on a ration designed to measure their ability to perform in the feed lot. Type scores were made and weights were taken at the beginning and the end of each feeding period. Semen was collected from the bulls at the end of each of the three treatment periods. The treatment groups are presented in Table I and discussed in the following paragraphs.

Winter Period

The treatments during this period were identical to those described by Hobbs and Anderson (1962) and Knapka (1963) and designated as follows:

A - Bulls in this group were fed 2 lbs. of alfalfa hay, 10 lbs. of corn silage and 10 lbs. of concentrates per head daily. The concentrate mixture consisted of 61 per cent ground shelled corn, 8 per cent

TABLE I

MANAGEMENT REGIMES AND WEIGHT GAINS

Period and Dates	Days in Period	No. Animals	Treatment	Average Daily Gain (lb.)	Average Body Weight at the End of Each Period (lb.)
Winter					
11-14-63 to 3-26-64	133	5	A	2.898	977.4
11-14-63 to 3-26-64	133	33	B	2.432	887.3
11-14-63 to 3-26-64	133	10	C	2.032	800.3
Pasture					
3-27-64 to 6-4-64	69	10	DA	--	1058.3
3-27-64 to 7-12-64	109	22	BA	1.331	950.9
4-9-64 to 7-20-64	102	16	HS	--	968.9
Fall					
7-13-64 to 10-20-64	99	22	Full Feed)		
7-20-64 to 10-20-64	92	5	Full Feed)	3.238	1284.4
9-19-64 to 10-20-64	32	11	Full Feed)		
6-5-64 to 10-20-64	137	8*	Maintenance Groups**	2.715	1168.1

*Two animals removed between the pasture and fall periods.

**Bulls were fed 1 lb. of concentrates per 100 lbs. body weight. The concentrates consisted of ground shelled corn and cottonseed meal in the ratio of 8:1. The bulls were fed on orchard grass-Ladino clover pasture.

cottonseed meal (CSM), 2 per cent animal fat, and 3 per cent molasses per head per day.

B - Bulls in this group were full-fed corn silage, and also fed 2 lbs. of alfalfa hay, 4 lbs. of ground shelled corn, and 1.5 lbs. of protein supplement per head per day.

C - The bulls in this group were fed similarly to the B group except that they received only 1 lb. of ground shelled corn per head per day.

Pasture Period

The pasture treatments were as follows:

DA - Received a self-fed ration of concentrates (the same as in the winter period) and hay in the ratio of 3:1.

BA - Received orchard grass-Ladino clover pasture plus 1 lb. of a 4:1 (ground shelled corn:CMS) concentrate per 100 lbs. of body weight. These bulls had previously been in the B and C groups in the winter period.

HS - Bulls selected for herd sire duty did not receive any supplemental feed while they were breeding cows. These bulls had been in A and B groups during the winter period.

Fall Period

Bulls in groups BA and HS were gradually placed on a full-fed, self-fed ration of concentrates (the same as in the winter period) and hay in the ratio of 3:1. Bulls which were in the DA group during the pasture period were fed 1 lb. of concentrates per 100 lb. of body weight. The concentrate consisted of ground shelled corn and CSM in the ratio of 8:1. The bulls were fed on an orchard grass-Ladino clover pasture.

Trace mineralized salt, dicalcium phosphate, and water were supplied free choice throughout the experiment.

The average daily gains and body weights of the bulls for each group during each period are shown in Table I (page 14).

Methods of Collection

The finger electrode method described by Austin (1961) was used to collect the electroejaculated semen. A regular artificial vagina rubber cone and two graduated centrifuge tubes were used in each collection. Usually a variable amount of clear fluid preceded the semen-containing fraction during electroejaculation. This pre-spermatozoa fraction was discarded during this experiment.

Methods of Evaluation

Immediately after each semen collection, the color and volume of each sample was recorded. The initial progressive spermatozoa motility was microscopically evaluated. A sample of each collection was reserved and later the spermatozoa were counted in a hemocytometer counting chamber. The ratio of live-dead spermatozoa was determined by counting spermatozoa which had been stained by the Eosin-Fast Green staining technique. These same stained cells were also examined for morphological forms.

The detailed procedures for each of these evaluations were as follows:

Subjective motility estimates were made according to modifications of the techniques used by Larson (1960). A drop of pure semen contained

in a loop of wire measuring 0.2 mm. in diameter was placed on a warm glass slide (thermostatically maintained at 37-38°C. in a microscope stage incubator) along with an equal amount of 2.9 per cent sodium citrate solution (2.0 g. $Na_3C_6H_5O_7 \cdot 2H_2O$ in 100 ml. of glass-distilled water). The diluted semen was covered with a 22 mm. square and 0.17 mm. thick cover glass and examined under the low power (150 X) of a compound microscope immediately after collection. A single loop was used throughout the experiment to achieve uniform samples because the type of movement observed is thought to be influenced by the size and depth of the droplet examined. The percentages of motile spermatozoa were recorded by two technicians working independently and the averages used.

Concentration of spermatozoa was estimated in the hemocytometer useing techniques modified from Salisbury (1943), Herman and Madden (1963), and Smith and Mayer (1955). The detailed procedures were as follows:

1. The vial of semen was slowly inverted ten times for thorough mixing.
2. Semen in the amount of 0.05 mm. was drawn into a standard red cell dilution pipette.
3. A small bubble of air was drawn into the pipette and the end of the pipette was wiped clean.
4. The pipette was filled to 1.01 mm. with diluting fluid (2 g. NaCl and 1 g. Eosin B added to 100 ml. glass-distilled water).
5. The pipette was agitated by bumping against the heel of the hand for at least 2 minutes until thoroughly mixed.

6. The first several drops were discarded to remove the incompletely mixed solution.
7. The cover glass was placed over the ruled field of the hemocytometer slide and a drop of the solution was allowed to flow under the cover glass.
8. The spermatozoa were allowed to settle for at least 1 minute.
9. The count was made using a magnification of 150 X; when the concentration was extremely high, a magnification of 600 X was used.
10. Spermatozoa in a total of 80 small squares (1/20 x 1/20 mm.), as used in counting erythrocytes, were counted. When the total number of spermatozoa thus counted was less than 20, the 400 squares of the whole field were examined.
11. The number of spermatozoa counted multiplied by 10,000 equals the concentration of spermatozoa per cubic millimeter of semen. When the spermatozoa were counted in 400 squares, the number was multiplied by 2000.

The differentiation of live from dead spermatozoa was made by modification of the methods of Mayer, Squiers and Bogart (1947) and Herman and Madden (1963). Detailed procedures follow:

1. One drop of stain (0.8 g. Eosin B and 2 g. of Fast Green in 3 per cent sodium citrate) from a glass rod was placed on a clean glass slide.
2. One drop of semen was placed in the stain and thoroughly mixed with the stain.

3. The edge of a second slide was drawn up to the mixture at a 30° angle and quickly pushed away thus causing a thin film of the mixture to form over the first slide.
4. The slide was rapidly dried over a hot plate.
5. The oil immersion objective (1000 X) of the microscope was used in counting the spermatozoa.
6. A total of 100 spermatozoa were counted and the live spermatozoa percentage computed. Those spermatozoa which remained unstained were considered to have been alive and those spermatozoa which were stained, if only in part, were considered dead.

Various kinds of morphology of spermatozoa were observed by applying modifications of the methods of Salisbury and Mercier (1945), Herman and Madden (1963), Swanson and Boyd (1962), and Rollinson (1951). A total of 100 spermatozoa were counted from the stained slide used in the live-dead spermatozoa dtermination. Morphologically abnormal spermatozoa were counted and the percentage recorded. Abnormalities observed and the criteria used in evaluating them were the following:

1. Bent tail--The tail of the spermatozoon is bent anteriorily to a greater or lesser extent but not touching itself.
2. Looped tail--The tail of the spermatozoon is looped back upon itself and may have a protoplasmic droplet enclosed in the loop.
3. Coiled tail--The tail of the spermatozoon is coiled around itself in a spring-like manner.

4. Broken head--The tail of the spermatozoon has been separated from the head.

5. Protoplasmic droplet--The tail of the spermatozoon has a droplet of protoplasm encasing it.

The method of least squares for analysis of data with disproportionate subclass numbers (Harvey, 1960; Steel and Torrie, 1960) utilizing the following mixed linear model was considered to be appropriate for analysis of semen characteristics data during the winter period:

$$Y_{ijk} = u + B_i + T_j + (BT)_{ij} + b_1(W_{ijk} - \overline{W}) + b_2(A_{ijk} - \overline{A}) + e_{ijk}$$

Y_{ijk} = the semen characteristics of the k-th bull on the j-th treatment.

u = overall mean for Y_{ijk}.

B_i = the effect of i-th breed.

T_j = the effect of j-th treatment.

$(BT)_{ij}$ = interaction effects.

b_1 = regression of semen characteristic on initial weight.

$(W_{ijk} - \overline{W})$ = the initial weight for k-th bull on j-th treatment and i-th bull, as a deviation from the average initial weight.

b_2 = regression of semen characteristic on initial age.

$(A_{ijk} - \overline{A})$ = the initial age for k-th bull on the j-th treatment and i-th breed, as a deviation from the average initial age.

e_{ijk} = the random error.

The same model that was used in the winter period was applied in the analysis of the data for the pasture and fall periods. Also, the winter treatment (WT) effect and the interaction between breed and winter treatment (B x WT) were considered in the analysis of the data.

Interclass correlation coefficients between variables were computed on a within-treatment and within-breed basis for each of the three periods.

CHAPTER IV

RESULTS AND DISCUSSION

Two hundred and eighty-four semen samples were collected from the 27 Angus, 10 Hereford, and 11 Polled Hereford bulls. There were 96 samples in the winter period, 96 in the pasture period, and 92 in the fall period. Two animals were eliminated because one was sold after the pasture period and the other changed treatments twice during the fall period.

The various semen characteristics for the three breeds of bulls on three levels of management were studied. These characteristics or qualities included semen volume, concentration of spermatozoa per milliliter, and total number of spermatozoa, motility, percentages of live and dead spermatozoa, total number of live spermatozoa, and percentage of total abnormal spermatozoa. The spermatozoa classified as abnormal included those with looped, coiled, and bent tails; broken heads; and those with protoplasmic droplets. The arithmetic means for various semen characteristics for each period are summarized in Table II, Table III, and Table IV. The fact that some of the means showed large differences without significance was not unexpected. Some of these differences resulted from uncontrolled environmental and genetic factors. Large amounts of interbreed and intrabreed variations existed. The data are discussed in relation to the results obtained from the analysis of variance and according to experimental phase in the following paragraphs.

TABLE II

THE ARITHMETIC MEANS AND THE EFFECTS OF VARIOUS FACTORS ON SEMEN CHARACTERISTICS AT THE END OF WINTER

	Volume (ml.)	Conc. (billions spermatozoa per ml.)	Total Spermatozoa (in billions)	Motility %	Live Sperma-tozoa %	Total Live Spermatozoa (in billions)	Total Abnormal Spermatozoa %
Arithmetic mean	5.806	0.237	1.376	44.20	39.57	0.541	34.72
Breed							
Angus	-0.46	-0.01	-0.03	1.52	12.71	0.09	-0.48
Hereford	0.32	-0.08	-0.05	0.64	-15.91	-0.29	4.87
Polled H	0.14	0.09	0.08	-2.16	3.20	0.20	-4.39
Treatment							
A	-0.72	-0.05	-0.47	1.50	6.59	-0.27	-1.43
B	0.13	-0.01	-0.05	-1.20	-1.44	-0.07	-2.12
C	0.59	0.06	0.52	-0.30	5.15	0.34	3.55
Breed-Treatment							
Angus-B	-0.12	0.005	0.17	1.31	18.17	0.30	-11.35
Angus-C	0.12	-0.005	-0.17	-1.31	-18.17	-0.30	11.35
Hereford-A	0.15	0.060	0.41	1.82	-8.82	0.16	-2.63
Hereford-B	-0.26	0.090	0.42	0.94	4.70	0.13	-0.08
Hereford-C	0.11	-0.150	-0.83	-2.76	4.12	-0.29	2.71
Polled H-A	-0.15	-0.060	-0.41	-1.82	8.82	-0.16	2.63
Polled H-B	0.38	-0.095	-0.59	-2.25	-22.77	-0.43	11.43
Polled H-C	-0.23	0.155	1.00	4.07	13.95	0.59	-14.06

TABLE II (CONTINUED)

	Volume (ml.)	Conc. (billions spermatozoa per ml.)	Total Spermatozoa (in billions)	Motility %	Live Sperma-tozoa %	Total Live Spermatozoa (in billions)	Total Abnormal Spermatozoa %
By weight	0.005	0.0005	0.004	-0.02	-0.05	0.002	0.033
By age	0.015	0.0003	0.004	0.02	0.22	0.004	-0.15

TABLE III

THE ARITHMETIC MEANS AND THE EFFECTS OF VARIOUS FACTORS ON SEMEN CHARACTERISTICS AT THE END OF PASTURE

	Volume (ml.)	Conc. (billions spermatozoa per ml.)	Total Spermatozoa (in billions)	Motility %	Live Sperma-tozoa %	Total Live Spermatozoa (in billions)	Total Abnormal Spermatozoa %
Arithmetic means	8.302	0.309	2.543	46.68	54.71	1.423	27.01
Breed							
Angus	-2.57	0.03	-0.40	-1.22	4.25	-0.27	0.97
Hereford	1.98	-0.06	-0.27	4.34	-4.43	-0.05	-0.44
Polled H	0.59	0.03	0.67	-3.12	0.18	0.32	-0.53
Winter treatment							
A	-2.24	0.10	0.16	-3.45	-4.89	0.08	9.13
B	-0.10	0.01	0.22	4.15	5.00	0.21	-0.98
C	2.34	-0.11	-0.38	-0.70	-0.11	-0.29	-8.15
Summer treatment							
DA	-3.93[a,b]	0.18	-0.005	-8.03	-13.23	0.08	3.61
BB	1.84[a]	0.04	0.940	7.10	6.37	0.56	-1.52
HS	2.09[b]	-0.22	-0.935	0.93	6.86	-0.64	-2.09
Breed-Winter treat.							
Angus-B	-1.62	0.02	0.18	-1.84	3.15	0.32	-5.04
Angus-C	1.62	-0.02	-0.18	1.84	-3.15	-0.32	5.04
Hereford-A	2.48	0.02	0.84	5.24	2.10	0.59	-6.99
Hereford-B	-1.51	0.10	0.75	0.55	5.41	0.54	1.37
Hereford-C	-0.97	-0.12	-1.59	-5.79	-7.51	-1.13	5.62

TABLE III (CONTINUED)

	Volume (ml.)	Conc. (billions spermatozoa per ml.)	Total Spermatozoa (in billions)	Motility %	Live Sperma-tozoa %	Total Live Spermatozoa (in billions)	Total Abnormal Spermatozoa %
Polled H-A	-2.48	-0.02	-0.84	-5.24	-2.10	-0.59	6.99
Polled H-B	3.13	-0.12	-0.93	1.29	-8.56	-0.86	3.67
Polled H-C	-0.65	0.14	1.77	3.95	10.66	1.45	-10.66
Breed-Summer treat.							
Angus-DA	0.46	-0.13	-0.76	-2.26	-6.33	-0.74	6.05
Angus-BA	-0.38	0.02	-0.18	4.29	10.02	0.47	1.47
Angus-HS	-0.08	0.11	0.94	-2.03	-3.69	0.27	-7.52
Hereford-DA	0.62	0.05	0.61	1.90	1.58	0.46	-2.38
Hereford-BA	-1.28	-0.02	-0.32	0.79	0.28	-0.36	-4.67
Hereford-HS	0.66	-0.03	-0.29	-2.69	-1.86	-0.10	7.05
Polled H-DA	-1.08	0.08	0.15	0.36	4.75	0.28	-3.67
Polled H-BA	1.66	0.00	0.50	-5.08	-10.30	-0.11	3.20
Polled H-HS	-0.58	-0.08	-0.65	-4.72	5.55	-0.17	0.47
By weight	0.007	0.00002	0.001	0.04	0.01	-0.0001	-0.004
By age	-0.014	0.002	0.012	-0.13	-0.08	0.006	0.099

[a,b]Means superscripted with the same letter are significantly different ($P < 0.05$), others not significantly different ($P > 0.05$).

TABLE IV

THE ARITHMETIC MEANS AND THE EFFECTS OF VARIOUS FACTORS ON SEMEN CHARACTERISTICS AT THE END OF FALL

	Volume (ml.)	Conc. (billions spermatozoa per ml.)	Total Spermatozoa (in billions)	Motility %	Live Spermatozoa %	Total Live Spermatozoa (in billions)	Total Abnormal Spermatozoa %
Arithmetic means	10.930	0.340	3.718	46.16	65.71	2.440	29.70
Breed							
Angus	0.036	0.02	0.17	2.32	5.77[a]	0.09	-0.96
Hereford	-0.133	-0.02	-0.41	5.27	14.21[b]	0.13	-0.50
Polled H	0.097	0.00	0.24	-7.59	-19.98[a,b]	-0.22	1.40
Winter treatment							
A	-1.96	-0.01	-0.53	11.42	5.04	-0.19	1.18
B	1.23	0.04	0.92	2.53	-0.01	0.61	-1.76
C	0.73	-0.03	-0.39	-13.95	-5.03	-0.42	0.58
Summer treatment							
BA	-0.90	0.10	0.83	0.89	-6.36	0.56	-15.31
DB	0.63	-0.05	-0.30	3.34	0.42	-0.44	9.82
HS	0.27	-0.05	-0.53	-4.23	5.94	-0.12	5.49
Breed-Winter treat.							
Angus-B	0.65	0.04	0.54	-5.76	-1.52	0.11	3.69
Angus-C	-0.65	-0.04	-0.54	5.76	1.52	-0.11	-3.69
Hereford-A	0.92	0.02	0.75	0.08	-1.07	0.45	0.36
Hereford-B	-0.25	0.01	-0.02	3.86	2.24	0.16	-2.49
Hereford-C	-0.67	-0.03	-0.73	-3.94	-1.17	-0.61	2.13

TABLE IV (CONTINUED)

	Volume (ml.)	Conc. (billions spermatozoa per ml.)	Total Spermatozoa (in billions)	Motility %	Live Sperma-tozoa %	Total Live Spermatozoa (in billions)	Total Abnormal Spermatozoa %
Polled H-A	-0.92	-0.02	-0.75	-0.08	1.07	-0.45	-0.36
Polled H-B	-0.40	-0.05	-0.52	1.90	-0.72	-0.27	1.20
Polled H-C	1.32	0.07	1.27	-1.82	-0.35	0.72	1.56
Breed-Summer treat.							
Angus-DA	1.16	-0.17	-1.60	-1.87	8.71	-0.81	7.18
Angus-BA	-0.26	0.19	1.71	12.52	14.67	1.44	-6.23
Angus-HS	-0.90	-0.02	-0.11	-10.65	-23.38	-0.63	-0.95
Hereford-DA	1.07	0.20	2.84	-1.56	-7.42	1.55	-1.59
Hereford-BA	-0.74	-0.18	-2.30	-7.84	-3.75	-1.52	11.16
Hereford-HS	-0.33	-0.02	-0.54	9.40	11.17	-0.03	-9.57
Polled H-DA	-2.23	-0.03	-1.24	3.43	-1.29	-0.74	-5.59
Polled H-BA	1.00	-0.01	0.59	-4.68	-10.92	0.08	-4.93
Polled H-HS	1.23	0.04	0.65	1.25	12.21	0.66	10.52
By weight	0.009	0.0005	0.009	0.05	0.02	0.007	-0.05
By age	-0.019	0.002	0.017	0.02	-0.11	0.002	-0.006

^{a,b}Means superscripted with the same letter are significantly different ($P < 0.05$), others are not significantly different ($P > 0.05$).

In studying the results of this experiment, the reader should note the following factors:

1. All evaluations of semen quality are based on average values of that factor for each group of bulls.
2. The biological significance of the protoplasmic droplet and the bent tail of a sperm cell have not been clearly established.
3. In this study changes in sperm morphology, semen volume, and percentages of live spermatozoa were great enough within certain groups to cause statistical significance but were not great enough to imply lowered fertility for that group.

Semen Collected at the End of the Winter Period

For all semen characteristics studied except the presence of protoplasmic droplets, no significant differences due to variation among management phases occurred. The number of protoplasmic droplets was significantly greater ($P < 0.01$) in spermatozoa from bulls on A treatment than for those on B and C treatments. The numbers of protoplasmic droplets were not significantly different in spermatozoa from bulls on the two remaining treatments.

During this period, significantly fewer spermatozoa with bent tails were observed in semen of Polled Hereford bulls ($P < 0.05$) than in semen of the other two breeds. However, no significant differences were detected between the Hereford and the Angus bulls.

There was, for the winter evaluation, significant interaction between breed and treatment in both the percentage of live spermatozoa and the percentage of broken-headed spermatozoa in the semen.

Semen Collected at the End of the Pasture Period

During this period, the volume of semen from the bulls on the DA group was significantly less ($P < 0.05$) than the volume of semen from the bulls in the BA and HS groups, but there were no statistical differences between bulls on the latter two groups. Semen from bulls seeing herd sire duty during this period had significantly more ($P < 0.05$) spermatozoa with bent tails than semen from bulls on the other two treatments.

Semen Collected at the End of the Fall Period

No significant differences were observed in semen quality during this period among the bulls on the two levels of nutrition. The average percentages of live spermatozoa in semen of Polled Hereford bulls, though considered to be sufficient for breeding, was significantly lower ($P < 0.05$) than that in the semen of Angus and Hereford bulls. No statistical difference was revealed in this characteristic between the Angus and Hereford bulls.

Interclass correlation coefficients between variables were computed on a within-treatment, within-breed basis for each of the three periods (Tables V, VI, and VII). Highly significant positive correlations at each of the three periods existed between concentration of spermatozoa in the semen sample and total spermatozoa numbers ($r = 0.931$, 0.910, 0.947, respectively), spermatozoa motility ($r = 0.583$, 0.499, 0.411, respectively), and total number of live spermatozoa ($r = 0.879$, 0.874, 0.738, respectively). Motility of spermatozoa showed a highly

TABLE V

CORRELATION COEFFICIENTS BETWEEN SEMEN CHARACTERISTICS AT THE END OF WINTER

	Semen Characteristics (r)[a]							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Body wt.								
(2) Age	0.613**							
(3) Volume	0.355	0.298						
(4) Concentration	0.320	0.176	0.168*					
(5) Total spermatozoa	0.408*	0.200	0.412*	0.931**				
(6) Motility %	-0.192	-0.153	-0.084	0.583**	0.461*			
(7) Live %	0.065	0.126	0.038	0.390*	0.293	0.464*		
(8) Total live	0.441*	0.209	-0.299	0.879**	0.896**	0.485**	0.588**	
(9) Total abnormal %	0.251	-0.398*	-0.171	-0.360	-0.361	-0.264	-0.067	-0.230

[a]r = coefficient of correlation.

*Significant ($P < 0.05$).

**Highly significant ($P < 0.01$).

TABLE VI

CORRELATION COEFFICIENTS BETWEEN SEMEN CHARACTERISTICS AT THE END OF PASTURE PERIOD

	Semen Characteristics (r)[a]							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Body wt.								
(2) Age	0.475*							
(3) Volume	0.050	-0.248						
(4) Concentration	0.093	0.210	-0.138					
(5) Total spermatozoa	0.044	0.082	0.197	0.910**				
(6) Motility %	-0.097	-0.279	0.020	0.499**	0.502**			
(7) Live %	-0.113	-0.159	-0.101	0.391*	0.330	0.784**		
(8) Total live	-0.062	0.015	0.028	0.874**	0.909**	0.589**	0.584**	
(9) Total abnormal %	0.149	0.318	-0.182	-0.411*	-0.493**	-0.492**	-0.321	-0.498**

[a]r = coefficient of correlation.

*Significant ($P < 0.05$).

**Highly significant ($P < 0.01$).

TABLE VII

CORRELATION COEFFICIENTS BETWEEN SEMEN CHARACTERISTICS AT THE END OF FALL

	Semen Characteristics (r)[a]							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Body wt.								
(2) Age	0.191							
(3) Volume	0.279	-0.164						
(4) Concentration	0.233	0.234	-0.031					
(5) Total spermatozoa	0.296	0.164	0.224	0.947**				
(6) Motility %	0.336	-0.063	0.033	0.441*	0.326			
(7) Live %	0.122	-0.285	0.086	-0.141	-0.167	0.580**		
(8) Total live	0.305	0.037	0.234	0.738**	0.705**	0.741**	0.427*	
(9) Total abnormal %	-0.194	0.074	0.129	-0.315	-0.226	-0.295	0.088	-0.237

[a] r = coefficient of correlation.

*Significant ($P < 0.05$).

**Highly significant ($P < 0.01$).

significant positive correlation with percentage of live spermatozoa ($r = 0.464, 0.784, 0.580$, respectively), and with total number of live spermatozoa ($r = 0.485, 0.589, 0.741$, respectively). Total numbers of spermatozoa showed a highly significant, positive correlation to total number of live spermatozoa ($r = 0.896, 0.909, 0.905$, respectively). A highly significant, positive correlation existed between percentage of live spermatozoa and the total number of live spermatozoa ($r = 0.588, 0.584, 0.487$, respectively). This investigation agrees with the report (Bishop et al., 1955) that there were high positive correlations between concentration of spermatozoa and total number of spermatozoa, between concentration of spermatozoa and motility, and between motility and percentage of live spermatozoa.

Negative correlations existed between per cent abnormal spermatozoa and each of the following factors: concentration of spermatozoa, total numbers of spermatozoa, motility, percentages of live spermatozoa, and total numbers of live spermatozoa but the correlations were not statistically significant except at the end of the pasture period. In general, it may be concluded that the incidence of abnormal spermatozoa showed a high degree of independence from the other characteristics examined. There was evidence that the incidence of some forms of morphologically abnormal spermatozoa is related to the incidence of dead spermatozoa.

Methods used in semen collection and evaluation in artificial insemination in beef cattle have usually been based on research obtained with dairy cattle. However, Herrick and Self (1962) stated that the

concentration of spermatozoa in dairy bull semen is higher than in beef bull semen. Ejaculate volume differed significantly ($P < 0.001$) between Friesian, Shorthorn, Ayrshire, Guernsey, Jersey, Hereford, and Red Poll (Bishop et al., 1955). The differences in percentage of bent tails at the end of the winter period and percentage of live spermatozoa at the end of the fall period between Polled Hereford and Angus, and between Polled Hereford and Hereford would indicate that further study of this problem on the basis of breeds is necessary. An insufficient number of samples prohibits making definite conclusions.

Definite deficiency of a specific nutrient is seldom found under normal management conditions with beef cattle. Excessively low or high planes of nutrition may affect fertility indirectly (Herrick and Self, 1962). Bulls underfed (60 per cent TDN recommended in Morrison's Feeding Standard) to 46 months of age did not recover appreciably in bone, endocrine, or testicular growth during a full year after they were increased to a 100 per cent TDN intake (Van Demark, Mauger and Fritz, 1964a, 1964b). Low levels (70 per cent of National Research Council's Recommended TDN) of feeding were observed to delay the onset of semen production 3 to 5 months in comparison to 100 per cent, 115 per cent, and 130 per cent of the NRC recommended TDN (Flipse and Almquist, 1961). But Bratton (1959) has concluded that body development and the onset of semen production in young bulls may be accelerated by a high level of feeding (140 to 160 per cent TDN recommended in Morrison's Feeding Standard) and retarded by a low level of feeding (60 to 70 per cent TDN recommended in Morrison's Feeding Standard) during early calfhood and

the low level of feeding did not result in damage to the spermatozoa producing tissue or permanently delay the onset of semen production. In the present experiment, the feeding level probably was not low enough nor excessively high to have a detrimental effect.

Body weight was highly related to the age of bull (+0.61), total number of spermatozoa (+0.408), and total number of live spermatozoa (+0.441) at the end of the winter period. This might be caused by immaturity of bulls, for body weight was not related to any semen characteristics at either the end of the pasture period or the fall period.

The effect of age of the bull on semen characteristics was not, however, detectable in any of the semen characteristics examined other than total abnormal spermatozoa ($r = 0.395$) at the end of the winter period.

CHAPTER V

SUMMARY

Semen quality was determined by several methods for young Angus, Hereford, and Polled Hereford bulls on three different regimes of management and nutrition. A total of 284 electroejaculated semen samples from the 48 bulls were examined. Semen volume, total number of spermatozoa, percentages of live spermatozoa, motility, and percentages of abnormal spermatozoa were evaluated. The significance of these data was determined statistically by the use of the least-squares analysis and by computing interclass correlation coefficients.

There was no significant differences in total number of spermatozoa, total live spermatozoa, motility of spermatozoa, total abnormal spermatozoa, or percentages of abnormal spermatozoa with broken heads, looped tails, or coiled tails due to the different methods of feeding or management of these beef bulls.

In semen samples collected at the end of the winter period, greater numbers of spermatozoa with protoplasmic droplets ($P < 0.01$) existed in semen from bulls on A group than on the B and C groups.

In semen collected at the end of the pasture period, the semen volume was significantly ($P < 0.05$) less for bulls on DA than for bulls on the other two regimes.

The only semen quality difference attributable to breed was found in semen collected at the end of the fall period when the percentage of

live spermatozoa was lower ($P < 0.05$) in samples from Polled Hereford bulls than from Hereford or Angus bulls. The decrease would not be expected to result in infertility among the Polled Herefords.

Positive correlations, which were highly significant, were found between the following factors: concentration of spermatozoa and total spermatozoa, concentration of spermatozoa and motility, concentration of spermatozoa and total live spermatozoa, motility and percentage of live spermatozoa, and total numbers of live spermatozoa. These correlations were found to be present in all three periods of the experiment.

Negative correlations were found between percentage of abnormal spermatozoa and each of the following factors: concentration of spermatozoa, total numbers of spermatozoa, motility, concentration of live spermatozoa, and total numbers of live spermatozoa. However, these negative correlations were not significant except at the end of the pasture period.

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