To the Graduate Council:

I am submitting herewith a thesis written by Kimberley Kirnan Hawkins entitled "The Relationships Between Reproductive Performance Traits of Beef Bulls in Natural Mating Systems and Subjective and Objective Parameters of Sperm and Related Structures." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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THE RELATIONSHIPS BETWEEN REPRODUCTIVE PERFORMANCE TRAITS OF BEEF BULLS IN NATURAL MATING SYSTEMS AND SUBJECTIVE AND OBJECTIVE PARAMETERS OF SPERM AND RELATED STRUCTURES

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Kimberley Kirnan Hawkins

August, 1979

1396898

ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation and gratitude to the many people who made the completion of her graduate study and thesis possible.

To Dr. John D. Smalling, for serving as Major Professor, for his time, advice, encouragement and compassion, for his receptiveness to thoughts and suggestions, and most of all, for his friendship.

To Dr. Don O. Richardson, for serving as a member of the graduate committee, and for all his patient and valuable help with the statistical analysis of this data.

To Dr. Hugo Eiler, for serving as a member of the graduate committee and for reviewing this manuscript.

To Dr. J. B. McLaren and Dr. J. R. McCurley, for their advice in the statistical analysis of these data.

To all my many friends in graduate school, especially Jim Fain and Carolyn Orr, for their friendship and support.

To my husband, Max F. Hawkins, for his never-ending patience, understanding and love, without whom I could not have succeeded.

To my mother and in memory of my father, to whom this thesis is dedicated, for constant love and encouragement, for giving me the ambition to succeed - the credit is theirs.

To Cathy Livingston, for her work in typing and illustrating this thesis.

ABSTRACT

Twelve Angus and 7 Polled Hereford bulls were used to determine the relationship of various semen quality attributes to various measures of reproductive efficiency.

The semen quality characteristics evaluated were: volume, concentration, motility, vigor, percent live, percent live normal, percent live immature, percent live normal, percent dead, percent dead normal, percent dead immature, percent dead abnormal and percent intact acrosomes. Scrotal circumference measurements were also taken.

Correlations between the semen quality characteristics indicated highly significant relationships between concentration, motility, vigor and percent live and percent dead cells. Sperm morphology classifications were also highly interrelated. Scrotal circumference was not consistently related to any of the ejaculate characteristics.

Reproductive performance was evaluated using 3 different expressions: the number of estrous cycles the females completed while in the presence of the bull, the mean calving interval for parous, lactating females assigned to each bull and the conception rate for each bull. All calculations were made in an attempt to avoid penalizing the bull for the parity status of the females.

Although these traits were highly correlated among themselves, few significant correlations existed with the semen quality characteristics.

The results of this study indicate the need for an extended study with larger numbers of observations, in order to accomplish more than just defining bulls with increased or impaired fertility.

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CHAPTER I

INTRODUCTION

Economic demands are constantly being placed on the Tennessee cattleman for improved efficiency of production. In order to maintain or shorten a 365-day calving interval, when a 42-day post-partum involution period is allowed, a bull must enable a cow to conceive within no more than two estrous cycles (in a natural mating situation). With 1,050,000 beef cows to be bred during the current year in the state of Tennessee (Hobson, 1979), sub-fertile bulls need to be eliminated in order to increase the number of calves weaned in cow-calf operations.

Although much research has been done to develop useful methods of evaluating semen quality in the artificial insemination industry, much less is known about the relationship of semen quality to natural breeding efficiency of beef bulls. With the large number of bulls used in natural mating situations, pressure is being exerted on the livestock industry to develop a practical, reliable method to identify sub-fertile bulls so that they can either be managed in such a way as to improve reproductive performance or else be replaced with more fertile bulls. If this can be accomplished, both Tennessee beef producers and the American beef cattle industry as a whole will be provided with a service previously thought impossible to obtain.

Until recently, no one significant method had been developed to determine the quality of a bull's semen. Standard evaluations have included estimations of concentration, motility and vigor, percent live

and dead spermatozoa, and percentages and types of structural abnormalities. These semen quality characteristics have all been found to be positively correlated with fertility, but no single measurement has been found effective in explaining large amounts of the variation in fertility among bulls.

With the discovery of the acrosomal cap and the positive relationship of the integrity of the cap to fertility, many new techniques have been developed to facilitate its study. One of the objectives of this study was to attempt to relate acrosomal characteristics to the potential fertility of the bull.

Scrotal circumference is also known to be a useful indicator of potential sperm output. It is also directly related to physiological age and weight of the bull. These measurements were also included in this study.

The objectives of this research were:

1. To characterize the distribution of prospective herd bulls as to morphology of sperm cells and integrity of the acrosomal caps.

2. To determine the relationship between the semen quality traits and the acrosomal characteristics with fertility of the bulls, as measured by natural breeding performance when determined by number of estrous cycles completed in his presence, change in calving intervals and percent cows conceiving.

3. To predict the success that a virgin bull can be expected to exhibit under natural breeding conditions.

CHAPTER II

LITERATURE REVIEW

I. EFFECTS OF SEX AND AGE ON REPRODUCTIVE EFFICIENCY

Reproductive inefficiency is often responsible for a loss of production in many beef cattle operations. The causes of this may be due to the environment, management, the livestock involved or to any combination of these; however, it is a common practice in many operations to place the blame on either the male or female alone.

Male Effects

Several researchers have reported that young bulls demonstrate a greater breeding efficiency than older bulls. Bowling et al. (1940) found a significant difference in number of services per conception because of variation in age. Bulls under 4 years of age showed a greater efficiency than bulls of any other age when bred to heifers. A gradual decrease in breeding efficiency was also noticed as the bulls increased in age; however, this difference was not significant until the bulls reached an average of six years of age.

In a study using the breeding records of the New York Artificial Breeders Cooperative, Tanabe and Salisbury (1946) found that between the ages of 1 and 3 years (inclusive), bulls had the highest breeding efficiency, while Collins et al. (1962) observed that while young bulls had higher rates of conception than did older bulls, 3 and 4 year old bulls had the highest rate of conception. Ruttle et al. (1975) observed that bulls 3 to 4 years old had greater semen volumes than

older bulls (8 to 9 years of age); however, no significant increase in abnormal cells occurred (because of the effects of age or body weight).

Lunstra et al. (1978) determined that the average age of puberty in Angus bulls was 295 days and in Hereford bulls was 326 days. Puberty was defined as the age at which a bull first produced an ejaculate containing a minimum of 50 million spermatozoa with at least 10% motility.

Almquist et al. (1976) used 27 Charolais bulls from puberty to 2 years of age to observe the effect of age on sperm concentration, motility and output. Significant increases were found as the bulls increased in age. These changes were similar to those reported in a trial conducted by Almquist and Cunningham (1967) using 17 Angus and Hereford bulls.

VanDemark (1956) also found that sperm cell producing capacity increased with age and body weight. It was also noted that a higher plane of nutrition and the accompanying increase rate of growth altered onset of puberty. Along similar lines, Baker et al. (1955) showed that sperm production per ejaculation in the growing bull was significantly correlated with age and weight. They attributed 25% of this variation in sperm numbers to growth associated with increasing age.

In Colorado, at the Central Plains Experimental Range, Rupp et al. (1977) used 660 2 year old nulliparous Hereford females and 17 Hereford bulls which were 20 to 50 months of age. This trial determined that even though some bulls cannot adequately serve large groups of heifers,

in most cases, bulls exposed to 60 heifers were as effective as bulls exposed to a smaller number of heifers. It was also reported that bulls with excessive libido tended to exhaust their sperm reserves. This evidence suggests that bulls must be selected for breeding ability as well as semen quality.

Female Effects

Several reports have indicated that heifers may require more service per conception than older females. Bowling et al. (1940) observed no significant difference between second to sixth conceptions (at which time the study was discontinued), while heifers required more services for conception that did females at any other age.

In a study using Brahman cattle in Florida, Plasse et al. (1968) determined that heifers conceived at an average of 65.5 days after first exposure, while cows conceived 45.5 days after first exposure, if they had calved at least one month before exposure. This difference was highly significant.

Wiltbank and Harvey (1963) found a significant difference in conception rate because of the variation in age. This experiment used Brahman and Angus cattle in Louisiana and indicated that the highest conception rate (82.1% conceiving in the first breeding period) was found in 5 to 10 year old cows; however, overall reproductive efficiency was greatest for cows in their fourth and fifth pregnancies, since these cows carried a significantly greater number of fetuses to term. These results were also observed by Tanabe and Salisbury (1946).

Tanabe and Almquist (1953) concluded that one cause of lowered reproductive efficiency in heifers was a high rate of embryonic mortality within the first month after conception.

In a trial conducted using 393 calving intervals of 168 Holstein cows, Whitmore et al. (1974) showed that cows bred at the first estrus after parturition demonstrated a lower fertility at first insemination than did cows bred at a later estrus. It was also noted that early breeding had no significant effect on subsequent fertility. Thatcher and Wilcox (1963), using the Florida Agriculture Experiment Station dairy herd, noticed a 12% improvement in reproductive efficiency associated with post-partum estrus activity.

Several other factors can prevent a cow from conceiving. Moberg (1976) pointed out that various conditions of environment and managerial stress have adverse effects on the reproductive efficiency of bovine females.

Slama et al. (1976) suggested that bulls with low conception rates might be the cause of a large percentage of the total number of services required for conception. The bull to which a cow was exposed was also the source of variation in calving interval in a study reported by Lasley and Comfort (1962). These results emphasize the need for a reliable indicator of the fertility of bulls.

II. RELATIONSHIP OF SCROTAL CIRCUMFERENCE TO SPERM OUTPUT AND TESTES WEIGHT

In recent years, scrotal circumference measurements have been the subject of much research. Several such studies suggest that they are reliable indicators of both total sperm output and testes weight.

Hahn et al. (1969) found that this measurement was easy to obtain, highly repeatable and also highly correlated with other testicular

measurements. Scrotal circumference was determined to be a useful indicator of potential and current sperm output and subsequent testicular development, although the magnitude of the relationship decreased with age. This was also observed by Underwood et al. (1978). Hahn et al. (1969) also found this to be highly correlated (r = .92) with testes weight. VanDemark (1956) obtained the same value for this correlation and Willett and Ohms (1957) recorded a slightly higher value (r = .94). These researchers also noticed a similar relationship between scrotal measurements and the age of the bull. A higher correlation (r = .68) was indicated for young bulls than for older bulls (r = .53) when scrotal circumference was related to total sperm output. Therefore, comparisons between bulls should be made on a constant-age basis.

Coulter et al. (1975) used 5,909 scrotal circumference measurements from Holstein bulls and 339 from Angus bulls to conclude that testes size increased at a rapid rate in young bulls, at a more limited pace in mature bulls and slightly decreased in old bulls. Angus bulls were observed to have larger testes until approximately 3 years of age at which time the Holsteins had larger testes. This suggests that Angus bulls may be earlier maturing.

Almquist et al. (1976) also obtained results indicating that testicular size increases more rapidly in young bulls. Scrotal circumference was found to increase 32% from puberty to 2 years of age, with three-fourths of this increase from puberty (41 weeks) to 65 weeks of age.

Scrotal circumference was demonstrated to be a useful indicator of puberty by Lunstra et al. (1978). A wide difference in age and

weight among breeds at puberty was observed; however, scrotal circumference measurements remained nearly constant at 27.9 $\pm$ .2 cm.

III. EFFECT OF SEMEN QUALITY AND SPERM MORPHOLOGY ON FERTILITY

In the past several decades, semen quality and sperm morphology have been the subject of a substantial amount of research effort. This work has been done in hope of discovering a qualitative or quantitative method of determining the potential fertility of a bull; however, the results have not been overly successful.

Semen Quality Effects

Motility, also expressed as percent motile sperm, and percent live sperm cells have been observed to be positively correlated with fertility in several studies.

Almquist et al. (1976) determined that sperm motility increased with age in Charolais bulls, with motile sperm output increasing significantly from 1 to 2 years of age. Concentration also showed a highly significant increase due to the variation in age. These results are in agreement with a study using Angus and Hereford bulls conducted by Almquist and Cunningham (1967).

Hafs et al. (1959) found that percent live sperm cells was highly correlated with percent motile sperm cells. They also suggested that a higher frequency of ejaculation aided in keeping the semen more uniform in quality from one ejaculation to the next.

In Mew South Wales, 1,000 inseminations were performed using semen previously evaluated for motility and percent live cells (Clark et al., 1973). This research indicated that percent motile cells and

percent live cells were highly correlated, as were conception rate and motility scores. At the same time, they cautioned that semen of high subjective quality could result in a wide range of fertility.

In an early experiment, Swanson and Herman (1944) demonstrated a highly significant curvilinear relationship between motility and conception rate.

Lasley (1951) recorded a significant correlation (r = .314) between fertility and percent motile sperm. He observed that for each 10% increase in percent motile sperm, fertility increased 4.6% , and concluded that percent motile sperm was a good indicator of fertility.

Linford et al. (1976) agreed that the best single test evaluation of semen was sperm motility. They stated that percent live cells was also a good indicator of fertility. Non-return to estrus was used as an indicator of conception and was found to be highly correlated with percent motility.

In a study using 48 semen samples from 6 bulls, Munro (1961) noticed a reduction in fertility (less that 74% conception) when percent live sperm cells dropped below 70%.

Although motility is widely used as an indicator of fertility, Neville et al. (1971) concluded that sperm cell concentration is a better indicator; however, neither characteristic is very reliable. Their conclusions were based on these six correlations: fertility to volume, .02; fertility to sperm cell concentration, .18; fertility to motility, .10; motility to volume, .02; motility to sperm cell concentration, .21; and sperm cell concentration to volume, .25.

Falk and Kaufman (1950) stated that fertilizing capacity of semen depends on the interrelation of volume, motility, concentration and morphology and concluded that all of these must be high in quality to insure fertilization.

Sperm Morphology Effects

Research has also shown that morphologically abnormal sperm cells are correlated with a reduction in fertility.

Falk and Kaufman (1950) indicated that good morphology was the only factor which could compensate, even to a small degree, for other small deficiencies in semen quality. Hence, they suggested that good morphology does more to insure fertility than any other aspect of semen quality.

In a study using 10,940 ejaculates, Carroll et al. (1963) determined that morphology ratings had a higher correlation to final semen classification (in this case, satisfactory, questionable and unsatisfactory) than did motility, concentration or percent live sperm cells. Their results found 82.9% of the bulls were satisfactory, 11.8% questionable and 4.9% unsatisfactory.

Swanson and Herman (1944) showed a significant negative correlation between morphologically abnormal cells and conception rate ($r = -.12$), while Munro (1961) also demonstrated that conception rate decreased as the number of abnormal cells increased. He stated that fertility was reduced to less than 66.6% conception when more than 23% of the cells were morphologically abnormal.

In an early study, Williams and Savage (1927) reported that bulls which were known to demonstrate good fertility had very uniform heads

and usually no more than 17% morphologically abnormal spermatozoa. They also suggested that acceptable numbers of abnormalities depended upon what types of abnormalities were present, for example, samples with large numbers of grossly misshapenheads were considered very undesirable.

Branton and Salisbury (1947) stated that the origin of morphologically abnormal cells was the testis. No significant difference was observed in the proportion of spermatozoa with attached droplets in any level of the reproductive tract. In the head of the epididymis, droplets were usually found on the neck of the sperm cells, but upon reaching the ampulla, the droplets were gone. This suggests that this characteristic is simply a morphological maturation stage present in all normal bulls.

Hafs et al. (1959) found no significant difference in the morphology of the spermatozoa when the bulls were ejaculated daily over a period of several weeks.

Several handling techniques must also be considered before semen is judged too harshly on morphological characteristics alone. Rao and Hart (1948) and Mercier and Salisbury (1947) agreed that morphological examination is most representative if it is done as soon after the semen is collected as possible. They also indicated that methods of collecting, handling and examination influence the number of abnormalities found in the sample. True abnormalities are usually lower in number than such studies indicate. Salisbury et al. (1942) found similar results and also stated that semen of individual bulls varies in ability to withstand these procedures. This emphasized the importance of collecting more than one sample.

IV. RELATIONSHIP OF ACROSOMAL CAP INTEGRITY TO FERTILITY

Many researchers have been encouraged to acquire more knowledge of the acrosome since its important role in fertility is now becoming better understood.

Origin and Structure

Blom and Birch-Andersen (1965) first used the term "acrosomal cap"; however, the presence of a sperm cap in bulls was identified earlier as the "galea capitis" by Blom (1946). The galea capitis was also observed by Bretschneider (1949, 1951), who stated that it was composed of inner and outer parts.

Hancock (1952) described the acrosome of the bull as being a cytoplasmic cap which covers approximately two-thirds of the anterior portion of the sperm head.

The equatorial segment was identified by Hancock (1957) as the area between the posterior straight border of the outer acrosome and the posterior concave border of the inner acrosome.

Saacke and Almquist (1964) also observed the equatorial segment to be a part of the acrosome. They agreed with Blom and Birch-Andersen (1965) that detachment of the acrosomal cap did not include the entire structure, but only the outer membrane.

The bovine sperm is illustrated in Figure 1.

Effects of Acrosomal Maturity on Collection Frequency

Several researchers have noticed gradual changes in the acrosomal cap of the bovine sperm head as aging occurred. Dickey et al. (1965)

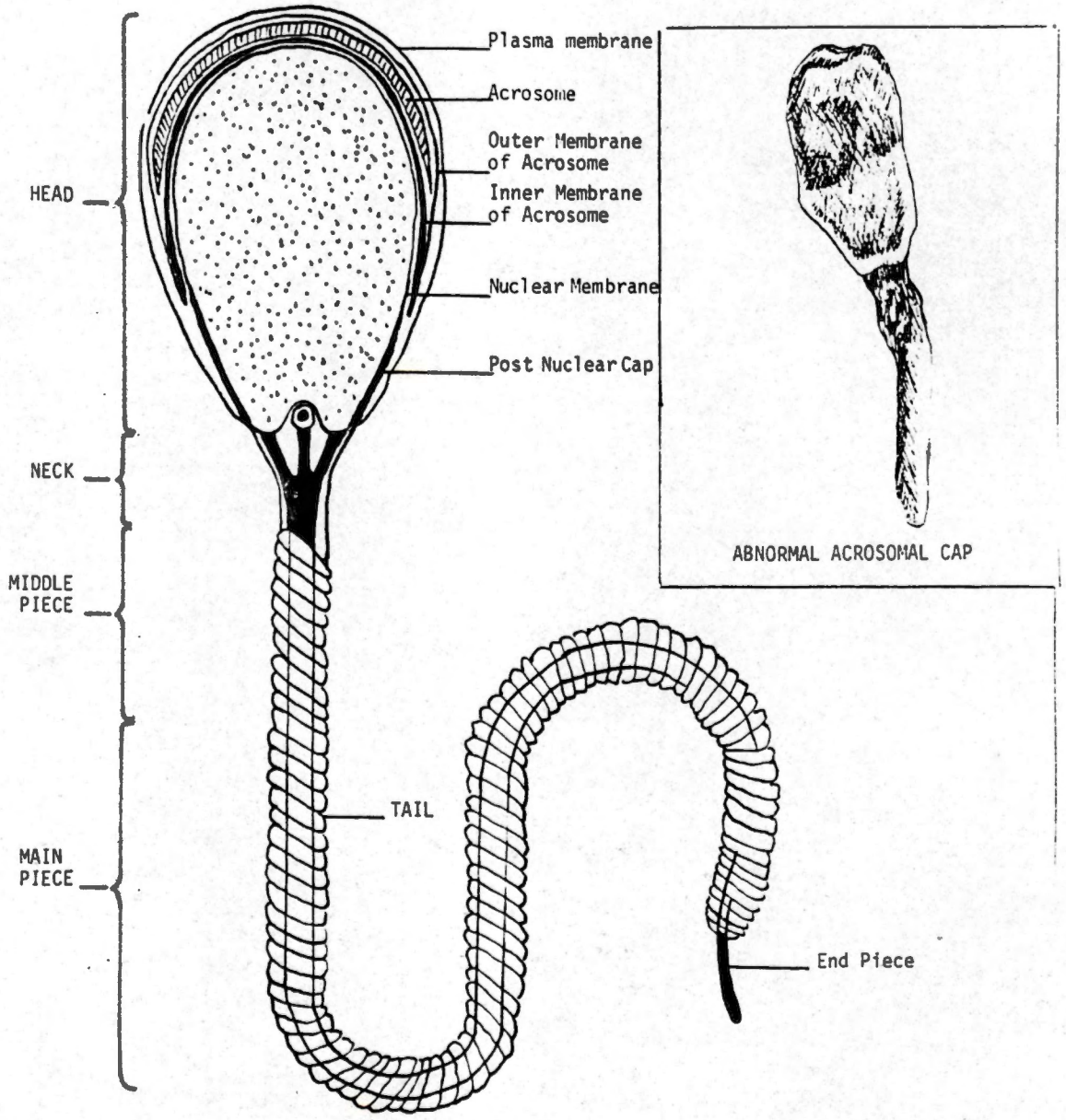


Figure 1. Diagrammatic representation of a spermatozoa.

observed these changes in the acrosomal cap of bull spermatozoa as it passed through the epididymis. The acrosome changed from a wavy, loosely attached structure (while the sperm cells were in the testes) to a closely adhering, smooth cap when the spermatozoa were ready to be ejaculated.

Hathaway and Hartree (1963) reported that in a small percentage of ejaculated bull and ram sperm cells, spontaneous detachment of the acrosome is a sign of approaching death of the sperm cell. Similarly, Lovell and Getty (1960) noted that as boar spermatozoa aged, the outer acrosome began to swell and wrinkle, and eventually began to detach in a large number of cases.

In bull semen collected after at least 3 weeks of abstinence, Blom (1946) recorded up to 18% spontaneous detachment of the acrosome. With subsequent regular collection of the bulls, this percentage was reduced to zero.

Wells et al. (1970) observed similar results in the first ejaculate of young bulls after a period of sexual rest. The semen was, in general, of poor quality, with 16.5% of otherwise "normal" cells possessing abnormal acrosomes. They also found that the number of cells with normal acrosomes increased significantly from the first collection to the sixth collection after sexual rest. The "aged" sperm cells showed a high percentage of both morphological and acrosomal abnormalities and less motility than did spermatozoa after more frequent ejaculations.

In a later study, Wells et al. (1971) confirmed previous results that frequent ejaculation reduced the percent of acrosomal

abnormalities. They also stated that a period of sexual rest of at least 4 weeks resulted in large increases of aged spermatozoa.

Morphology in Relation to Fertility

A few studies have described the sequence of changes in the sperm head which may occur during fertilization.

Saacke and Marshall (1968) described the deterioration of the acrosomal cap as the loss of the apical ridge, with a swelling of the acrosome, followed by the formation of the equatorial segment from the posterior region and then concluded with the detachment of the anterior acrosome. They stated that these changes occurred either immediately prior to or after death of the cell.

A type of acrosomal breakdown in which the plasma and outer membranes fuse and form vesicles was recorded by Bedford (1970). He concluded that this type of breakdown was observed in nearly all capacitated spermatozoa in close proximity to the ovum; however, only approximately 1% of degenerating sperm passed through a similar phase.

Jones (1973) found two morphologically distinct changes in the acrosome of boar spermatozoa. He stated that rapid swelling of the acrosome might result in vacuolation, while slow swelling could produce vesiculation. He also concluded that vesiculation rarely occurs in the epididymis, but could be induced *in utero*, suggesting that this final stage is morphologically similar to that which occurs during fertilization.

Very little research has been found that relates semen quality or sperm morphology to fertility which has included acrosome morphology.

Saacke (1970) used 24 ejaculates from 5 bulls to observe that objective cell counts (200 cells per sample) were highly repeatable when compared to microscopic estimates of percent motility. These comparisons were made on semen thawed for 8 hours at 37° C.

Saacke and White (1972) observed that acrosome retention was more highly correlated to fertility after 2 hours of post-thaw incubation ($r = .60$) than was motility ($r = .41$). After variation in fertility because of the differences in bulls had been removed, acrosome retention still maintained a significant relationship to fertility, whereas motility did not. Johnson et al. (1976) confirmed that percent intact acrosomes observed during the incubation period was correlated with fertility.

In a later study, Wells et al. (1973) found that the percentages of normal sperm, live cells, total percent aged acrosomes and normal cells with aged acrosomes were measurements which could significantly aid in the prediction of fertility when accomplished before freezing the semen.

Effects of Fixation and Staining

Several methods of fixation and staining have been studied in recent years. Early research by Wells et al. (1970, 1971) used 2.9% sodium citrate as a sperm cell suspension medium, without reporting any damage due to technique. However, in a more recent study, Johnson et al. (1976) reported that fresh, unfixed bovine semen could not be accurately evaluated for acrosomal cap integrity. They reported significant differences in acrosomal damage among duplicate, unfixed samples.

Johnson et al. (1976) suggested the use of a glutaraldehyde fixative (.25 ml of raw semen in 2 ml of .2% glutaraldehyde in phosphate-buffered saline). They reported that the percent intact acrosomes of fixed cells was significantly higher than for unfixed cells, without observing any difference in acrosomes due to length of storage or shipping. This is in agreement with Pursel and Johnson (1974). Johnson et al. (1976) suggested that this technique might be applicable under field conditions to test the potential fertility of young bulls and those used for natural mating purposes.

Two principle types of staining procedures have been used to evaluate acrosomal cap integrity. Saacke and Marshall (1968) and Saacke et al. (1968) used Giemsa stained smears to examine acrosomes, while Wells and Awa (1970) used a Fast green-Eosin B stain to observe the cells. They found the results using both stains to be highly correlated.

CHAPTER III

MATERIALS AND METHODS

This study used nineteen virgin bulls, 12 Angus bulls at the Plateau Experiment Station (PES), Crossville, Tennessee and 7 Polled Hereford bulls at the Tobacco Experiment Station (TES), Greeneville, Tennessee. The age of the bulls varied from 15 to 16 months at time of the initial collection.

Each bull was collected on two occasions, the first (March, 1978) several days before being exposed to a group of females and the second time, upon removal from the females (late June to early July 1978). At each collection, two samples of semen were taken for analysis. Scrotal circumference measurements were also made at this time, using a Lane scrotal tape calibrated in centimeters.

Semen Collection

All bulls were collected using a Plectron electro-ejaculator. Two ejaculates from each bull were collected in succession, with no rest period between samples. Precautions were taken to protect the spermatozoa against cold shock. As soon as each sample was collected, it was examined subjectively and slides were prepared for later analysis.

Subjective Semen Evaluation

As each semen sample was obtained, color and colume measurements were recorded. Color was determined visually and described as ranging from creamy to gray, with creamy being the most concentrated.

The volume measurement was taken directly from the graduated collecting tube.

The samples were then evaluated microscopically by placing a drop of pure semen from an innoculating loop onto a slide which had been previously warmed in a microscope stage warmer, and an equal drop of 2.9% sodium citrate solution (2.9 g $Na_2C_6H_5O_7.2H_2O$ and distilled water, Q.S. 100 ml solution). The diluted semen was then covered with a 22 mm square glass coverslip and examined under low and medium powers on a compound microscope. Subjective measurements of concentration, motility and vigor were subsequently recorded.

Concentration was estimated in tenths of billions per ml of semen collected.

Motility was estimated as the percentage of sperm cells which exhibited movement. This measurement was scored in increments of 5.

Vigor was scored on a scale of 0 to 4, with 0 being no movement at all and 4 being the most vigorous type of movement. The estimation of this score was determined by the speed and direction of movement of a majority of the spermatozoa in the sample.

Live-Dead Preparation

The live-dead slides which were made at the time of collection were stained with an Eosin-Nigrosin stain described by Swanson and Bearden in 1951, which combines 1 g of Eosin with 5 g of nigrosin in 100 ml of 2.9% sodium citrate. The stain was refrigerated between usages to prevent bacterial or fungal growth.

The slides were prepared by mixing a drop of pure semen with an equal drop of stain on a previously warmed glass slide. A thin smear

was made of this mixture in a manner similar to that of preparing a blood smear, and the slides were air-dried and taken to the laboratory for evaluation.

All slides were labeled with the collection date and coded for anonymity in evaluation.

Acrosome Preparation

Slides were also made at the time of collection for acrosome integrity evaluation. A semen sample was fixed by pipetting 0.25 ml of pure semen into a tube containing 2.0 ml of 0.2% glutaraldehyde in phosphate buffered saline (Johnson et al., 1976).

The slides were prepared by placing a drop of the fixed preparation on a warm glass slide and a thin smear made as previously described. These slides were then labeled similarly to the live-dead slides and taken back to the laboratory for staining and evaluation.

A stock solution of Giemsa stain was prepared using 0.5 g of Giemsa dissolved in 33.0 ml of glycerin and combined with 33.0 ml of methanol (Jones, 1967). One volume of the stock solution was combined with 2 volumes of methanol and 4 volumes of a 1% ethanolamine buffer to form a fresh mixture used in the actual staining procedure (Illisson, 1969).

The slides were stained for 20 minutes in the fresh Giemsa mixture, rinsed in distilled water for 2 minutes, restained in Giemsa for 10 minutes, rinsed in buffer for 5-20 seconds and finally rinsed in distilled water and allowed to air-dry (Doyle, 1979).

Objective Slide Evaluations

Two hundred sperm cells per live-dead slide were counted to determine the percentage of live and dead cells per semen sample. Sperm cells which remained unstained were considered to have been live cells, while those which were either partially or totally stained were labeled as dead cells.

Morphology of the spermatozoa was also recorded from the examination of the live-dead slides. Sperm cells were classified as either normal, immature or abnormal. Those cells which had normally shaped heads and fairly straight tails with no cytoplasmic droplet present were considered normal, while those which were similar but did have an observable cytoplasmic droplet present were labeled immature.

Abnormal spermatozoa were divided into several categories. Those with no tails were classified as broken heads. Those with tails which were bent sharply at an angle were considered to be bent, while the ones with tails coiled into a tight ball were categorized as coiled. The sperm cells with tails which were doubled back towards the head were listed as shoehook. There was also a category for those cells with misshapen heads. Illustrations of all cell types are shown in Figure 2.

The live-dead slides were examined on a compound microscope under the oil immersion objective.

Two hundred spermatozoa on each acrosomal slide were counted to determine the percentage of intact acrosomes (PIA). Each sperm cell was either classified as intact or non-intact, the latter being all

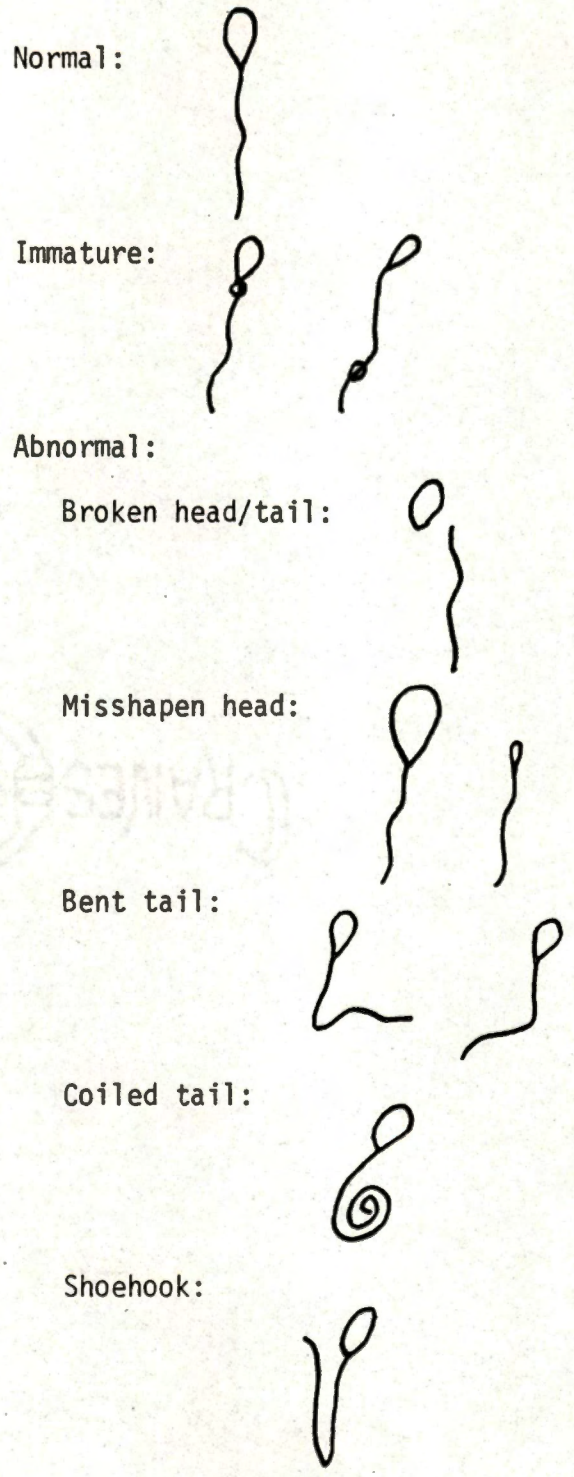


Figure 2. Sperm morphology.

cells from which the acrosome was either partially or completely torn away from the head.

These slides were examined on a Zeiss standard microscope under the oil immersion objective, using an interference green filter.

Females

A total of 236 females were exposed to the nineteen bulls previously mentioned. Of these, 127 Angus females at PES and 109 Polled Hereford females at TES were used in the study. A range of 7 to 14 females was exposed to each bull at PES, while 13 to 19 females were exposed to each bull at TES (see Table 1).

The females were chosen randomly from another study involving genetic selection, without regard to past reproductive performance, except cows which had failed to give birth for two consecutive years were removed from the herd.

Of the 127 females at PES, 27 were virgin heifers and 9 others had not given birth the previous year (1977), while at TES, of 109 females, 20 were virgin heifers and 8 others had gone without calving in 1977 (see Table 1).

The three methods which were used to estimate the performance of the bull were the number of estrous cycles which the females completed in the bull's presence, the calving interval of parous, lactating females and the percent conception rate of all females exposed to each bull.

To quantitatively measure the fertilizing capacity of the bull's semen, we chose the number of estrous cycles which the females completed

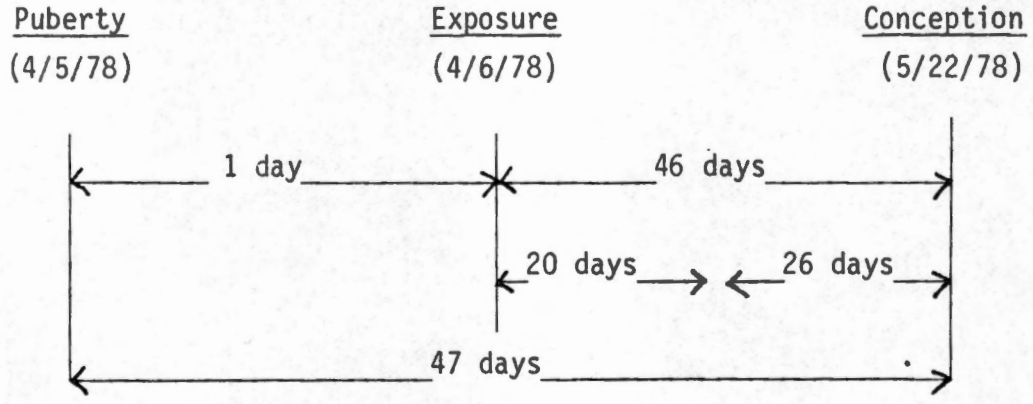
Table 1. Livestock profile

	Bulls		Females			
	Herd No.	Birthdate	Parous Lactating	Parous Non-Lactating	Virgin Heifers	Total
TES	4-57	1/20/77	14	0	2	16
	4-137	1/23/77	13	1	5	19
	4-197	1/23/77	9	1	5	15
	4-277	1/25/77	11	1	3	15
	4-297	1/25/77	10	2	1	13
	4-397	1/27/77	11	1	2	14
	4-1077	2/19/77	13	2	2	17
	n = 7		81	8	20	109
PES	5-417	1/20/77	10	0	1	11
	5-557	1/22/77	10	0	3	13
	5-577	1/22/77	8	0	5	13
	5-617	1/22/77	7	0	2	9
	5-637	1/22/77	6	2	2	10
	5-757	1/24/77	7	2	1	10
	5-777	1/24/77	10	1	2	14
	5-977	1/26/77	14	0	2	9
	5-1117	1/29/77	3	3	3	9
	5-1317	2/2/77	5	1	3	9
	5-1397	2/2/77	5	0	2	7
	5-1557	2/5/77	6	0	1	7
	n = 12		91	9	27	127
	N = 19		172	17	47	236

in the presence of the bull. To minimize the effects of parity status of the female on fertility, a procedure described by Irwin in 1976 was used. Date of conception for each female was calculated by subtracting 280 days for Angus females (285 for Polled Hereford females) from the date of parturition (Wheat and Riggs, 1958). An involution period of 42 days was also allowed, and it was assumed that females were physiologically exposed to both previous and current bulls the same number of days.

Virgin heifers were assumed to reach puberty at an average of 366 days after birth for Angus heifers and 415 days for Polled Hereford heifers (U.S.M.A.R.C., 1976). The number of days from the estimated date of puberty to the first day of exposure to the bull was calculated and divided by 21 (average length of estrous cycle). The number of days overlapping into the exposure period was then calculated and subtracted from the total number of days from the first exposure to the bull to the calculated conception date. The remaining days were divided by 21 to represent the apparent number of estrous cycles completed by the heifer in the presence of the bull (see Figure 3).

Lactating females were divided into two groups. For those females with involution periods of 42 days or more, number of estrous cycles was calculated by subtracting 42 from the total number of days from the last parturition to the day of first exposure to the bull. Average estrous cycle length (21 days) was subtracted out until less than 21 days remained, if greater than 21 days were still left to the time of the first exposure. This number remaining represented part



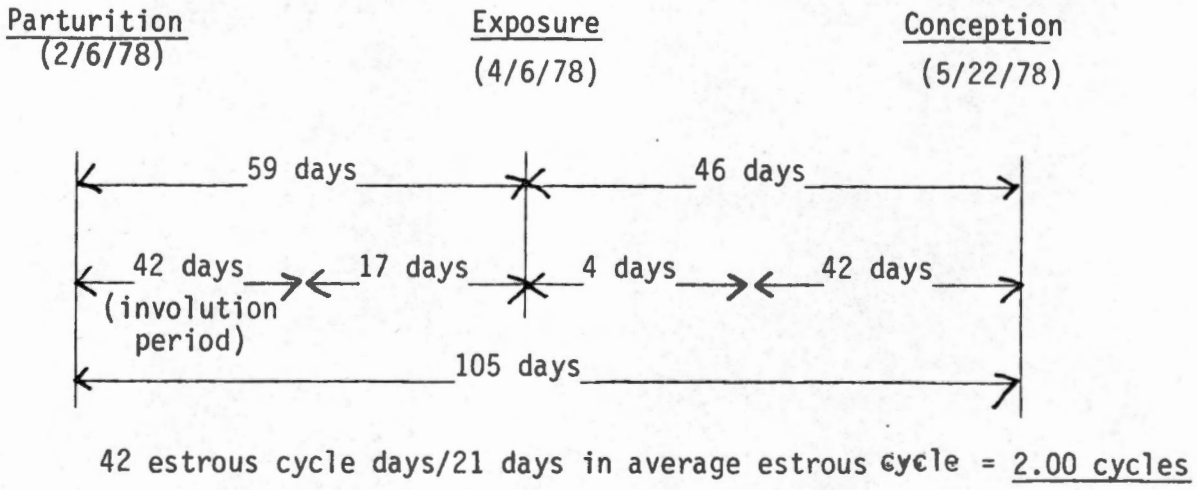
26 estrous cycle days/21 days in average estrous cycle = 1.24 cycles

Figure 3. Heifer 4/727 exposed to bull 4-197.

of an estrous cycle which the female entered before being exposed to the bull, and ended in his presence. The number of days this cycle overlapped with exposure time was subtracted from the number of bull exposure days to the time of possible conception. The days remaining after this calculation were the total estrous cycle days, which, when divided by 21, gave the number of estrous cycles the cow completed before conceiving. This type of calculation is illustrated in Figure 4.

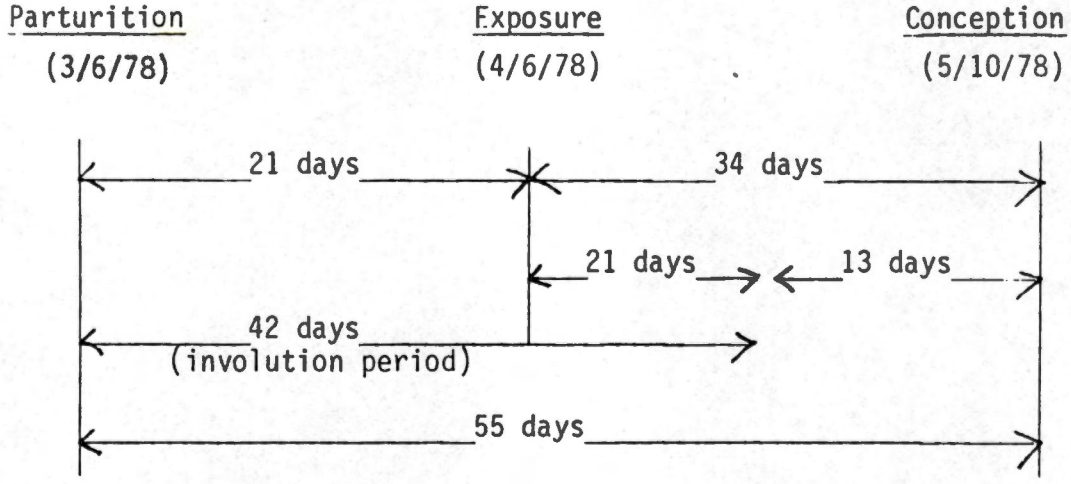
For lactating cows which had an involution period of less than 42 days, the procedure was to subtract 42 from the total number of days from the previous parturition to possible conception. This left the number of estrous cycle days, which, when divided by 21, again gave the number of estrous cycles completed by the female in the presence of the bull before conception occurred (Figure 5). This complex procedure also minimizes the effect of involution period on fertility.

Non-lactating cows were assumed to be cycling, and an average date at which lactating cows had reached parturition was used to calculate a constant date (2/15/78 for PES cows and 2/10/78 for TES cows). The number of days from the constant date to the day of first exposure to the bulls was calculated and divided by 21 to give the number of estrous cycles completed before exposure. The number of days which the last estrous cycle overlapped into the exposure period was again subtracted from the total number of days from first exposure to to the possible date of conception. The remaining days, divided by



42 estrous cycle days/21 days in average estrous cycle = 2.00 cycles

Figure 4. Lactating cow 4-542 exposed to bull 4-57.



13 estrous cycle days/21 days in average estrous cycle = .62 cycles

Figure 5. Lactating cow 4-746 exposed to bull 4-57.

21, again represented the number of possible estrous cycles the female completed in the presence of the male. An example of this is shown in Figure 6.

For those females which did not conceive, the numbers of estrous cycles were calculated in the same way in which they were calculated for those that did conceive, except that the estrous cycles were calculated to the end of the exposure period, as shown in Figure 7.

Two other measurements of fertility for each bull were also calculated. Calving interval (which in reality is a variation of the previous calculation) for parous, lactating cows was expressed as the total number of days between two successive parturitions. A more commonly used, but less reliable measurement, percent conception, was calculated as the number of females which conceived divided by the number of females to which the bull was exposed.

Statistical Procedures

The statistical analysis of the data was performed using the Statistical Analysis System (SAS) procedures.

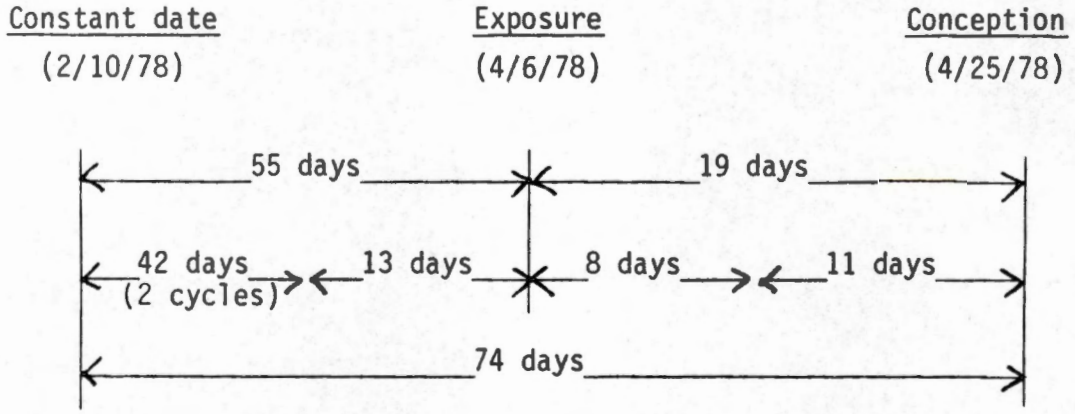
Residual correlations were first obtained on all semen quality variables in the data set, using the model:

$Y_{ij} = \mu + b_i + e_{ij}$, where:

b_i is the effect associated with experiment station location and,

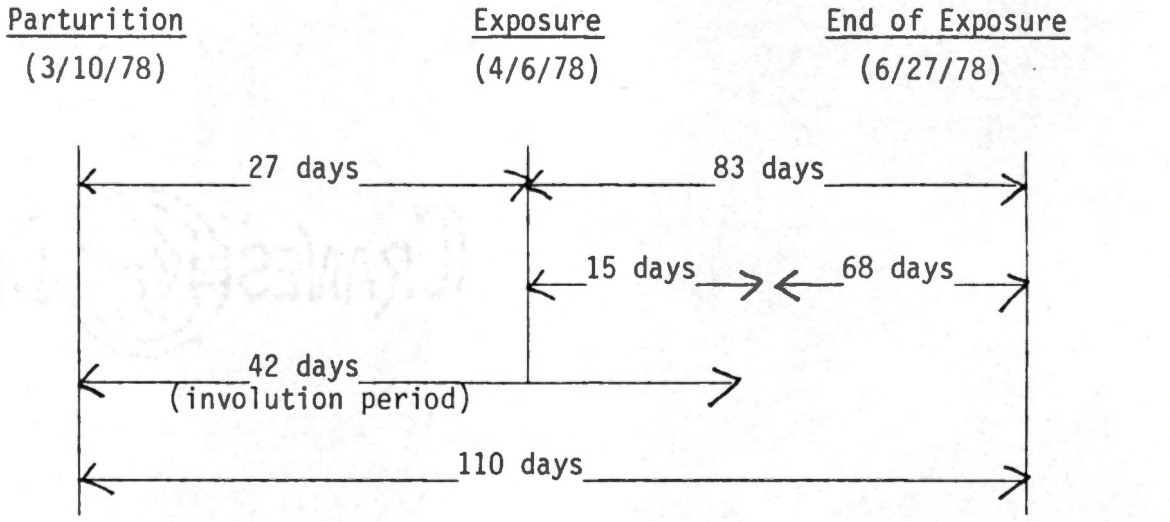
e_{ij} is the random error associated with each observation.

Residual correlations were also obtained on the reproductive performance variables and the semen quality characteristics using the model:



11 estrous cycle days/21 days in average estrous cycle = .52 cycles

Figure 6. Non-lactating cow 4-325 exposed to bull 4-197.



68 estrous cycle days/21 days in average estrous cycle = 3.24 cycles

Figure 7. Lactating cow 4-506 exposed to bull 4-57.

$Y_{ijkl} = \mu + b_i + c_j + d_k + e_{ijkl}$ where:

b_i is the effect associated with experiment station location,

c_j is the effect associated with collection day,

d_k is the effect associated with sample number and,

e_{ijkl} is the random error associated with each observation.

The Maximum R^2 Improvement technique for the stepwise regression procedure was used to determine which variable should most likely be included in a prediction equation for each of the three reproductive performance traits.

CHAPTER IV

RESULTS AND DISCUSSION

Specific semen characteristics were evaluated as measures of quality, while their possible effects on breeding efficiency of the bull were determined using 3 reproductive performance traits.

Tables 2 and 3 show the arithmetic means, by collection day, for the semen quality characteristics, while Table 4 shows the arithmetic means of the reproductive traits.

Relationships of Semen Quality Characteristics

As shown in Table 5, several of the semen quality characteristics were significantly correlated.

While volume was not significantly correlated with any of the semen quality characteristics, subjective concentration (although an estimation rather than a precise measurement) was found to be significantly correlated with several of the other traits. It was highly correlated ($P < .01$) with motility ($r = .73$) and vigor ($r = .70$) estimations.

Concentration was also significantly correlated with the morphology characteristics. It was positively correlated ($P < .01$) with percent live cells ($r = .56$) and percent live normal cells ($r = .50$) and with percent live abnormal cells ($r = .26$, $P < .05$). As this might suggest, concentration was negatively correlated with dead cell characteristics: percent dead cells ($r = -.56$), percent dead normal cells ($r = -.55$) and percent dead abnormal cells ($r = -.36$). These

Table 2. Arithmetic means of subjective semen quality characteristics and scrotal circumference

	Volume (ml)	Concentration (bil./ml)	Motility (%)	Vigor (0-4)	Scrotal Circumference (cm)
TES					
1st collection	5.08 ± 2.59	.46 ± .24	49.62 ± 19.73	2.46 ± .97	30.12 ± 1.56
2nd collection	4.85 ± 2.04	.57 ± .23	52.92 ± 22.41	3.00 ± 1.04	31.46 ± 1.45
Station mean	4.97 ± 2.29	.51 ± .24	51.20 ± 20.68	2.72 ± 1.02	30.76 ± 1.63
PES					
1st collection	6.90 ± 3.08	.48 ± .20	47.95 ± 20.16	2.82 ± .96	32.93 ± 1.97
2nd collection	7.91 ± 3.51	.62 ± .24	60.68 ± 27.27	3.41 ± .91	33.5 ± 2.52
Station mean	7.40 ± 3.30	.55 ± .23	54.32 ± 24.56	3.11 ± .97	33.22 ± 2.25
Both Stations					
1st collection	6.22 ± 3.00	.47 ± .22	48.57 ± 19.73	2.69 ± .96	31.89 ± 2.27
2nd collection	6.83 ± 3.38	.60 ± .24	57.94 ± 25.59	3.26 ± .96	32.78 ± 2.39
Overall mean	6.52 ± 3.18	.54 ± .23	53.19 ± 23.12	2.97 ± 1.00	32.33 ± 2.36

Table 3. Arithmetic means of objective semen quality characteristics

	% Live				% Dead				% Intact Acrosomes
	Total	Normal	Immature	Abnormal	Total	Normal	Immature	Abnormal	
TES									
1st collection	61.31 ± 9.35	39.15 ± 13.33	9.70 ± 7.67	12.46 ± 5.56	38.69 ± 9.35	23.46 ± 8.42	1.62 ± 1.33	13.62 ± 6.54	67.77 ± 10.32
2nd collection	51.92 ± 23.01	39.83 ± 16.80	9.17 ± 9.42	8.92 ± 4.27	41.67 ± 22.82	27.17 ± 21.06	3.42 ± 7.04	12.08 ± 4.06	71.45 ± 11.94
Station mean	59.68 ± 17.01	39.48 ± 14.78	9.44 ± 8.31	10.76 ± 5.21	40.12 ± 16.87	25.24 ± 15.57	2.48 ± 4.94	12.88 ± 5.43	69.46 ± 11.00
PES									
1st collection	49.64 ± 20.16	34.05 ± 17.32	5.82 ± 5.48	10.23 ± 7.34	50.36 ± 24.37	33.36 ± 17.56	1.55 ± 1.79	14.95 ± 9.83	66.05 ± 8.44
2nd collection	71.32 ± 24.91	55.68 ± 24.62	5.95 ± 9.63	9.68 ± 3.20	28.68 ± 24.91	19.27 ± 17.14	1.27 ± 2.05	8.05 ± 8.60	75.36 ± 11.56
Station mean	60.48 ± 26.71	44.86 ± 23.71	5.89 ± 7.73	9.95 ± 5.60	39.52 ± 26.71	26.32 ± 18.57	1.41 ± 1.91	11.50 ± 9.78	70.70 ± 11.06
Both Stations									
1st collection	53.97 ± 20.75	35.94 ± 15.95	7.26 ± 6.54	11.06 ± 6.74	46.03 ± 20.75	29.69 ± 15.46	1.57 ± 1.61	14.46 ± 8.67	66.69 ± 9.07
2nd collection	66.59 ± 24.77	50.09 ± 23.22	7.09 ± 9.54	9.41 ± 3.57	33.26 ± 24.66	22.05 ± 18.69	2.00 ± 4.50	9.47 ± 7.51	74.06 ± 11.65
Station mean	60.19 ± 23.52	42.91 ± 20.96	7.17 ± 8.09	10.25 ± 5.44	39.74 ± 23.49	25.93 ± 17.43	1.80 ± 3.35	12.00 ± 8.45	70.26 ± 10.97

Table 4. Arithmetic means of estrous cycles, calving intervals and conception rates

	Estrous cycles completed in presence of bull (No.)	Calving interval (Days)	Conception rate (%)
TES	.59 ± .05	365.30 ± 4.62	85.21 ± 9.66
PES	.67 ± .17	353.69 ± 8.43	85.15 ± 17.97
Overall mean	.64 ± .15	357.90 ± 9.17	85.17 ± 15.40

Table 5. Residual correlation coefficients between semen characteristics and selected variables

	Estrous cycles	Calving interval	Conception rate	Volume	Concentration	Motility	Vigor	Scrotal Circumference	% live Cells	% live normal	% live immature	% live abnormal	% dead cells	% dead normal	% dead immature	% dead abnormal	% intact acrosomes
Estrous cycles	1.0000	-.4408	-.5100**	-.0059	.0522	.0088	-.0152	-.1706	.0710	-.0113	.0877	.2145[a]	-.0702	-.0798	-.0176	-.0313	-.1149
Calving interval		1.0000	.4504**	.0278	.0415	.1205	.1329	.0221	.2353*	.1754	.0150	.3244**	-.2370*	-.2719*	-.0967*	-.0932	.2459*
Conception rate			1.0000	.2509*	.0406	-.1001	.0363	.4596**	-.0118	.0213	-.0694	-.0499	.0088	.0161	-.0377	.0388	.0885
Volume				1.0000	-.0768	-.1130	-.0166	.1422	-.0484	-.0036	-.0777	-.1140	.0481	.0365	.0567	.0491	-.0310
Concentration					1.0000	.7347**	.6999**	.1917[a]	.5592**	.5001**	.1672	.2566*	-.5570**	-.5452**	-.2197[a]	-.3643**	.7699**
Motility						1.0000	.8708**	.0449	.6738**	.6856**	-.0051	.3068**	-.6719**	-.5987**	-.3182**	-.5412**	.9048**
Vigor							1.0000	.1859	.6236**	.6524**	-.0569	.3100**	-.6253**	-.5456**	-.3226**	-.5028**	.8015**
Scrotal Circumference								1.0000	-.0594	-.0336	-.0425	-.0828	.0581	.0205	-.1423	.2014[a]	.1706
% live cells									1.0000	.8922**	.2907*	.5031**	-.9997**	-.9444**	-.3707**	-.7315**	.6551**
% live normal										1.0000	-.1262	.2168[a]	-.8923**	-.7693**	-.4084**	-.7593**	.6424**
% live immature											1.0000	.3000**	-.2901*	-.4126**	.0809	-.0161	.0728
% live abnormal												1.0000	-.5022**	-.5594**	-.1389	-.2449*	.2490*
% dead cells													1.0000	.9432**	.3719**	.7347**	-.6539**
% dead normal														1.0000	.3329**	.4922**	-.6173**
% dead immature															1.0000	.0725	-.2950**
% dead abnormal																1.0000	-.4704**
% intact acrosomes																	1.0000

*Significant at $P < .05$ level
**Significant at $P < .01$ level
[a]Approaches significance at $P < .10$ level

correlations were all highly significant ($P < .01$). Concentration was not significantly correlated with percentages of immature cells ($r = .17$ for percent live immature cells), although it did approach significance ($P < .10$) for percent dead immature cells ($r = -.22$).

Motility and vigor were very highly correlated ($r = .87$, $P < .01$), as well as being significantly correlated with morphology traits. Both were positively related to live cell percentages - and negatively related to dead cell percentages. Correlations with percent live cells ($r = .67$ and $r = .62$ for motility and vigor, respectively), percent live normal cells ($r = .69$ and $r = .65$) and percent live abnormal cells ($r = .31$ for both motility and vigor) were all highly significant ($P < .01$), as were correlations with percent dead cells ($r = -.67$ and $r = -.63$ for motility and vigor, respectively), percent dead normal cells ($r = -.50$ and $r = -.56$) and percent dead abnormal cells ($r = -.54$ and $r = -.50$). Although motility and vigor were both highly correlated ($P < .01$) with percent dead immature cells ($r = -.32$, in both cases), neither trait was significantly related to percent live immature cells ($r = -.01$ and $r = -.05$, respectively).

All of the morphology traits were significantly interrelated. Percent live cells was highly positively correlated ($P < .01$) with percent live normal cells ($r = .89$), percent live immature cells ($r = .29$) and percent live abnormal cells ($r = .50$), while, at the same time, it was highly negatively correlated ($P < .01$) with percent dead cells ($r = -.999$), percent dead normal cells ($r = -.94$), percent dead abnormal cells ($r = -.73$) and percent dead immature cells ($r = -.37$).

Although percent live normal cells was not significantly correlated with percent live immature cells ($r = -.13$), the relationship with percent live abnormal cells did approach significance ($P < .10$). Percent live normal cells was highly negatively related to percent dead cells ($r = -.89$), percent dead normal cells ($r = -.77$), percent dead abnormal cells ($r = -.76$) and percent dead immature cells ($r = -.41$).

Percent live immature cells and percent live abnormal cells was significantly correlated ($P < .01$) and each was also highly related ($P < .01$) with percent dead cells ($r = -.29$ and $r = -.50$, respectively) and percent dead normal cells ($r = -.41$ and $r = -.56$, respectively). Percent live abnormal cells was significantly correlated ($P < .05$) with percent dead abnormal cells ($r = -.24$); however, percent live immature was not ($r = -.02$).

Dead cell characteristics were also significantly related. Percent dead cells was highly correlated ($P < .01$) with percent dead normal cells ($r = .94$), percent dead immature cells ($r = .37$) and percent dead abnormal cells ($r = .73$). Percent dead normal cells was highly positively correlated ($P < .01$) with percent dead immature cells ($r = .33$) and percent dead abnormal cells ($r = .49$); however, percent dead abnormal cells and percent dead immature cells were not significantly related ($r = .07$).

Percent intact acrosomes were significantly related to some of the other semen quality characteristics. Concentration, motility and vigor were all highly correlated with percent intact acrosomes ($r = .77$, $r = .90$ and $r = .80$, respectively).

Sperm morphology characteristics showed varying levels of correlation with percent intact acrosomes. Percent live cells and percent live normal cells were highly correlated ($P < .01$) with percent intact acrosomes ($r = .65$ and $r = .64$, respectively), and less significantly ($P < .05$) with percent live abnormal cells ($r = .25$).

Dead cell characteristics were negatively related to percent intact acrosomes. Correlations with percent dead cells ($r = -.65$), percent dead normal cells ($r = -.62$), percent dead immature cells ($r = -.30$) and percent dead abnormal cells ($r = -.47$) were all highly significant ($P < .01$).

Relationships of Scrotal Circumference Measurements

Scrotal circumference measurements were not significantly related to any of the semen quality characteristics; however, the correlations with vigor ($r = .19$), concentration ($r = .19$), percent dead abnormal cells ($r = .20$) and percent intact acrosomes ($r = .17$) all approached significance ($P < .10$). Scrotal circumference was highly correlated ($P < .01$) with conception rate ($r = .46$), approached a significant correlation ($P < .10$) with estrous cycles completed ($r = -.17$), but was not significantly related to calving interval ($r = .02$).

Relationships of Reproductive Performance Traits

The reproductive performance traits of number of estrous cycles completed in the presence of the bull (hence referred to as "number of estrous cycles"), calving interval and conception rate were all highly related. Number of estrous cycles was highly correlated

($P < .01$) with both calving interval ($r = -.45$) and conception rate ($r = -.51$). Calving interval and conception rate were also highly correlated ($r = .45$, $P < .01$).

The reproductive performance traits showed no significant relationship with concentration, motility or vigor, and relatively few significant correlations with the sperm morphology characteristics.

Conception rate was not significantly correlated with any of the semen quality traits.

Number of estrous cycles was only found to approach significance ($P < .10$) when correlated with percent live abnormal cells ($r = .22$).

Calving interval did demonstrate slightly more significant relationships. It reached a significant level ($P < .05$) when related to percent live cells ($r = .24$), percent dead cells ($r = -.24$), percent dead normal cells ($r = .27$) and percent intact acrosomes ($r = .25$). Calving interval was highly correlated ($P < .01$) with percent live abnormal cells ($r = .32$).

Prediction Equations for Number of Estrous Cycles

The Maximum R^2 Improvement technique was used with the effect of experiment station location forced into the equation. In this case, 7.27% of the variation in number of estrous cycles was accounted for by station location.

The first variable added was percent live abnormal cells, which explained 4.07% more of the variation ($R^2 = .1134$). The 3-variable model expressing the most variation ($R^2 = .1400$) included the variables station, motility and percent intact acrosomes (with motility replacing percent live abnormal cells, for the time being).

Percent live abnormal cells was again added in the 4-variable model. This prediction equation explained 2.66% more of the variation than did the previous one.

The optimal equation was most likely the 5-variable equation, which included the variables station, concentration, motility, percent live abnormal cells and percent intact acrosomes. This model explained 20.00% of the variation in number of estrous cycles which the females completed in the presence of the bull, while the 16-variable model only accounted for 7.52% more variation, as shown in Table 6.

Prediction Equations for Calving Interval

Station location accounted for 37.05% of the difference in calving interval. The first variable entered was percent live abnormal cells, explaining 3.25% more of the variation.

The 3-variable equation, which added the variable percent intact acrosomes, only increased it 1.65% (R^2 = .4495), but the 4-variable model added 4.72% (R^2 = .4967). This model included motility.

Again, the optimal prediction equation included the variables station, concentration, motility, percent intact acrosomes and percent live abnormal cells. This model explained 52.52% of the variation in calving interval, while the 16-variable model only explained 4.67% more variation. This is demonstrated in Table 7.

Prediction Equations for Conception Rate

In this instance, station location had no effect at all (R^2 = .0000). The first variable entered, scrotal circumference,

Table 6. Maximum R^2 improvement equations for the variable estrous cycles

	Prediction Equations	R^2	R^2
1 - Variable model	STA	.0727	.0727
2 - Variable model	STA + .0054 LA	.1134	.0307
3 - Variable model	STA + .0035 MOT - .0081 PIA	.1400	.0266
4 - Variable model	STA + .0030 MOT + .0054 LA - .0077 PIA	.1765	.0365
5 - Variable model	*STA + .0027 MOT - .0096 PIA - .0050 LA + .1529 CONC*	.2000	.0235
6 - Variable model	STA + .0026 MOT - .0093 PIA + .0054 LA + .1829 CONC + .0140 COL	.2128	.0128
16 - Variable model	STA + .0035 MOT - .0106 PIA + .0190 LA + .2259 CONC + .0252 COL -.0114 SC + .0020 VOL - .0050 VIG - .0011 DI - .0251 LI +.0009 DA - .0010 DN - .0260 LN - .0177 D + .0076 L	.2752	.0624

*Considered to be the optimal equation for this variable.

Table 7. Maximum R^2 improvement equations for the variable calving interval

	Prediction Equations	R^2	ΔR^2
1 - Variable model	STA	.3705	.3705
2 - Variable model	STA + .4205 LA	.4330	.0625
3 - Variable model	STA + .3650 LA + .1112 PIA	.4495	.0165
4 - Variable model	STA + .4410 LA + .4970 PIA - .2060 MOT	.4967	.0472
5 - Variable model	*STA + .4649 LA + .6294 PIA - .1862 MOT - 10.5603 CONC*	.5252	.0285
6 - Variable model	STA + .4449 LA + .6083 PIA - .1825 MOT - 12.4830 CONC - .8993 COL	.5385	.0133
16 - Variable model	STA - .2280 LA + .5395 PIA - .2076 MOT - 11.8522 CONC - .9052 COL - .8949 LI - .2157 DN - .7236 LN + .7326 L - .1447 DA + .6020 VIG + .0765 SC + .1586 D - .0115 DN	.5619	.0234

*Considered to be the optimal equation for this variable.

accounted for 20.55% of the variation. This is in agreement with the high correlation between conception rate and scrotal circumference.

Volume was included to increase the explanation of this difference by 3.46% (R^2 = .2401), and color was also included, raising it another 4.39%.

In the 5-variable model, motility was entered and percent intact acrosomes replaced volume to account for 4.71% more variation. Percent dead abnormal cells was included in the 6-variable equation (R^2 = .3621) and volume was re-entered for the 7-variable equation to give the optimal prediction equation in this instance, explaining 39.00% of the variation in conception rate. The 16-variable model only accounted for 5.50% more variation. These equations are illustrated in Table 8.

Conclusions

Many of the semen quality correlations were similar in results to those of other researchers.

The high positive correlations between percent live cells, motility and vigor were as expected, as were the high negative correlations between percent dead cells, motility and vigor.

The interrelationships between live cell characteristics and dead cell characteristics were again as expected. Percent live cell abnormalities increased as percent live cells did, but this is attributed to the fact that total percent live cells increased.

The 3 reproductive performance traits were all highly related to each other, but not very significantly related to any of the semen quality characteristics. Of these, calving interval began to show

Table 8. Maximum R^2 improvement equations for the variable conception rate

	Prediction Equations	R^2	R^2
1 - Variable model	STA	.0000	.0000
2 - Variable model	STA + 3.460 SC	.2055	.2055
3 - Variable model	STA + 3.2557 SC + .9926 VOL	.2401	.0346
4 - Variable model	STA + 3.3860 SC + 1.1853 VOL - 2.4389 COL	.2840	.0439
5 - Variable model	STA + 3.5356 SC + .8346 PIA - 3.3820 COL - .1391 MOT	.3310	.0470
6 - Variable model	STA + 3.5516 SC + .7887 PIA - 2.7394 COL - .5567 MOT - .4049 DA	.3621	.0311
7 - Variable model	*STA + 3.4476 SC + .6875 PIA - 3.1019 COL - .5066 MOT - .3957 DA + .9177 VOL*	.3900	.0279
8 - Variable model	STA + 3.3870 SC + .7741 PIA - 3.3724 COL - .5532 MOT - .4070 DA + .8592 VOL - .2719 LI	.4080	.0180
16 - Variable model	STA + 3.1526 SC + 1.0446 PIA - 3.9740 COL - .6456 MOT + 1.2110 DA + .8927 VOL + .3824 LI + .8558 LA + .8122 VIG - .5988 D - 9.5397 CONC + .4041 L + .6048 LN + 1.6568 DN + 1.089 DI	.4449	.0369

*Considered to be the optimal equation for this variable

hints of relationships - perhaps with a larger number of observations, more significant correlations for all three characteristics could be obtained. Percent intact acrosomes followed this same pattern, showing relatively few correlations of significance.

Scrotal circumference also showed little significance. It was highly correlated with conception rate - again, perhaps with more numerous data, better results might arise.

The prediction equations presented appeared reliable for these date; however, it is doubtful that any one equation could be used to predict the fertility of a bull under these conditions.

CHAPTER V

SUMMARY

This study was an attempt to determine the relationship between reproductive performance traits of beef bulls in natural mating situations and subjective and objective semen quality characteristics.

The semen quality traits determined were: concentration, motility, vigor, volume, percent live cells, percent live normal cells, percent live immature cells, percent live abnormal cells, percent dead immature cells, percent dead abnormal cells, and percent intact acrosomes.

The reproductive performance variables calculated were: (1) the possible number of estrous cycles that all females completed in the presence of the bull, (2) the mean calving interval for parous, lactating cows and (3) the conception rate for the females exposed to each bull. The parity status of the females varied and all calculations were made as is to avoid penalizing the bull for the female's lack of conception because of this status.

Residual correlations between the semen quality traits and the reproductive performance variables were calculated. Highly significant correlations were obtained between the semen quality traits of conecntration, motility, vigor and percent live and percent dead cells, as well as among the various sperm morphology classifications.

The 3 fertility traits were all highly interrelated, but only approached significance when correlated with the semen quality traits.

Possibly, a larger number of observations could increase these correlations to a level of significance.

The Maximum R^2 Improvement technique presented prediction equations reliable for these data; however, these would not necessarily be reliable for larger quantities of data.

The results of this study indicate the following:

(1) bulls may be characterized according to the qualitative aspects of sperm cell morphology and acrosomal integrity,

(2) a range of semen quality traits exists in which it is difficult to separate small, subtle differences between bulls of average fertility,

(3) it is possible, however, to identify bulls which lie outside the limits of this range of semen characteristics,

(4) an extended study is needed, using more observations, to define these boundaries and perhaps reduce them, and

(5) with more refined techniques and larger data sets, perhaps broader implications of the results could be made.

In analyzing and interpreting a data set of this nature, the realization must be made that both the male and the female play large roles in the reproductive cycle, and only with large numbers of observations can differences due to one or the other become readily apparent.

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VITA

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She was married on June 9, 1979 to Max F. Hawkins.