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I am submitting herewith a thesis written by Charles Michael Irwin entitled "Relationships Between Sperm Cell Morphology and Changing State of the Acrosome and Their Possible Effect on Fertility of Bulls Mated in Natural Breeding Systems." 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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RELATIONSHIPS BETWEEN SPERM CELL MORPHOLOGY AND CHANGING STATE OF THE ACROSOME AND THEIR POSSIBLE EFFECT ON FERTILITY OF BULLS MATED IN NATURAL BREEDING SYSTEMS

A Thesis

Presented for the

Master of Science

Degree

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Charles Michael Irwin

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ABSTRACT

Twelve Angus, 2 Hereford, and 1 Polled Hereford bull were used to determine the effects of extended semen collection, and subsequent semen incubation, on qualitative semen characteristics, as well as the possible effects of these semen characteristics on the reproductive efficiency of the bulls. All semen samples were obtained by electroejaculation.

The semen characteristics evaluated were the percent of morphologically normal sperm cells with normal acrosomes (NN), the percent of morphologically normal sperm cells with abnormal acrosomes (NA), the percent of morphologically abnormal sperm cells with normal acrosomes (AN), the percent of morphologically abnormal sperm cells with abnormal acrosomes (AA), and the percent of live sperm cells.

Correlations were obtained between the semen characteristics and the various factors of the collection and evaluation procedures that could have influenced the semen characteristics. Highly significant ($P < .0001$) positive correlations were found between sperm cells of the NN classification and the percent of live spermatozoa, and between sperm cells of the NA classification and the percent of dead spermatozoa. A highly significant ($P < .0001$) negative correlation was found between sperm cells of the NN classification and those of the NA classification.

Regression analysis revealed that collection day, bull, incubation time, and the percent of dead sperm cells were the factors explaining most of the variation in the semen characteristics evaluated.

Fertility was defined as the calculated number of estrous cycles each female could have completed in the presence of a herd sire. All

lactating females were computationally allowed 42 days of involution when calculating their possible number of estrous cycles. All estrous cycles were calculated in a manner to avoid penalizing a bull for a female's lack of conception in an estrous cycle that could have started prior to exposure to a bull and terminated in his presence.

The effects of semen characteristics on fertility were analyzed by two methods. One was the regression of the percent of normal sperm cells with normal acrosomes and the percent of normal sperm cells with abnormal acrosomes on the calculated number of estrous cycles of only the females that calved as a result of exposure to a herd sire. The second analysis used the same semen characteristics regressed on the calculated number of estrous cycles of all females exposed to a bull.

Based on their different rates of improvement in the semen characteristics, the bulls were divided into two groups. Group I (5 bulls) produced semen of higher quality than Group II (8 bulls).

Regression analysis of fertility revealed that the semen characteristics in the NN and NA categories of Group I, for the overall incubation period, and after 8 hours of incubation, were significant ($P < .05$) in accounting for the greatest amount of variation in fertility within the Group I bulls. No significance was obtained in the analysis of Group II or of both groups combined.

The results of the study indicate that improved techniques exist to aid in the characterization of bulls on sperm cell morphology and changing state of the acrosome. These techniques could be used to separate bulls of either increased or impaired fertility from bulls of

average fertility. Separation of bulls with more subtle differences in fertility may be difficult to accomplish.

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CHAPTER I

INTRODUCTION

Economic and social pressures have placed demands on American livestock producers to improve the efficiency of production. Recognizing the reproductive and genetic factors involved have lead to the rapid growth and development of artificial insemination, particularly in the dairy industry. The beef industry has been more deliberate in accepting and developing improved methods for artificial insemination.

An understandable effect of the artificial insemination program has been the increased demand for, and the production of, bulls proven superior in genetics and efficiency. These bulls must be able to produce large quantities of fertile spermatozoa that can withstand freezing and storage at -196° C, post-freeze thawing, and still produce acceptable conception rates.

Due to this selection pressure, bulls in artificial insemination centers produce semen that is above the average quality of semen produced by bulls in natural matings. However, the large number of bulls used in natural mating will ultimately exert pressure on the livestock industry to attempt the development of a practical, reliable method for judging the semen characteristics of a bull in relation to his potential fertility. If this can be accomplished, the beef cattle industry will realize goals that many have thought to be improbable to attain. Until recently, no significant method has been developed to characterize a bull's semen and utilize this to judge his potential fertility. Most evaluations have included estimations of percent live spermatozoa, their

rate of movement (motility and vigor), percentages and types of structural abnormalities, and concentration measurements. Many researchers have found these semen characteristics to be positively correlated with fertility, and with the exception of recent acrosome work, no single semen quality assay has been effective in explaining a significant amount of the variation in fertility among bulls.

With the understanding that the acrosome has an important part in the fertilization process, many researchers have been encouraged to acquire more knowledge of the acrosome. Recent improvements in laboratory techniques, histochemical techniques, differential stains and microscopy have facilitated the study of acrosome characteristics. As a result, inclusion of acrosomal characteristics makes it possible to relate the state of the acrosome to the potential fertility of a bull.

The objective of this study was to investigate the efficacy of a recently developed differential acrosome staining technique (Wells and Awa, 1970), and its application in determining the aspects of changing semen characteristics and state of the acrosome on the potential fertility of beef bulls in natural mating systems.

CHAPTER II

REVIEW OF LITERATURE

Poor reproductive efficiency in beef cattle is a major factor in causing economic losses for beef cattlemen. The causes of reproductive inefficiency are many and varied. They result from management and environmental and physiological interactions; however, it is left to management to attempt control of all factors affecting livestock production.

As of now, the complexity and partial solution of the problem could depend on the individual cattleman's knowledge of his herd, and the degree to which he applies his knowledge of livestock production and management.

A major fault in any beef cattle production system is to consider the reproductive problem as being due solely to either the male effects or the female effects on reproduction. Both sides must be analyzed, within an individual herd, and an attempt made to assess the contributions of both sexes to the problem.

In this light, the first part of this review of literature will examine some of the aspects of the effects of age in each sex upon reproduction.

A. EFFECTS OF GENOTYPIC SEX AND AGE ON REPRODUCTIVE EFFICIENCY

Male Effects

Tanabe and Salisbury (1946) reported that young bulls between the age of 1 to 3 years, inclusive, showed the highest breeding efficiency

of all age groups. Within this group, the 2-year-old bulls possessed the peak in conception rates, which had a gradual decline with increasing age of the bull. The data of this study was obtained from the breeding records of the New York Artificial Breeders Cooperative from its beginning in June, 1940, until June, 1944. This included 12,621 total services of registered Holstein-Friesian heifers and cows to 41 bulls that ranged in age from 1 to 12 years. Morgan and Davis (1938) similarly observed that young bulls under 2 years of age required the smallest number of services for each conception. The number of services per conception varied little from 2 to 8 years of age.

A trial conducted by Almquist and Cunningham (1967) on 17 Angus and Hereford bulls between 1 and 2 years of age noted that with increasing age highly significant increases were observed in ejaculate volume, weekly output of motile spermatozoa, and sperm concentration.

Bowling et al. (1940) analyzed the data from the herd records of the Dairy Department of the West Virginia Agricultural Experiment Station. The data included 43 bulls ranging in age from 1 to 15 years. These workers found that the age of the bull had a significant effect upon the number of services per conception in the case of heifers. Bulls under 4 years of age, when bred to heifers, showed a greater efficiency than bulls of any other age. As the bulls increased in age, a gradual decrease in breeding efficiency resulted, but this decrease did not become significant until the bulls reached an average age of 6 years. Bulls, 13 years old and over, were significantly less effective as breeders than bulls 6 years of age.

McCullough et al. (1950) secured breeding records for yearly average 60-to-90-day non-returns (conception rate) for 164 Holstein, 72 Jersey, and 117 Guernsey sires. These bulls had been used 2 or more full consecutive years and were owned by 18 artificial insemination associations representing 17 states. The bulls ranged from 2 to 13 years of age when they were first used. When the data on these bulls were arranged into age groups, no uniform trend was found for either improved or lessened conception rate with an increase in age. Baker and Quesenberry (1944), doing a range study involving beef cattle, reported no significant effect due to the age of the bulls. These results are in contrast with the usual ideas and conclusions from other studies, possibly because of fewer numbers of older bulls, due to a more intense culling rate at younger ages. Generally, it is thought that a sire becomes less useful with advancing age, particularly beyond 6 or 7 years of age. Factors that could now reduce the affect due to advancing age of sires used in artificial insemination are improvements in techniques and semen extenders used in artificial insemination centers. It is possible to select bulls with high conception rates at ages ranging from 2 to 13 years of age.

Female Effects

Many researchers have indicated that heifers require more services per conception than more mature females. Increased embryonic mortality may be the cause of decreased reproductive efficiency in heifers. Tanabe and Almquist (1953) concluded that there appeared to be a high fertilization rate among infertile heifers with subsequent high embryonic death within the first month of pregnancy. In this study, 96 heifers

were inseminated and slaughtered within the first 30 days of gestation to determine the occurrence of embryonic mortality.

Armstrong and Shandys (1961) conducted a study, involving 5,674 cows and heifers exposed to breeding during the year, to determine the importance of management practices on calving percentage, and on the economics of production. It was noted that the average conception rate for heifers was 12 percent lower than for cows. Donald and Russell (1968) noted an 11 percent higher conception rate of cows over heifers.

Rhoad (1944) found little difference between cows and heifers in the number of services for conception, based on records from 12 years of pasture matings. The date of conception was indirectly determined by counting back 282 days from the time of birth of the calves. This allowed the calculation, using an average length of estrus (20 days), of the expected number of services the female received. He found that 52 percent of the females became pregnant in the first 20 days of the breeding season, 80 percent pregnant at the end of 40 days, and 90 percent pregnant by the end of 60 days.

Erb and Holtz (1958), in a study which covered a 30-year period (1920-1950) and involved 9,994 breeding periods of 2,607 Holstein-Friesian cows at Carnation Farms, found that clinically normal heifers apparently fertilized as efficiently as normal cows in their second and third reproductive periods (51.3 versus 52.4 percent fertilizations per service). Similarly compared, normal heifers had a significantly greater total embryonic loss, and fewer fertilizations and services carried to term.

Plasse et al. (1968) used 3,938 Brahman calves, from four ranches in Florida, with limited breeding seasons, to evaluate reproductive

performance of their dams. This study revealed that heifers conceived 65.5 $\pm$ 1.7 days after first exposure to a bull, while cows which had calved at least 1 month before the breeding season started conceived after 45.5 $\pm$ 1.1 days; the difference being highly significant. It was further noted that 4- to 5-year-old cows were more likely to fail to calve than older cows. The interval from exposure to conception was greater the longer the breeding season lasted.

Temple et al. (1964), from a report involving cattle in the Southeast, Texas, and Oklahoma regions, removed more 2- and 3-year-old cows, because of reproductive deficiencies, than for any other age group. Four and 5-year-old cows were the next highest group showing non-conception, but as cows reached 5 years of age, reproduction increased.

In Louisiana, Wiltbank and Harvey (1963) found a definite age difference in conception rate in a study involving Brahman and Angus cattle. In this experiment, age of cow and whether or not the dam was nursing a calf were noted. Conception rate was as follows:

1. 3- to 4-year-old wet cows, 58.2 percent;
2. 3-year-old heifers, 62.3 percent;
3. Wet cows over 10 years of age, 63.4 percent;
4. 5- to 10-year-old wet cows, 73.4 percent;
5. 2-year-old heifers, 75.1 percent;
6. Dry cows over 10 years of age, 76.9 percent;
7. 3- to 4-year-old dry cows, 79.5 percent; and
8. Cows with the highest conception rate were 5- to 10-year-old dry cows, 82.1 percent conceiving in the first breeding period of 21 days.

It was also evident that normal cows, in their fourth and fifth reproductive periods, had the highest percentage of services carried to term because of a marked decline in the estimated embryonic loss, as compared to younger cows. Also, cows in their fourth and fifth reproductive periods carried significantly more services to term than did older cows. They concluded that fertilization per service and embryo loss declined markedly with increasing age. The finding that cows reached their peak in reproductive efficiency at approximately the fourth pregnancy was agreed upon by Davis (1951), Ehlers et al. (1954), Olds et al. (1949), and Tanabe and Salisbury (1946).

Cunha et al. (1967) used the British breeds, Brahman and British-Brahman crosses, and found that British and crossbred cows were fairly constant in reproduction from 2 to 8 years of age, but the Brahmans were very erratic in their breeding behavior. British cows 4 years old and older at the time of breeding, up to and including 9-year-old cows, had a similar reproductive performance, with an overall conception rate of 83 percent. After 10 years of age, conception rate dropped to 75 percent.

During a 10-year period, Lasley and Comfort (1962) found that the bulls to which cows were exposed were a highly significant ($P < .05$) source of variation in the calving intervals of the cows.

Combined Effects

Tanabe and Salisbury (1946) observed that the relationship between the age of cows, or bulls, and the conception rate was not a simple linear regression. The bulls and cows were classified into 3-year age groups, and it was noted that young bulls between the ages of 1 and 3, and cows between the ages of 4 and 6, possessed a higher breeding

efficiency than any other group.

Bowling et al. (1940) indicated that when no consideration was given either to the age of the bull or the age of the female to which he was mated, the heifers that were bred for first calving required a larger number of services per conception than did females of any other group. After the first conception, however, the number of services per conception did not vary to a marked degree, up through the sixth conception, which was as far as the study was carried. This study included 706 females.

Hilder et al. (1944) found that young bulls were more efficient than older bulls when bred to cows of all ages, but their greatest margin over older bulls was on first-gestation females. Seven of these bulls (averaging 3 years of age), when bred to virgin heifers, obtained 164 conceptions from 279 services, an average of 1.70 services per conception. This average was significantly smaller than the average (1.90) for all bulls under 5 years of age, when bred to heifers.

Morgan and Davis (1928) concluded that among bulls of varying ages mated with cows of varying ages, the 2-year-old bulls mated to 2-year-old females required the fewest number of services per conception (1.80), as compared with the number of services per conception (2.52) for bulls of all ages. Bulls 3 years of age and older lacked efficiency when mated with females under 2 years of age. Cows and bulls between the ages of 2 and 8 years showed little influence of age upon the number of services required for conception. When bred to bulls of all ages, the number of services required for conception of the 2,090 cows of varying ages was 3,041 for 1,375 conceptions, or 2.21 services per conception.

Lindley et al. (1959) found from 31 bulls used for 6 years, a significant negative correlation ($r = -.62$) existed between the age of the bull and semen motility. Differences between the age of the bull and the age of the cow, in the number of services per conception, were both significant ($P < .05$), with the most efficient matings being from 5-year-old bulls and 4-year-old cows.

B. EFFECTS OF SEMEN QUALITY AND SPERM MORPHOLOGY ON FERTILITY

In the past 50 years, a great deal of research effort has been devoted to the study of semen characteristics and their possible effects on fertility. There are several reasons for this search, one of which is the relative ease in collecting millions of germ plasm cells from the bull and subjecting the cells to a variety of subjective and objective analytical procedures. Second, is the fact that, genetically, the bull contributes 50 percent of the genetic makeup of the calf crop, and a higher percentage of the herd's productiveness when his daughters become a part of the breeding herd. Being able to collect and examine semen from many bulls affords the opportunity to compare bulls in categories other than the number of calves their respective breeding groups produced. From these qualitative and quantitative semen comparisons, research workers have hoped to find a reliable method of judging a bull's potential fertility, even though it is recognized that, due to numbers alone, the cow herd represents a greater source of biological variation in reproduction. However, by virtue of the genetic contribution of a single animal (the bull), it is still possible to exert more selection pressure for improvement in livestock production through the male.

Natural Mating

Williams and Savage (1927) summarized their clinical observations in the following manner: (1) the dimensions of sperm heads from good fertility bulls are remarkably uniform; (2) no highly efficient bulls should produce more than 17 percent abnormal sperm; and, (3) permissable numbers of abnormal sperm in an ejaculate depend largely on the morphological types of abnormalities present.

Falk and Kaufman (1950) stated that fertilizing capacity of an ejaculate depended upon the interrelationships of volume, motility, concentration, and morphology. To insure fertilization, all these should necessarily be of top quality. They pointed out that the only important factor which could compensate for any deficiencies present was good morphology. Hence, they felt that morphology has a higher stabilizing influence for fertility than any other aspect of semen quality.

Salisbury and Van Demark (1961) stated that the proportion of the abnormal spermatozoa to normal spermatozoa in ejaculates determined the fertility of the bull. Also, some reduced fertility was indicated by morphologically normal spermatozoa that were deficient in DNA.

Salisbury et al. (1942), Mercier and Salisbury (1947b), and Rao and Hart (1948) all reported that the morphological examination of semen was most representative if done as soon as collected. Methods of collection, technique of handling, and examination influenced the proportion of morphologically abnormal spermatozoa observed. Faulty laboratory procedures increased the anomalies, and they concluded that true abnormalities were generally lower than reported.

Salisbury et al. (1942) showed that, at least in some cases, the semen of individual males varied in its capability to withstand laboratory examination procedures. They indicated that when the proportion of morphologically abnormal spermatozoa alone was used as the criterion of fertility, the importance of more than one sample of semen from a male could not be overemphasized.

Rao and Hart (1948) concluded that semen containing protoplasmic droplets in relatively low numbers (less than 1 percent) should be considered normal.

Bioly and Smith (1958) found the distribution of spermatozoa with protoplasmic droplets, from slaughter bulls in 11 paired and 9 single epididymides, was 62, 42, and 84 percent, respectively, in the caput, corpus, and cauda epididymis. The variation between the right and left epididymides of the same bull was less than among bulls. About 6 percent of the spermatozoa in the ampullae had droplets. They also reported that the percent epididymal spermatozoa with protoplasmic droplets changed when fluids from the seminal vesicles were added. In the corpus and the cauda epididymis, the percent of spermatozoa with distal protoplasmic droplets, and with proximal droplets, decreased significantly ($P < .01$) when seminal fluid was added.

Branton and Salisbury (1947) studied the morphology of spermatozoa from different levels of the male reproductive tract, and reported no significant differences in the proportion of abnormal spermatozoa, and that abnormalities affecting the heads of the spermatozoa were the predominant types. They concluded that the origin of morphologically abnormal spermatozoa was the testis. Also, no statistically significant

difference was observed between bulls in the proportion of spermatozoa with attached droplets at any given level of the tract. In the head of the epididymis, the protoplasmic droplets were usually located on the neck of the spermatozoa, and in the ampulla the droplets were usually absent. This led to the conclusion that these were morphological maturation stages and were the same for all normal bulls in the development of the spermatozoa.

Some research has found that fertility appears to decrease with an increase in the number of morphologically abnormal spermatozoa. Swanson and Herman (1944) found a significant negative correlation between percent morphologically abnormal sperm and conception rate ($r = -.12$). They also reported a highly significant curvilinear correlation between motility and conception rate ($r = .97$).

An early study attempting to predict fertility under natural breeding conditions was conducted by Lasley and Bogart (1943). Twelve Hereford bulls were utilized to define some of the factors influencing reproductive efficiency. They reported a curvilinear correlation ($r = .708$) between concentration and fertility. Fertility varied little when the percentage of live sperm per ejaculate ranged from 50 to 90 percent. Fertility decreased as the percentage of live sperm fell below 50 percent.

Wiltbank (1963) found that bulls having a 70 percent conception rate had 5 to 9 percent abnormal spermatozoa, while bulls with a 60 percent conception rate had 10 to 18 percent abnormal spermatozoa, and those with a 52 percent conception rate had 20 to 28 percent abnormal spermatozoa. Bulls with more than 50 percent abnormal spermatozoa had

an average conception rate of 36 percent.

Carroll et al. (1963) examined 10,940 ejaculates for semen quality. The characteristics determined were motility, concentration, morphology, and percentage live sperm. Semen was quality graded as follows:

1. very good,
2. good,
3. fair, or
4. poor.

They found that morphology had the greatest correlation to the final semen classification. A numerical score was used in the final classification:

1. 60 and above was considered satisfactory,
2. 30 to 60 questionable, and
3. below 30 unsatisfactory.

Their results indicated that 82.9 percent of the bulls were satisfactory, 11.8 percent questionable, and 4.9 percent were unsatisfactory.

Wiltbank et al. (1965) grouped bulls into four categories based on semen characteristics. These categories were as follows:

1. good motility,
2. poor motility,
3. moderate abnormal sperm, and
4. excess abnormal sperm.

They found that only bulls of the fourth category showed a marked decrease in fertility, as compared to other bulls. Motility had the highest correlation when used to predict fertility ($r = .40$). Their conclusion was that semen quality is related to the percentage of abnormal spermatozoa and percent motile spermatozoa.

Onks (1972) found a highly significant correlation (r = .994) between conception rate and calving rate (P .01). Both conception rate and calving rate were negatively correlated to percent abnormal sperm (r = -.508, -.526, respectively). Thus, abnormal spermatozoa do have an inverse effect upon bull performance. A significant correlation (r = .489) was found between motility and the number of cycles before conception. The day of conception was also significantly correlated with motility (r = .364), and days from parturition to conception showed a significant correlation (r = .526) with percent abnormal spermatozoa. Percent abnormal spermatozoa accounted for the most variation in conception rate (R^2 = .258).

Onks (1972) found that a 2.5 percent increase in abnormal spermatozoa caused a 1 percent drop in conception rate, while a 2.7 percent decrease in motility caused an increase of an extra heat cycle before conception. A drop in motility caused the day of conception to increase by one day. He concluded that: (1) the quality of beef bull semen had an effect upon the reproductive performance of beef cows; and, (2) percent motility and percent abnormal spermatozoa played the largest part in determining the degree of reproductive efficiency an individual bull possesses.

Season of the year has been proven to affect fertility in females by Mercier and Salisbury (1947a), who found a significant correlation between season and fertility (P <.05). Conception rate was highest in summer and lowest in the winter and spring. The highest conception rate was during July, while the poorest was in February. The reason stated for this is the length of daylight, which was more closely associated

with conception rate than temperature.

Johnston and Branton (1953) found significant correlations between temperature and fertility. Bulls were collected weekly for a year. Fertility was based on 60- to 90-day non-return rates, and contrary to Mercier and Salisbury (1947a) and Anderson (1945), they found fertility highest during the winter, spring, and early summer. Significant correlations ($P < .01$) were found between fertility and maximum temperature ($r = -.46$), fertility and minimum temperature ($r = -.45$), and fertility and vapor pressure ($r = -.55$). They concluded that adverse temperatures affect the fertility of both the male and female bovine.

Artificial Insemination

Ludwick et al. (1948) found a significant correlation between incubation time and conception rate ($r = .84$), where incubation time was the time required for sperm to lose all activity at 37.8° C, and conception rate was based on 60- to 90-day non-return rates.

Lasley (1951) found a significant correlation ($r = .314$) between fertility and percent motile sperm. For each 10 percent increase in percent motile sperm, fertility increased 4.6 percent, and he concluded that motility of spermatozoa is a good indicator of semen quality.

Buckner et al. (1954) reported that 90 percent of the variation in fertility of bulls could be accounted for by using a combination of seven tests. They were:

(1) methylene-blue reduction time,

(2) drop of progressive motility in 120 minutes at 4° C,

(3) initial total motility,

(4) pH drop in 1 hour,

(5) drop of progressive motility at 45° C,

(6) total motility after 2 days, and

(7) initial total motility by dilution rate.

During this study, the among-bull correlations, in most instances, were higher than the total or within-bull correlations. They concluded that fertility prediction among bulls is more reliable than prediction on a within-bull basis. The best single estimates for predicting differences in fertility of bulls were progressive mobility after incubation for 16 hours at 38° C ($r = .90$), total motility after the same treatment ($r = .86$), and methylene-blue reduction time ($r = .72$).

Cupps et al. (1959) graphed breeding efficiency as compared to motility, live spermatozoa, and morphologically abnormal spermatozoa. They felt that conception rate may be impaired when less than 43 percent of the sperm are living, less than 37 percent are motile, or more than 35 percent are abnormal.

During a 9-year breeding period, about 1,000 inseminations per year were made using semen from 51 bulls, 200 inseminations from 17 ejaculates per bull (Clarke et al., 1973). Conception rates were calculated for each bull based on 180-day non-returns. The mean value for estimated percentage motile sperm significantly increased ($P < .001$) over time. Conception rate and motility score were significantly correlated (Spearman's rho = .306, $P < .025$), but conception and estimated percentage motile sperm were not (rho = .187, $0.20 > P > .10$).

Clark et al. (1973) stated that the significant correlation between conception rate and motility score was unimportant, as the scores only ranged from 4.6 to 5 for motility estimated on a 1 to 5 scale, 5

being the highest score. Conception rates ranged from 44 to 75 percent, leading to the conclusion that semen of a relatively high subjective quality would give a wide range of fertility and that semen fertility can best be assessed from actual conception rates under field conditions.

Neville et al. (1971) found correlations ($P < .01$ if $r \pm .15$) between fertility and semen characteristics to be: (1) fertility to volume, .07; (2) fertility to sperm cell concentration, .18; and, (3) fertility to motility, .10.

Other correlations were: (1) motility to volume, .02; (2) motility to sperm cell concentration, .21; and, (3) sperm cell concentration to volume, .25.

They concluded that sperm cell concentration is the best predictor of semen quality, but none of the characteristics is a good indicator of fertility, not even motility, which is the most widely accepted indicator of semen quality.

In one study using 6 bulls (8 semen samples per bull), Munro (1961) found a reduction in fertility (<74 percent conceptions) occurred when the incidence of live spermatozoa dropped below 70 percent. The abnormality percentages, ranging from 10.1 to 32 percent, did not appear to have any influence on fertility. Later, in the same study, Munro (1961), using an average of 10.5 semen samples from 17 bulls, found the mean conception rate declined as the incidence of primary and secondary abnormalities increased. Reduced fertility (<66.6 percent conceptions) was usually accompanied by a total primary and secondary abnormality incidence of over 23 percent, especially when the percentage of primary abnormalities was 18 percent or higher. He concluded that fertility

was usually reduced when either the incidences of live spermatozoa fell below 70 percent, or those of primary and secondary abnormal spermatozoa rose above 23 percent, with primary abnormalities having the greatest effect on fertility.

In other species, Singleton and Shelby (1972) found a significant correlation ($r = .29$, $P < .05$) between percent normal sperm from boars and fecundity in gilts (the number of embryos 25 days post-insemination, expressed as a percent of corpora lutea). A significant correlation also existed between sperm concentration and fecundity ($r = .61$, $P < .01$). They concluded that:

1. The semen characteristics studied are not good indicators of boar fertility, accepting the fact that when the semen characteristics are above or below some optimum level, they might affect fertility; and,

2. The significant variation that existed between boars was not large enough to greatly affect fecundity in the gilt.

Hulet et al. (1965) found in sheep, that motility score and percentage motility were significantly correlated with fecundity ($r = -.25$ and $.22$, $P < .05$). Fecundity was defined as the percentage of lambs born of ewes that lambed in each mating group. Four morphological semen traits were also significantly correlated with fecundity ($P < .01$). The percentage live normal cells ($r = .33$), percentage abnormal cells ($r = .40$), percentage abnormal necks ($r = .42$), and percentage abnormal middlepieces ($r = .44$). The correlation between ram fertility and fecundity of ewes, within age groups, was 0.23 and highly significant. The correlation was highest ($r = .30$) in ewes 4 years of age and older.

They concluded that the correlations between semen quality and fecundity signifies that the correlation between ram fertility and ewe fecundity might be attributed mainly to the physiological fertilizing capacity of the ram's semen. They also suggested that the fertilization of one ovum, in ewes producing two ova, is partly independent of the fertilization of the other ovum.

C. THE ACROSOME

Origin

Blom (1946) demonstrated the presence of a sperm cap in the bull and stallion and identified it as the "galea capitis." This term was also used by Bretschneider (1949, 1951) who stated that this structure of the mature spermatozoa of the bull is composed of inner and outer parts.

The term "acrosome cap" was first adopted by Blom and Birch-Andersen (1965).

The acrosome appears to originate from the golgi apparatus of the spermatids during spermiogenesis. Leblond and Clermont (1950) demonstrated the presence of a periodic acid--Schiff (PAS) reactive material in the acrosome and golgi apparatus of the spermatids of adult rats. Greeson (1951) suggested that the acrosome is formed from the proacrosome and the vacuoles surrounding it. Gumbo (1966) stated that the golgi apparatus might secrete the acrosomal material.

Bishop and Walton (1952) stated that after the formation of the proacrosome, which lead to the formation of the acrosome cap, the golgi remnant migrated to the posterior end of the maturing spermatid and was

shed from the cell with the residual cytoplasm of the droplet.

Wu and McKenzie (1955) felt that the acrosome and galea capitis were two different structures and disagreed with the conclusion of Hancock (1952) that the terms acrosome and galea capitis have been used to describe the same structure in bull sperm.

Structure

Hancock (1952, 1957a) described the acrosome of the bull and boar as a cytoplasmic cap covering about two-thirds of the anterior portion of the sperm head. It was believed that the acrosome was composed of two parts, the larger outer acrosome which had a straight posterior border coinciding with the anterior border of the post-nuclear cap, and the inner acrosome that possessed a concave border directed caudally with its convex border following the outline of the front of the sperm head.

Hancock (1957a, 1957b) stated that the area between the posterior straight border of the outer acrosome, and the posterior concave border of the inner acrosome, should be called the equatorial segment. This was also the opinion of Lovell and Getty (1960). Rahlman (1961) suggested that the acrosomic cap of the bull overlaped the post-nuclear cap. These reports are not as conclusive on the acrosome structure, as more recent work points out.

Blom and Birch-Andersen (1965) found the nucleus to be covered by three membranes, the cell membrane, the acrosome, and the nuclear membrane. They stated that the acrosome cap covered about 60 percent of the anterior part of the nucleus in bull sperm. They described the acrosome as an electron-dense, double-walled structure. The apical body

was a characteristic thickening along the anterior edge of the sperm head and was composed of a more or less closed fold, the apical fold, and a thickening containing a smaller body of thinner density called the apical vacuole.

The anterior part of the acrosome was found to consist of a double-walled sac containing a substance of low electron density. The outer membrane showed a well defined swelling, which was the apical body, while the inner membrane was closely adherent to the nucleus. The perforatorium was found at the tip of the nucleus beneath the inner membrane of the anterior of the acrosome. The posterior portion of the acrosome appeared to be identical with the equatorial segment. The saggital sections of the sperm head clearly showed this segment to be a part of the acrosome, in which the outer and inner membranes were closely parallel, containing a layer of high electron density. They suggested that the outer membrane of the anterior part of the acrosome was identical with the galea capitis of Blom (1946), and only this membrane was shed spontaneously, artificially in vitro, or during fertilization. Following this occurrence, the inner membrane remained on the perforatorium and the anterior part of the nucleus, while the equatorial segment remained attached to the midsection of the sperm head. They suggested that the detachment of the galea capitis released acrosomal enzymes.

The findings of Saacke and Almquist (1964) were in agreement with the description of the structure of the acrosome given by Blom and Birch-Andersen (1965). Saacke and Almquist (1964) confirmed the presence of the apical body at the tip of the sperm head but could not confirm the presence of the apical vacuole within the apical body. They suggested

that they might not have been able to obtain identical sagittal sections as those of Blom and Birch-Andersen (1965), or that the spermatozoa might show individual variation with respect to the structure of the apical body. The equatorial segment was also found by Saacke and Almquist (1964) to be a part of the acrosome, and they agreed with Blom and Birch-Andersen (1965) that the detachment of the acrosome cap included only the outer membrane and not the entire structure.

A schematic drawing outlining the structure of the bovine sperm head is presented in Figure 1.

Perforatorium

Clermont et al. (1955) demonstrated the presence of the perforatorium, which was distinct from the acrosome. They considered the rat perforatorium to be a thickening of the nuclear membrane that fitted tightly over the apex of the nucleus. They stated that it did not stain with the periodic acid-Schiff technique (while the acrosome did), and it was resistant to alkaline hydrolysis (while the acrosome was not). Similarly, Bishop and Austin (1957) found this structure in the spermatozoa of the cotton rat.

Nicander and Bane (1962) and Bane (1963) studied the perforatorium in the bull, boar, ram, dog, rabbit, and guinea pig, and found small amounts of electron-dense material between the acrosome and the nuclear membranes of all these species, mainly along the anterior part of the sperm head. These findings were confirmed by Blom and Birch-Andersen (1965).

White (1962) stated that the loss of the acrosome exposed the perforatorium, which might have some unidentified enzyme that facilitated

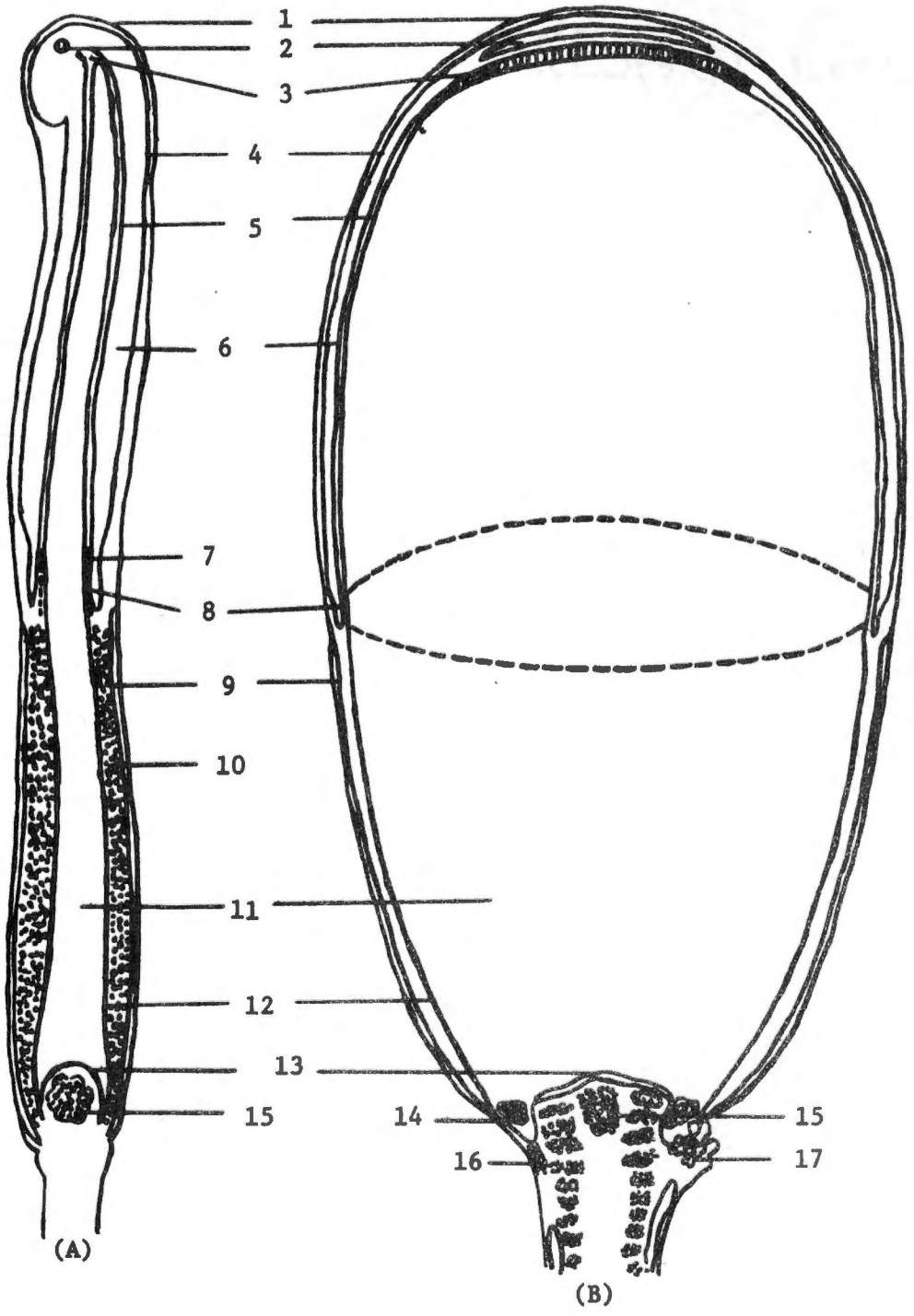


Figure 1. Schematic drawing of the normal bull sperm head. (A) Median-sagittal section; (B) true coronal section.

1. Cell membrane; 2. apical vacuole; 3. perforatorium; 4. outer membrane of the acrosome cap; 5. inner membrane of the acrosome cap; 6. acrosome cap (contents); 7. intermediate layer; 8. equatorial segment; 9. post-nuclear cap; 10. intermediate layer; 11. nucleus; 12. nuclear membrane; 13. basal plate; 14. basal knob; 15. centriole; 16. evaginated nuclear membrane; 17. evaginated nuclear membrane (convoluted type). (Blom and Birch-Andersen, 1965)

sperm passage through the zona pellucida.

Environment and Aging Effects

Bedford (1963) found a significant ($P < .01$) reduction in the size of the rabbit acrosome during the passage of the sperm cells from the head of the epididymis to the tail of the epididymis. He proposed that the acrosome of the rabbit matured in the epididymis and not in the testis, as he found a significant correlation between the width of the acrosome and the position of the cytoplasmic droplet on the midpiece. The acrosome contracted during epididymal passage, becoming more closely apposed to the nuclear surface. This action was suggested to have been a prerequisite for the acrosome to have acquired maturity and fertilizing capacity.

With similar results, Dickey et al. (1965) found gradual changes in the acrosome cap of bull sperm, which took place in the caput, corpus and cauda epididymis. This resulted in changing the acrosome from a wavy, loose-fitting structure in testicular sperm, to a smooth structure, closely adherent to the nucleus in ampullary and ejaculated sperm.

Hancock (1952, 1957a) stated that the detachment of the acrosome was a sign of the death of the sperm cell.

Hathaway and Hartree (1963) observed spontaneous detachment of the acrosome in a small proportion of ejaculated sperm cells from bulls and rams.

Iype et al. (1963) found that the acrosome was completely lost from bull and buffalo sperm cells suspended in water, subjected to cold shock (0° C), and subsequently rewarmed to room temperature.

Lovell and Getty (1960) stated that as boar spermatozoa age, the outer acrosome became enlarged and swollen and in later stages became wrinkled. Further aging resulted in a great percentage of acrosomal detachment. These authors noted a large number of motile bacteria in the seminal fluid after 24 hours of storage and suggested that the detachment of the outer acrosome may have been due in part to bacterial or enzymatic action.

Onuma (1964), studying acrosomal abnormalities in bulls and boars, found that the percentage of acrosomal abnormalities increased with storage and aging. He also concluded that the susceptibility of the acrosome to temperature shock was greater in boars than in bulls.

Pursel et al. (1972), using unextended boar semen, found that sperm acrosomes were altered, and sperm motility was reduced by cold shock at 0°, 5°, 10°, and 15° C for 10 minutes. The sperm acrosomes developed cold shock resistance during 2.5 and 4.5 hours of incubation at 30° C. Acrosomes of whole ejaculate spermatozoa were more susceptible to cold shock than were the acrosomes of the sperm rich fraction.

Pursel et al. (1974) reported that when boar spermatozoa were stored at 15° and 25° C, the apical ridges enlarged, and their smooth, crescent shape became irregular. The acrosomes deteriorated more rapidly when stored at 25° C than at 15° C.

In agreement with Onuma (1964), Jones (1973b) found that the plasma membrane of bull spermatozoa ranked higher than those of the boar or ram in withstanding osmotic shock due to pressure changes or changes in buffer concentration. They were the least susceptible to damage from handling in the laboratory or preparation for artificial insemination.

Awa (1968) found that bovine sperm cells incubated at 37° C for 12 hours or stored undiluted at 5° C produced a gradual elevation of the acrosomal cap, loosing the smooth outline and becoming wrinkled and irregular in shape. He found the equatorial segment became more visible upon aging the sperm cells. Incubated sperm cells (37° C) deteriorated rapidly to 70 percent elevated acrosomes in 8 hours.

Abstinence and Regular Collection

Blom (1946) observed up to 18 percent spontaneous detachment of the acrosome (galea capitis) in fresh semen of Danish bulls collected after 3 or more weeks of abstinence. This percentage was reduced to zero with subsequent regular collection of semen. The same phenomenon was also reported by Blom (1946) in stallions, and he concluded that the acrosomal detachment was due to a prolonged stay of the spermatozoa in the epididymis and represented the first stages of acrosomal disintegration.

Wondafrash (1968) found that of 17 Angus and 23 Hereford bulls in a state of sexual rest, most of the bulls averaged not more than 18 percent morphological abnormalities of sperm cells. He found initial ejaculate quality was low. It was also reported that significant differences ($P < .10$) were found between breeds in percent abnormal cells and tailless cells per ejaculate. Hereford bulls emitted semen of lower quality than did the Angus bulls. There were no breed differences in the distribution of abnormal acrosomes, while the percentage associated with each cell type was highly significant ($P < .01$). The percentage of acrosomal abnormalities in each cell type was significantly different ($P < .01$) from other cell types. It was stated that the percentage of abnormal acrosomes in normal cells should be evaluated for its potential

in improving results from semen evaluation.

Awa (1968) studied the semen of a Jersey bull that had been in a state of sexual rest for 6 months and found 19.5 percent capless (missing or detached acrosome) sperm cells.

Wells et al. (1970) confirmed the findings of Wondafrash (1968) and found that the first ejaculate from young bulls in a state of sexual rest produced low quality semen (51.2 percent live cells, 82.1 percent normal cells, 17.9 percent abnormal cells, and 7.9 percent tailless cells). Sixteen and one-half (16.5) percent of the normal cells, 44.5 percent of the morphologically abnormal cells, and 76.7 percent of the tailless cells possessed abnormal acrosomes.

In a group of five sexually rested bulls collected once a week for 6 weeks, Wells et al. (1970) found that the number of normal cells with normal acrosomes changed from 73.1 percent at the first collection, to 92.9 percent by the sixth collection. The total percentage of abnormal acrosomes dropped significantly ($P < .05$) from 29.3 percent in the first collection, to 12.7 percent by the sixth collection. There were significant ($P < .05$) differences among bulls in this measurement. They suggested the distribution and weekly changes in acrosomal characteristics of normal cells implies that this ejaculate characteristic shows a higher variability than the level of normal cells. The aged (in vivo) semen from sexually rested bulls possessed marginal motility, a high percentage of abnormal cells, a high percentage of acrosomal abnormalities, and a significant percent of normal cells having abnormal acrosomes. They concluded that acrosome evaluation should be added to routine semen evaluation procedures.

Subsequently, Wells et al. (1971) found that frequent ejaculation reduced the percent of abnormal acrosome characteristics. Bulls ejaculated four times weekly exhibited a steady decline in acrosomal abnormalities, with less variation than a once-a-week ejaculation group. The differences between the groups was 11.8 percent and 23.7 percent, respectively. A highly significant effect ($P < .005$) was found between groups. The bulls ejaculated four times weekly were found to maintain 90 to 95 percent normal acrosomes, while the once-a-week group maintained 75 to 80 percent normal acrosomes. They stated that the degree of aging was typically lower in second ejaculates and that a period of rest of 4 weeks or more resulted in large increases of aged acrosomes. They concluded that if aging of the acrosome is related to the fertility of a bull, then practical use of frequent ejaculations would result in a greater harvest of potentially useful sperm cells.

Acrosomal Enzymes

McClean and Rowlands (1942) found a close correlation between the dispersion of the cumulus oophorus and the concentration of hyaluronidase. They suggested that the gel surrounding the ovum contains hyaluronic acid, which is liquified by the enzyme so that the effective sperm can penetrate the ovum. They also implied that certain types of sterility may be a result of a deficiency of the enzyme.

Johnston and Mixner (1947) found hyaluronidase in bull seminal plasma immediately following ejaculation. They reported that the sperm cells were the source of the enzyme. Storing the semen at 5° C for 144 hours gave a 400 percent increase in concentration of hyaluronidase.

Storage at 37° C for 72 hours gave a 645 percent increase in concentration. In both cases, motility decreased, very drastically in the latter instance.

Rao and Berry (1949) stated that the cytoplasmic cap is probably involved in the secretion of hyaluronidase.

Austin (1960) treated rabbit semen with digitonin, a substance capable of removing the acrosome from sperm heads, and also treated another sample with formalin, a substance known to preserve the acrosome on the sperm heads. The supernatant from the first sample, which contained the digitonin, had 22 units of hyaluronidase per ml, compared with 2.4 units per ml in both untreated semen and the supernatant of semen treated with formalin. Austin (1960) felt that hyaluronidase might be released from the acrosome during sperm capacitation through changes in the properties of the acrosome.

Hathaway and Hartree (1963) found that the disruption of the acrosome was accompanied by the release of hyaluronidase and suggested that at least part of the enzyme is present in the acrosome.

Mann (1964) stated that the acrosomal material is not starch, glycogen, or hyaluronic acid but suggested it might be the enzyme hyaluronidase, a labile PAS-reactive enzyme known to be present in mammalian spermatozoa. Mann (1964) also reported that the content of the enzyme per sperm differs among species. It is highest in the bull and rabbit, less in humans and boars, very little in the dog, and practically none in fowl and reptiles.

Garner et al. (1972a) obtained acrosomal extracts of ejaculated bovine spermatozoa that were found to contain multiple molecular forms

of acrosin, a trypsin-like proteinase. Acrosin extracts from homogenized testicular tissue contained only one acrosin fraction, while the differences in acrosin fractions from the epididymis suggested that the testicular acrosin fraction was modified during passage through the epididymis. Four different acrosin fractions were found in the cauda epididymis. Acrosin fractions from spermatozoa flushed from the vas deferens were similar to acrosin fractions found in ejaculated spermatozoa , suggesting that multiple forms of acrosin are formed prior to ejaculation.

Polakoski and McRorie (1972) found that acrosin from rabbit acrosomal extracts exhibited amidase, esterase, peptidase, and proteinase activity. Acrosin showed a high degree of specificity for arginyl-peptide bonds. Substrate levels of L-arginine completely inhibited acrosin. They also confirmed the presence of a sulfhydryl-requiring proteinase in acrosomal extracts.

Garner et al. (1972b) further reported on the relative stability of acrosin (proteinase) compared to acrosomal proteins and suggested that it is detrimental to acrosomal proteins during <u>in vitro</u> storage of bull spermatozoa. They stated that intra-acrosomal proteolysis may contribute to the depression of fertility from stored spermatozoa.

Abnormality and Heritability

Many research workers have studied the incidence of acrosome abnormality, its heritability, and its influence on fertility.

Evidence that acrosome abnormality is inherited was first presented by Hancock (1949), who found that about 88 percent of the spermatozoa of 7 related Holstein-Friesian bulls had acrosomal deformities.

The 7 bulls were used in 6 different herds on a total of 108 females, with no conceptions resulting. Two bulls were common in the pedigree of all these sterile animals. Teunissen (1947) also reported that 82 to 96 percent of the spermatozoa of a sterile bull had an abnormal formation at the anterior tip of the acrosome. This defect was also found in the spermatozoa of 60 other bulls which were either of low fertility or were sterile.

These findings were confirmed by Rollinson and Makinson (1949) and Rollinson (1951a,b,c), who also found that this acrosomal abnormality was frequently associated with infertility. He found that a particular bull with 5 percent abnormal acrosomes was sterile and suggested that 1 percent or more abnormalities of the acrosome in any bull would make him infertile.

Hancock (1953) and Donald and Hancock (1953) demonstrated the knobbed acrosome as an abnormality in the spermatozoa of 17 Holstein-Friesian bulls. They believed that this condition was due to an autosomal, sex-linked recessive gene, since 16 of these bulls were related to 3 common-male ancestors.

A case in a Swedish Yorkshire boar was reported by Bane (1961) in which 92 to 95 percent of the acrosomes had a backward-pointing, tongue-shaped segment along their anterior edge. Another type of abnormality was found in spermatozoa of this boar in that 5 to 6 percent of the sperm heads were malformed and had an abnormal distribution of the acrosomal substance on the heads of the sperm cells.

Buttle and Hancock (1965) described the knobbed acrosome as a cause of sterility in boars and found that boars having this characteristic emitted a slightly higher percentage of dead spermatozoa than normal

boars. They found few sperm cells on ova from sows mated to affected boars, and they thought that the failure of fertilization was due to the failure of defective spermatozoa to attach to the ova.

Bane and Nicander (1966) gave a similar interpretation of the cause of sterility in boars after they observed a high percentage of defective acrosomes in sterile boars.

The revelation of the apical body in the acrosome cap of bull spermatozoa by Saacke and Almquist (1964) and by Blom and Birch-Andersen (1965) provided a convincing interpretation of the knobbed acrosome. The apical body of sperm cells of sterile bulls is changed into a cyst-like structure with inclusion bodies, 6 to 8 times the normal size of the apical body. The perforatorium in such cells is bent. Blom and Birch-Andersen (1965) concluded that the result of this malformation is an obstruction of the sloughing off of the galea capitis (acrosome), thus preventing the penetration of the sperm cell into the ovum. The bent perforatorium may also interfere with penetration of the ovum.

Saacke et al. (1966) studied inherited acrosomal abnormalities of bull sperm and classified them as follows: (1) the knobbed acrosome with an intensely stained region at or near the apex of the sperm head, (2) the ruffled acrosome with irregular staining intensity, and (3) the incomplete acrosome with a portion of the cap missing.

They also found that two sons of a subfertile Holstein bull possessed knobbed, ruffled, and incomplete acrosomes in their spermatozoa. They reported that asymmetry of the equatorial segment was associated with many abnormal acrosomes. Irregular acrosomic granule spreading within the young spermatids was the most prevalent abnormality

in the testis of the three related bulls.

Saacke et al. (1968) stated that histological examinations of the testes revealed that these acrosomal defects were readily detectable in early spermiogenesis. The study also revealed indications of abnormal nuclear morphology with concurrent deformity of the acrosomal cap. It was found that for the Holstein sire and the two affected sons, the incidences of sperm with knobbed, ruffled, and incomplete acrosomes were 5, 8, and 20 percent, respectively.

Saacke et al. (1968) also studied a slightly subfertile Jersey bull whose semen initially contained 22 percent ruffled acrosomes. It was suggested that sperm with defective acrosomes could initiate fertilization and prevent normal cells from participating in fertilization.

Acrosome Reaction

Some workers have categorized the sequence of events that occur during the acrosomal reaction.

Saacke and Marshall (1968) using electron micrographs provided evidence that deterioration of the acrosome of bull sperm occurred in the following sequence:

1. Loss of the apical ridge and breakdown of the cell membrane, with a slight to severe swelling of the anterior acrosome.

2. Acrosome ruffling followed by breakdown of the outer membrane of the anterior acrosome, while the posterior region remains intact as the equatorial segment.

3. A loss of moderately electron-dense ground substance in the anterior acrosomal cap.

The sequence of acrosome alteration observed with differential interference-contrast microscopy was as follows:

1. The loss of the apical ridge with a slight to severe swelling of the acrosome, and appearance of the nuclear ring (this deterioration occurred as a sharp transition after cessation of motility).

2. Formation of the equatorial segment with continued swelling of the acrosome.

3. Loss of the anterior acrosome (outer membrane) by dissolution or detachment, and retention of the posterior acrosome (equatorial segment).

They felt the acrosome alterations are either post-mortem or occur immediately before death of the cell, due to the fact that only spermatozoa that had lost motility gave evidence of acrosomal alteration.

The question arises as to whether the acrosome changes observed in vitro were the true acrosome changes that occur in the female reproductive tract (capacitation) or a false reaction.

Saacke and Almquist (1964) observed that the shedding of the galea capitis (acrosome) of bovine spermatozoa might be involved as an early state in capacitation. However, they added that the changes in the acrosome cap should not be considered solely as capacitation, because some other structural and/or chemical changes are likely involved in this process.

Hadek (1969) reported that the spermatozoa of the golden hamster incubated in utero, after normal mating, did show changes in morphology. These spermatozoa exhibited a swelling of the acrosomal cap, and gradual detachment of the cell membrane, as well as separation of the acrosomal

layers. The cell membrane and acrosomal cap started to disintegrate long before the spermatozoa reached the zona pellucida.

Hadek (1969) also observed that rabbit spermatozoa exposed to oviduct fluid showed an enlargement of the sperm head profile, with rounding and caudal displacement of the acrosomal cap. This also occurred before penetration of the zona pellucida. The cell membranes were absent, and the acrosomal layer was in the process of disintegration as the spermatozoa reached the zona pellucida.

Bedford (1970) describes the true acrosomal reaction as one in which the plasma membrane and outer acrosome membranes fuse and vesiculate. He reported that this type of acrosomal breakdown is seen in less than 1 percent of degenerating sperm, in contrast to capacitated sperm in the vicinity of the ovum, which almost always appear with vesiculation of the membranes.

According to Zamboni (1971), the inner and outer membranes of the acrosome vesiculate and form fenestrae through which the hyaluronidase is released at the cumulus. After the spermatozoon has digested a path through the cumulus oophorus, only the inner acrosome layer remains. The inner acrosomal layer containing a "zona lysin" for the depolymerization of the zona pellucida. Thus, the spermatozoon gains access to the vitellus for the completion of the fertilization process.

Jones (1973a) reported that he observed two morphologically dissimilar types of changes in the acrosome of the boar. He stated that it was not possible to specify whether vesiculation and vacuolation of the acrosomes are different physiological processes but that they might be the same cytolytic process occurring at different rates. Possibly, rapid

swelling of the acrosome could produce vacuolation, while slow swelling results in vesiculation. He stated that the process of vesiculation observed rarely occurs in the epididymis but could be induced in utero, and the final stage is morphologically similar to the true acrosomal reaction which possibly takes place during fertilization by mammalian spermatozoa.

The true, or physiological, acrosome reaction apparently involves the progressive breakdown and fusion between the outer acrosome membrane and the plasma membrane, forming a series of vesicles around the anterior half of the sperm head. Fenestrae form between the points of fusion and provide openings for the dispersion of acrosomal contents. The vesiculated plasma membrane/acrosome complex is generally lost from the sperm head before penetration begins.

Morphology in Relation to Fertility

Little research has been executed on the subject of semen characteristics, or sperm morphology, with the inclusion of acrosome morphology, in relation to fertility.

Saacke (1970) reported that alterations of the acrosome in bovine spermatozoa had only been observed in immotile cells. However, he felt that the alterations reflect either the environmental conditions around the sperm or the nature of the sperm itself.

Twenty-four ejaculates from five different bulls demonstrated a correlation between post-thaw (8 hours at 37° C) motile life and maintenance of the acrosome that was significant, but relatively low ($r = .56$). A highly significant difference existed among bulls and ejaculates within bulls relative to maintenance of the acrosome at 37° C post-thaw. Objective counts of 200 cells/sample for intact acrosomes were highly

repeatable (coefficient of variance = 6 percent) when compared to microscopic estimates of percent motility (CV = 25 percent). Evaluation of acrosomal maintenance was felt to be quite precise.

In a later study of 156 ejaculates from 16 Holstein bulls, Saacke and White (1972) examined the relationship of percent motility and percent intact acrosomes to fertility (percent 90-day non-returns) during a 10-hour post-thaw incubation period (37° C). Acrosome retention was more highly related to fertility ($r = .60$, at 2 hours, $P < .01$) than was motility ($r = .41$, at 2 hours, $P < .01$). Mean values for the entire incubation period were as follows: (1) intact acrosomes, $r = .60$, $P < .01$, and, (2) motility, $r = .42$, $P < .01$.

After statistically removing variation in fertility due to differences among bulls, acrosome retention at 2, 4, and 8 hours, and the mean acrosomal retention maintained a significant relationship to fertility ($r = .20$, 2 hours; $r = .22$, 4 hours; $r = .21$, 8 hours; $P < .05$), whereas motility did not maintain a significant relationship.

The measurements most highly related to fertility, on an ejaculate basis, were averaged for all ejaculates of each bull (5 to 13 ejaculates). Correlations with fertility of .56, .80, and .81 resulted for percent motility at 0 hours, percent intact acrosomes at 2 hours, and mean percent intact acrosomes at 10 hours, respectively. By obtaining the mean for a 10-hour incubation period or percent intact acrosomes at 2 hours of incubation (on 5 to 13 ejaculates), 65 percent of the variation (R^2) in fertility among males could be explained.

It was also noted that primary abnormalities had a significant negative correlation to fertility ($r = -.34$, $P < .01$), and secondary

abnormalities (droplets) also had a significant negative correlation to fertility ($r = -.37$, $P < .01$). Bulls above average in fertility (> 66 percent 90-day non-returns) averaged 12.8 percent abnormals, while bulls below average in fertility averaged 20.5 percent abnormals. Except for motility estimations, the major differences were due to variations from bull to bull, rather than from ejaculate to ejaculate within a given bull.

Wells et al. (1973), using 5 to 8 ejaculates from 42 bulls with a mean fertility of 71 percent 90-day non-returns, found that percentages of normal sperm, live cells, normal cells with aged acrosomes, and total percent aged acrosomes, were pre-freeze measurements that added significantly to the prediction of fertility. Significant post-freeze measurements were percentages of motile sperm, normal sperm, normal sperm with aged acrosomes, and total percentages of aged and non-aged acrosomes. All variables together accounted for 36 percent of the variation among bulls for fertility.

Effect of Laboratory Technique

In more recent studies, Johnson et al. (1976) confirmed that the greatest number of intact acrosomes were observed during the incubation intervals most highly correlated with fertility (Saacke and White, 1972).

Johnson et al. (1976) reported that the percent of intact acrosomes for bovine spermatozoa fixed with buffered glutaraldehyde was higher ($P < .01$) than for unfixed spermatozoa. This agrees with the findings of Pursel and Johnson (1974).

Reminiscent of earlier work (Salisbury et al., 1942; Salisbury and Mercier, 1945; Rao and Hart, 1948; Mercier and Salisbury, 1947b; Pursel et al., 1972; Pursel and Johnson, 1974), Johnson et al. (1976)

found that laboratory technique, creating stress, can induce morphological damage in the spermatozoa. In the latter instances, the stress of making thin smears was found to produce excess acrosome damage in unfixed boar spermatozoa.

Johnson et al. (1976) stated that fresh, unfixed bovine spermatozoa stored in 2.9 percent sodium citrate could not be adequately evaluated for intact acrosomes. They found great differences in acrosome damage between duplicate estimations of unfixed samples. They reported that in unfixed samples, 23.3 percent required reestimations of the state of the acrosome, compared to only 8.9 percent in fixed samples.

This is somewhat in contrast with Awa (1968), Wondafrash (1968), Wells et al. (1970), and Wells et al. (1971) who used 2.9 percent sodium citrate as a sperm cell suspension medium and did not report any observations of what might have been technique-induced acrosome damage.

Johnson et al. (1976) felt that 2.9 percent sodium citrate did not protect the acrosome from the stress encountered in smear preparation and evaluation. Their final conclusion was that proper fixation of raw semen might have suitable application in evaluating the potential fertility of beef bulls used in natural matings.

CHAPTER III

MATERIALS AND METHODS

Bulls

The bulls in this study were 15 herd bulls (12 Angus, 2 Hereford, and 1 Polled Hereford) used at the Blount and Alcoa Farms of The University of Tennessee Agricultural Experiment Station, Knoxville and Alcoa. The bulls ranged in age from 20 to 92 months at the time of collection, which was in October and November of 1974. Thirteen of the bulls were exposed to females during at least one of three breeding periods (the fall of 1973 or the spring or summer of 1974).

Four of the Angus bulls were maintained at Blount Farm, while the remainder were housed at the Alcoa Farm. Initially, the bull groups were collected on alternate days, two semen samples collected from each bull, twice a week. This schedule was later changed to avoid interference with another research project. Mechanical malfunction of the electro-ejaculator caused each farm group of bulls to miss one collection day. This resulted in a gap of 7 days in the collection schedule for each group. For the Alcoa group bulls, the break came early in the schedule, and for the Blount Farm bulls, the break came during the latter part of the schedule. The end result was a 5-week collection schedule with 9 collection days yielding a potential of 18 semen samples from each bull. The collection schedule is presented in Table I, while other pertinent information on the bulls is presented in Table II.

TABLE I

SEMEN COLLECTION SCHEDULE

Alcoa Farm	Blount Farm
10/17/74	10/18/74
10/21/74	10/22/74
10/24/74[a]	10/25/74
10/28/74	10/29/74
10/31/74	11/01/74
11/04/74[b]	11/04/74[b]
11/08/74	11/08/74[a]
11/11/74	11/11/74
11/15/74	11/15/74
11/20/74	11/20/74

[a]Mechanical malfunction of electro-ejaculator.

[b]Bull groups switched to the same collection day.

TABLE II

BULL PROFILE

Bull	Breed	Location	Birth Date	Age (Mo.) Breeding	Age (Mo.) Collection	Number of Females Exposed	Total Bull Exposure Days
173	Angus	Alcoa	2/11/73	15	20	8	90
252	Angus	Alcoa	2/08/72	26	32	33	182[a]
411	Angus	Blount	3/02/71	38	43	16	112
417	Angus	Alcoa	2/01/67	86	92	42	185[a]
521	Angus	Blount	1/28/71	40	44	22	26
773	Angus	Alcoa	1/29/73	15	20	12	90
837	Polled Hereford	Alcoa	5/28/68	70	76	21	95
855	Angus	Alcoa	11/29/68	64	70	33	90
1049	Angus	Alcoa	1/25/69	62	68	39	192[a]
1762	Angus	Blount	2/28/72	26	31	16	105
2022	Angus	Alcoa	3/20/72	24	30	21	89
2472	Hereford	Alcoa	9/23/72	18	24	17	90
2631	Angus	Alcoa	8/23/71	32	37	25	91
1029	Hereford	Alcoa	2/04/69[b]		68		
3191	Angus	Blount	12/28/71[b]		33		

[a]Bulls used during two breeding periods.

[b]Bulls not used as herd sires during the breeding season.

Method of Semen Collection

All bulls were collected with the Plectron electro-ejaculator. These were range bulls, and, as such, no sexual preparation was attempted. The two semen samples were collected in succession without allowing for time for rest between samples.

Precautions were taken to insure against cold shock of the spermatozoa at all times.

As soon as a sample was collected, aliquots were diluted on a 1:5 or 1:10 ratio (dependent on sperm cell concentration) in culture tubes containing 2.9 percent sodium citrate, and incubated at 37° C in a water bath for 8 hours.

Subjective Semen Evaluation

The color and volume of each semen sample was recorded as it was obtained. The initial progressive motility of each sample was microscopically evaluated and recorded. Subjective motility estimations were made by placing a drop of pure semen from an innoculating loop, measuring 0.2 mm in diameter, on a warm glass slide, along with an equal amount of 2.9 percent sodium citrate solution (2.9 g, $Na_2C_6H_5O_7 \cdot 2H_2O$ 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 low power (150X) and medium power (400X) of a compound microscope. The glass slides were thermostatically maintained at 37° C in a microscope stage incubator. The same innoculating loop was used throughout the study.

Subjective motility was estimated as the percentage of sperm cells in a sample that exhibited motility and was scored in increments of 5.

The subjective vigor scores were dependent on the rate and direction of movement of the majority of sperm cells that were motile. The scale of vigor was from 0 to 4, with 4 being the most vigorous rate of movement.

Concentrations of spermatozoa were subjectively estimated in counts of tenths of a billion sperm cells per ml of semen collected.

Acrosome Evaluation

The differential acrosome slides were made with the Wells-Awa stain (Awa, 1968; Wells and Awa, 1970) at the time of collection and after 8 hours of incubation. The stain for this technique was made by mixing 1 volume of a 1 percent solution of water soluble eosin-B, 2 volumes of a 1 percent solution of water soluble fast green FCF with 1 and 7/10s volumes (1.7) of ethyl alcohol. The pH, generally 6.8 to 7.0, was checked and if needed, buffered to 7.0 by the use of a 1/10 (.1) molar solution of $NaHCO_3$ (one drop per ml of stain). The stain was freshly mixed on every collection day.

The acrosome slides were made according to the following procedure:

1. A small drop of the diluted semen was placed on a clean, warm slide and mixed with an equal volume of the Wells-Awa stain. After a period of 1 to 2 minutes, allowing the stain to set, a thin smear was made in the same manner of making a blood-cell smear.

2. The smear was quickly dried over a small electric heater and coded with the bull number, collection number, and hours of incubation (0 or 8 hours).

3. A quick microscopic check was made of the quality of the stained slide, and subsequent slides were immediately made if the quality

of the slide was judged not to be acceptable.

All slides were examined by collection day, rather than by bull, in an effort to avoid biased estimations.

Live-Dead Evaluation

The live-dead slides were also made at the time of collection and after 8 hours of incubation. The stain used was that described by Swanson and Bearden (1951) combining 1 gram of eosin-B with 5 grams of nigrosin in 100 ml of sterilized 2.9 percent sodium citrate. As recommended, the stain was refrigerated, when not in use, to help control possible bacterial or fungi growth. The live-dead slides were made in the same manner as those for the acrosome evaluation.

Objective Cell Counts

Two hundred sperm cells per acrosome slide were evaluated to determine the percentages of acrosomal characteristics of each ejaculate. Spermatozoa were classified in the following categories:

1. Morphologically normal sperm cells with normal acrosomes (NN).
2. Morphologically normal sperm cells with abnormal acrosomes (NA).
3. Morphologically abnormal sperm cells with normal acrosomes (AN).
4. Morphologically abnormal sperm cells with abnormal acrosomes (AA).

Normal or abnormal cell types were assigned based on the gross morphology of the cell. Any defects of the sperm head were considered as primary abnormalities. Defects of the mid-piece and tail were considered as secondary abnormalities. All primary and secondary abnormalities were classified as morphologically abnormal cells.

This sytem allows classification of most types of morphological abnormalities, along with, and other than, acrosome characteristics. A cell with a normal acrosome was delineated as one which possessed a smooth, closely adherent acrosome. A sperm cell with an abnormal acrosome was defined as one in which the acrosome was either absent, elevated, knobbed, or in any stage of disintegration.

Also, 200 sperm cells per live-dead slide were counted to ascertain the percentage of live and dead cells per ejaculate, an indication of cell viability. Spermatozoa remaining unstained were considered to have been alive, and any spermatozoa stained completely, or in parts, were classifified as dead.

Using both types of staining procedures afforded the opportunity to determine the association between various abnormalities of cell type, acrosome defects, and the percent live cells.

All examinations were made with a Bausch and Lomb Dyna Zoom laboratory microscope, using a blue filter.

Acrosomal evaluations were made under the oil immersion objective (1000X), while live-dead evaluations were typically made at 400X magnification.

Colorimeter Evaluation

A Klett-Summerson photoelectric colorimeter was used to measure the turbidity of pure semen for each sample collected that was not contaminated with urine or dirt. Sperm dilutions in 2.9 percent sodium citrate were 1:10 or 1:40. The readings of the colorimeter were converted to concentration estimates from conversion tables calculated by E. W. Hupp (personal communication).

Females

A total of 305 females were exposed to 13 herd sires utilized in the semen study. Of these, 261 were Angus, 45 were Herefords, and 2 were Polled Herefords. The number of females exposed to each bull is presented in Table II, page 43. The field data on these females was obtained from the herd records of the Animal Science Department of The University of Tennessee, Knoxville. All variables for each individual were calculated twice to minimize error.

The possible effects of semen characteristics on fertility were first examined, utilizing only the females that calved from having been exposed to one of the herd sires. This left a total of 246 females: 211 Angus, 34 Hereford, and 1 Polled Hereford. There were 191 lactating cows (49 with less than 42 days of involution), 47 virgin heifers, and 8 non-lactating cows.

Calculation of Estrous Cycles

This procedure was used to minimize the effects of age and parity status of the female on fertility. Fertility, in this study, was defined as the apparent number of estrous cycles the individual female completed in the presence of the bull. It was felt that this characterization might represent a truer reflection of the physiological fertilizing capacity of the bull's semen, this capacity being qualitatively measured by the morphological cell types and changing state of the acrosome. The apparent number of estrous cycles could correspond to the possible number of services per conception for a bull with an individual female. However, since no observer was present to record the actual number of services a female received during any one particular

estrus, the variable is more accurately referred to as the possible number of estrous cycles the female completed until she conceived.

By not actually having the pasture mating witnessed by an observer, indirect methods were employed in determining the possible date of conception (Rhoad, 1944; Onks, 1972). The possible conception date was determined by calculating backward from the date of Parturition II (parturition as a result of exposure to 1 of the 13 herd sires). This calculation was based on the breed average for a gestation period of 279 days for Angus females, and 284 days for Hereford females (Anderson and Plum, 1965).

All females were assigned to one of three parity categories, based on their parity status before exposure to one of the herd sires. They were classified as follows: (1) lactating cows of all ages, (2) non-lactating cows of all ages, and, (3) virgin heifers being exposed for the first time.

The apparent number of estrous cycles for lactating cows was calculated in an appropriate manner, based on their involution period, which was simply the number of days from parturition prior to exposure to one of the herd sires (Parturition I), to the date of first exposure to one of the herd sires. However, if a lactating female had an involution period of 42 days or more, then 42 days was subtracted from the number of days from Parturition I to the first date of exposure to a herd sire. If the number of days remaining to the first exposure date was greater than 21, the average estrous cycle length (21 days) was subtracted until a figure of less than 21 days before first bull exposure was obtained. This remaining number of days represented part of an estrous cycle that

the female would enter before first bull exposure and which would terminate in his presence. This calculation then kept a bull from being penalized for the female's lack of conception in this cycle. The number of days that this estrous cycle overlapped into the number of bull exposure days was then subtracted from the number of days from first bull exposure to the possible conception date. This left a figure representing the number of possible estrous cycle days, which was then divided by 21 to obtain the apparent number of estrous cycles the female completed in the presence of the male. An example of this type of calculation is presented in Figure 2.

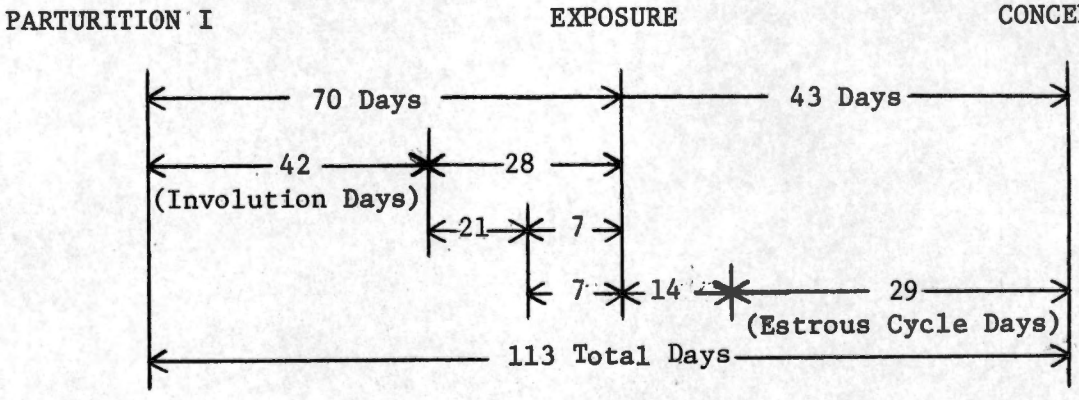


Figure 2. Estrous cycles of lactating cow 1436 exposed to bull 417.

Thus, the apparent number of estrous cycles for cow number 1436 is:

$$\text{ESTROUS CYCLES} = \text{ESTROUS CYCLE DAYS}/21$$

$$= 29/21$$

$$= 1.38$$

If a lactating female had an involution period of less than 42 days in length, 42 was subtracted from the number of days from Parturition I to the possible conception date. This gave the number of estrous cycle days the female completed in the presence of a herd sire. This figure divided by 21 gave the apparent number of estrous cycles completed to achieve conception. Figure 3 represents this type of calculation.

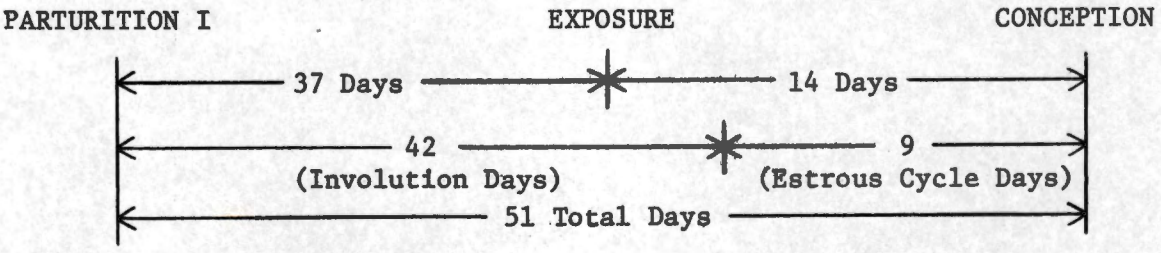


Figure 3. Estrous cycles of lactating cow 661 exposed to bull 2631.

Thus, the apparent number of estrous cycles for cow number 661 is:

$$\text{ESTROUS CYCLES} = \text{ESTROUS CYCLE DAYS}/21$$
$$= 9/21$$
$$= 0.43$$

By using this procedure, the effects of involution period were minimized to avoid penalizing a bull in an unjust manner. Late calving females and management decisions also influence whether or not some females have an adequate involution period before exposure to a bull. Thus, this procedure was used so that the number of females that biologically would take less than 42 days for involution should approximate the number taking more than 42 days, with the resulting average of 42 days for the breeding groups.

Virgin heifers were assumed to reach puberty upon cycling for the first time. Puberty for each heifer was calculated from the date of birth and was based on United States Meat Animal Research Center (U.S.M.A.R.C.) puberty data of 379 days for Angus heifers and 404 days for Hereford heifers. The number of days from the estimated puberty date to the first date of bull exposure was divided by an average 21-day estrous cycle. The number of days the last estrous cycle overlapped into the number of bull exposure days was then calculated. This figure was subtracted from the number of days from the first date of bull exposure to the possible date of conception. The remaining days divided by 21 represented the apparent number of estrous cycles the heifer completed in the presence of the bull. An example of this computation is presented in Figure 4.

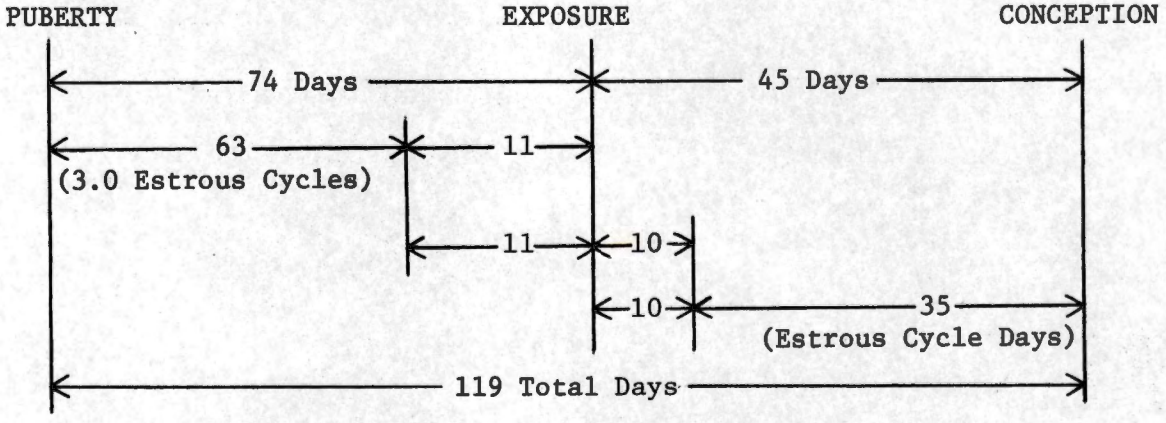


Figure 4. Estrous cycles of virgin heifer 883 exposed to bull 2022.

Thus, the apparent number of estrous cycles for heifer number 883 is:

ESTROUS CYCLES = ESTROUS CYCLE DAYS/21

= 35/21

= 1.67

Non-lactating cows were assumed to be cycling, and an average date by which lactating cows in their respective breeding groups were reaching Parturition I was used to calculate a constant date (9/30/73 for fall, 2/04/74 for winter, and 4/23/74 for spring cows). The number of days from the constant date to the date of first bull exposure was divided by 21. Again, the number of days the last estrous cycle overlapped into the number of bull exposure days was calculated. This figure was subtracted from the number of days from the date of first bull exposure to the possible date of conception. These remaining days, divided by 21, represented the apparent number of estrous cycles the non-lactating cow completed in the presence of the bull. Figure 5 represents this type of calculation.

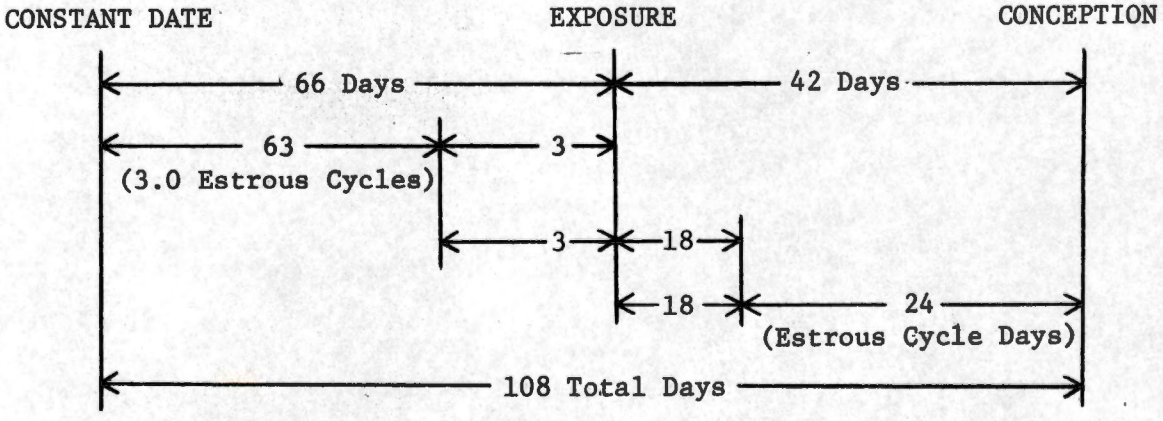


Figure 5. Estrous cycles of non-lactating cow 701 exposed to bull 1049.

Thus, the apparent number of estrous cycles for cow number 701 is:

ESTROUS CYCLES = ESTROUS CYCLE DAYS/21

= 24/21

= 1.14

The second analysis to determine the possible effect of changing semen characteristics on fertility utilized all lactating cows and virgin heifers exposed to the herd sires and the non-lactating cows that calved as a result of exposure to one of the herd sires. Non-lactating cows that did not calve following exposure to one of the herd sires were not used in either analysis since they were most probably barren and, as such, were not suitable test animals to be included in the data analysis.

This procedure gave a total of 296 females, of which 251 were Angus, 43 were Herefords, and 2 were Polled Herefords. Of this total, there were 225 lactating cows (60 with less than 42 days of involution), 63 virgin heifers, and 8 non-lactating cows.

For the females that did not calve as a result of exposure to one of the herd sires, the number of estrous cycles was calculated in the same manner as the females that did calve. The only difference was that the estrous cycles were calculated up to the last day of bull exposure.

Due to the nature of this data set, there was no way to determine the effects of possible conception and subsequent embryonic mortality on fertility.

Statistical Procedure

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

Correlations were first obtained on all variables in the data set. The initial regression model was set up using selected positively correlated variables in descending order of correlation, followed by selected negatively correlated variables in descending order of correlation. This was done to determine the effects of the independent variables on the semen characteristics classifying the morphological cell type and state of the acrosome. A total regression was obtained, and, subsequently, one variable was dropped from the model each time a regression was obtained to determine the change in R^2 (ΔR^2) explained by each variable.

The model used in the initial regression analysis was:

$$Y_{ijklmnopq} = u + b_i + c_{ij} + d_{ijk} + m_{ijkl} + t_{ijklm} + h_{ijklmn} + f_{ijklmno} + P_{ijklmnop} + e_{ijklmnopq}$$ where:

n is the overall mean,

b_i is the effect associated with the i-th bull,

c_{ij} is the effect associated with the j-th collection day in the i-th bull,

d_{ijk} is the effect associated with the k-th number of subsequent days of each collection day, from the first collection day in the ij-th bull-collection day subclass.

m_{ijkl} is the effect associated with the l-th motility score in the ijk-th bull-collection day-subsequent days subclass,

t_{ijklm} is the effect associated with the daily high ambient temperature on the approximate day of spermatogenesis on the sperm cells collected on each particular collection day in the ijkl-th bull-collection day-subsequent days-motility subclass,

h_{ijklmn} is the effect associated with hours of incubation time in the bull-collection day-subsequent days-motility-temperature at spermatogenesis subclass,

$f_{ijklmno}$ is the effect associated with the percent of dead sperm cells in the ijklmn-th bull-collection day-subsequent days-motility-temperature at spermatogenesis-hours of incubation time subclass.

$P_{ijklmnop}$ is the effect associated with the daily high ambient temperature on each collection day in the ijklmno-th bull-collection day-subsequent days-motility-temperature at spermatogenesis-hours of incubation time-percent dead sperm cells subclass,

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

A second regression analysis was obtained to determine the possible effects of the percent normal cells with normal acrosomes (NN) and the percent normal cells with abnormal acrosomes (NA) on the calculated number of estrous cycles of all females that calved as a result of exposure to one of the herd sires. The assumption was made that no change occurred in the qualitative semen characteristics of bulls in good health, during the time lapse from the breeding season to the semen collection period. The percentage of abnormal cells with normal acrosomes (AN) and abnormal cells with abnormal acrosomes (AA) were excluded from this procedure because of the relatively low percentages of these sperm cell classifications exhibited by the bulls during the semen evaluation period.

Two herd sires, bulls 1029 and 3191, were not included in this analysis due to the fact that they were utilized as clean-up bulls on females previously exposed to other bulls for fall breeding, and, as such, only a few females calved as a result of this exposure.

In the second regression analysis, the effects of the aforementioned semen characteristics on estrous cycles were determined for hour 1, hour 8, and all hours combined. This procedure was followed for all the bulls collectively and for the bulls divided into two groups (I and II). Bulls that had exhibited the ability to produce a minimum of 50 percent normal sperm cells with normal acrosomes (NN), for at least the last two days of semen collection, were utilized as Group I (5 bulls). The other bulls were classified as Group II (8 bulls).

The model for the second regression analysis was:

$$Y_{ijk} = u + n_i + c_{ij} + e_{ijk} \quad \text{where:}$$

u is the overall mean,

n_i is the effect associated with i-th percent of normal sperm cells with normal acrosomes.

c_{ij} is the effect associated with the j-th percent of normal sperm cells with abnormal acrosomes in the i-th percent of normal sperm cells with normal acrosomes.

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

A third regression analysis was run, using the same model and procedure as the second analysis, to determine the possible effects of the previously outlined semen characteristics on the calculated number of estrous cycles of all females exposed to the herd sires. Again, some females that were thought to have been barren were excluded from the analysis.

Student-Newman-Kuels Tests were performed to separate significantly different means between bulls. Means tested were the percent normal cells with normal acrosomes (NN), percent normal cells with

abnormal acrosomes (NA), percent abnormal cells with normal acrosomes (AN), percent abnormal cells with abnormal acrosomes (AA), percent live cells, calculated estrous cycles of all females that calved, and the calculated estrous cycles of all females exposed to the herd sires.

CHAPTER IV

RESULTS AND DISCUSSION

During a 5-week period, 266 semen samples were collected from 12 Angus, 2 Hereford, and 1 Polled Hereford bull. Specific semen characteristics were evaluated as measures of semen quality, and their possible effect on the breeding efficiency of the bulls was determined according to the calculated number of estrous cycles each female, used in the analysis of a bull, could have completed in the presence of the male.

The qualitative semen characteristics evaluated included the percentages of normal sperm cells with normal acrosomes (NN), normal sperm cells with abnormal acrosomes (NA), abnormal sperm cells with normal acrosomes (AN), abnormal sperm cells with abnormal acrosomes (AA), and live sperm cells. Abnormal cells included all cells with primary or secondary abnormalities. Abnormal acrosomes included all cells with acrosomes that were either absent, elevated, knobbed, detached, or in any state of disintegration.

Tables III and IV show the arithmetic means, by hour, for the qualitative semen characteristics and calculated fertility variables evaluated for each bull. The means are also represented by group. The standards for classifying bulls into groups have been previously discussed. These standards are also reflected in Figures 6, 7, 8, and 9, which illustrate the mean percentages of normal sperm cells with normal acrosomes for each bull, by group, for hours 1 and 8 on each collection day.

TABLE III

THE ARITHMETIC MEANS OF SEMEN CHARACTERISTICS FOR INDIVIDUAL BULLS, BY HOURS

Bull	Percent									
	Hour 1					Hour 8				
	NN	NA	AN	AA	Live	NN	NA	AN	AA	Live
Group I										
411	39.9	57.0	0.5	2.6	61.6	28.0	69.3	0.3	2.5	42.2
837	35.6	44.4	1.0	2.4	52.5	25.1	56.0	0.1	2.3	32.6
855	46.5	43.6	1.7	2.9	63.5	33.6	58.0	0.1	3.4	42.0
1049	45.3	50.6	1.2	2.9	66.3	31.2	66.2	0.1	2.7	43.1
2631	37.5	58.1	1.3	3.3	67.1	28.2	68.4	0.2	3.5	47.0
Group II										
173	26.7	68.4	0.9	4.0	61.7	17.9	78.0	0.1	4.1	34.6
252	36.4	60.3	0.4	2.1	76.8	26.9	70.3	0.1	2.8	54.5
417	34.1	62.1	0.7	2.8	59.1	22.5	74.4	0.1	3.1	41.3
521	31.3	59.2	0.9	3.0	62.1	18.6	73.3	0.1	2.5	30.0
773	25.8	70.4	1.0	3.0	48.2	16.9	79.8	0.1	3.3	26.5
1762	29.1	61.6	0.9	2.9	57.2	17.8	74.2	0.1	2.5	32.7
2022	30.8	58.8	1.7	3.3	59.2	22.1	68.9	0.1	3.5	39.9
2472	32.9	51.9	2.4	7.3	53.4	21.6	65.3	0.1	7.6	29.0
Group III[a]										
1029	31.2	63.0	1.9	3.9	63.4	22.9	72.5	0.1	4.6	39.3
3191	39.5	56.9	0.5	3.1	61.0	25.9	71.6	0.1	3.1	38.3
Overall Mean	34.8	57.8	1.1	3.4	60.9	23.9	69.7	0.1	3.4	38.2
Group I Mean	41.0	50.8	1.2	2.8	62.2	29.2	63.5	0.1	2.9	41.4
Group II Mean	30.9	61.6	1.1	3.7	59.7	20.5	73.0	0.1	3.7	36.1

[a]Bulls not used as herd sires during the breeding season.

TABLE IV

THE ARITHMETIC MEANS OF SEMEN CHARACTERISTICS AND FERTILITY FOR INDIVIDUAL BULLS

Bull	Percent Hours Combined NN	NA	AN	AA	Live	Estrous Cycles Females Calving	Females Exposed
Group I							
411	34.0	63.1	0.4	2.5	51.9	0.4	0.4
837	30.3	50.2	0.6	2.3	42.5	1.1	1.4
855	40.0	50.6	0.9	3.1	52.7	0.5	0.8
1049	38.3	58.4	0.7	2.8	54.7	0.4	0.4
2631	32.9	63.3	0.8	3.4	57.0	0.3	0.6
Group II							
173	22.3	73.2	0.5	4.0	48.1	1.6	1.6
252	31.6	65.3	0.2	2.8	65.6	1.1	1.6
417	28.3	68.3	0.4	3.0	50.2	1.7	2.1
521	25.0	66.2	0.5	2.8	46.1	-0.2	0.2
773	21.4	75.1	0.5	3.2	37.4	0.6	0.6
1762	23.4	67.9	0.5	2.7	45.0	0.7	0.9
2022	26.4	63.8	0.9	3.4	49.5	1.0	1.3
2472	27.3	58.6	1.2	7.4	41.2	1.1	1.5
Group III[a]							
1029	27.0	67.8	1.0	4.3	51.4	0.0	0.0
3191	32.7	64.2	0.3	3.1	49.6	0.0	0.0
Overall Mean	29.4	63.7	0.6	3.4	49.5	0.8	1.0
Group I Mean	35.1	57.1	0.7	2.8	51.8	0.5	0.7
Group II Mean	25.7	67.3	0.6	3.7	47.9	0.9	1.2

[a]Bulls not used as herd sires during the breeding season.

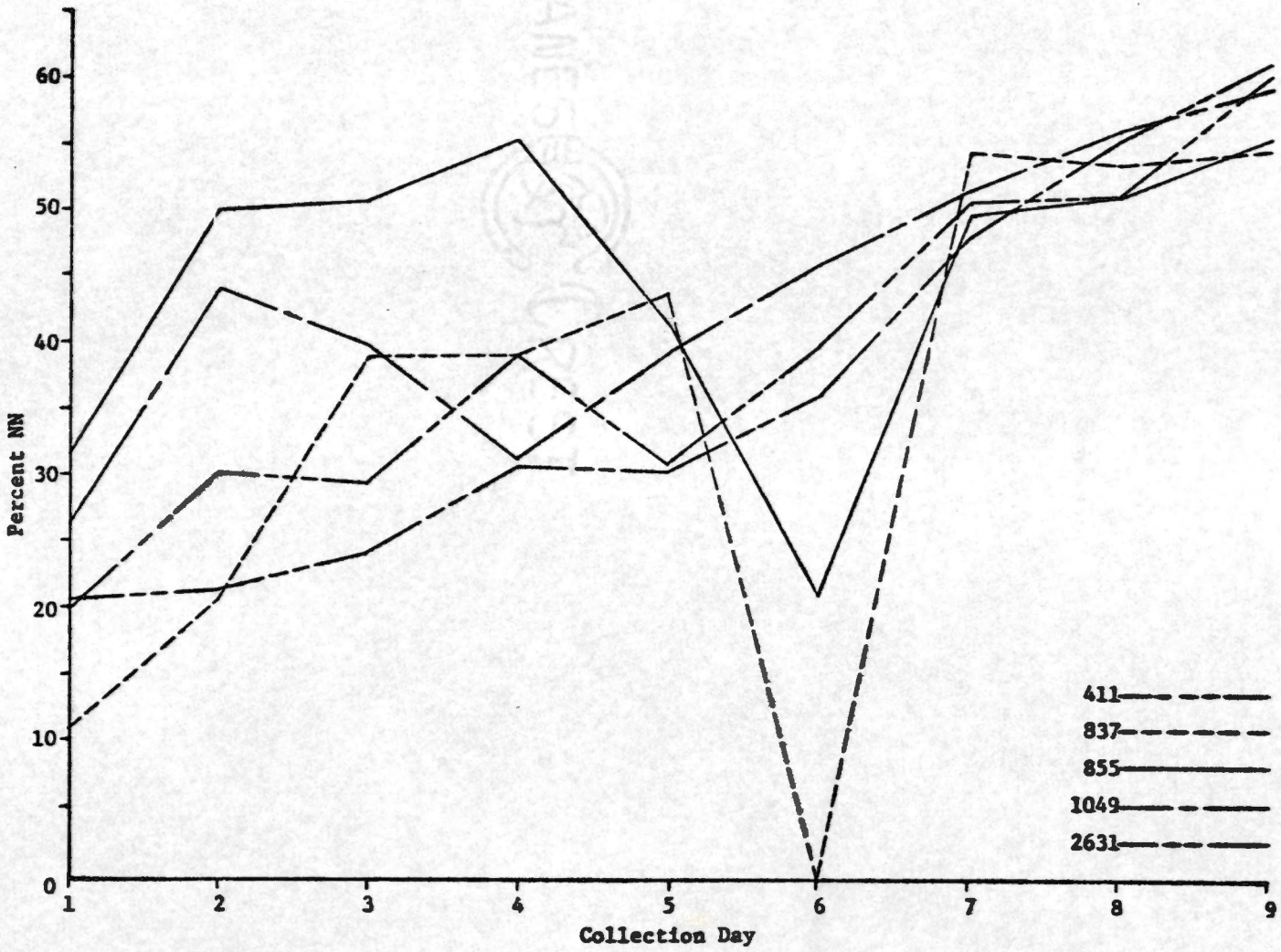


Figure 6. Daily variations of the NN classification for Group I (Hour 1).

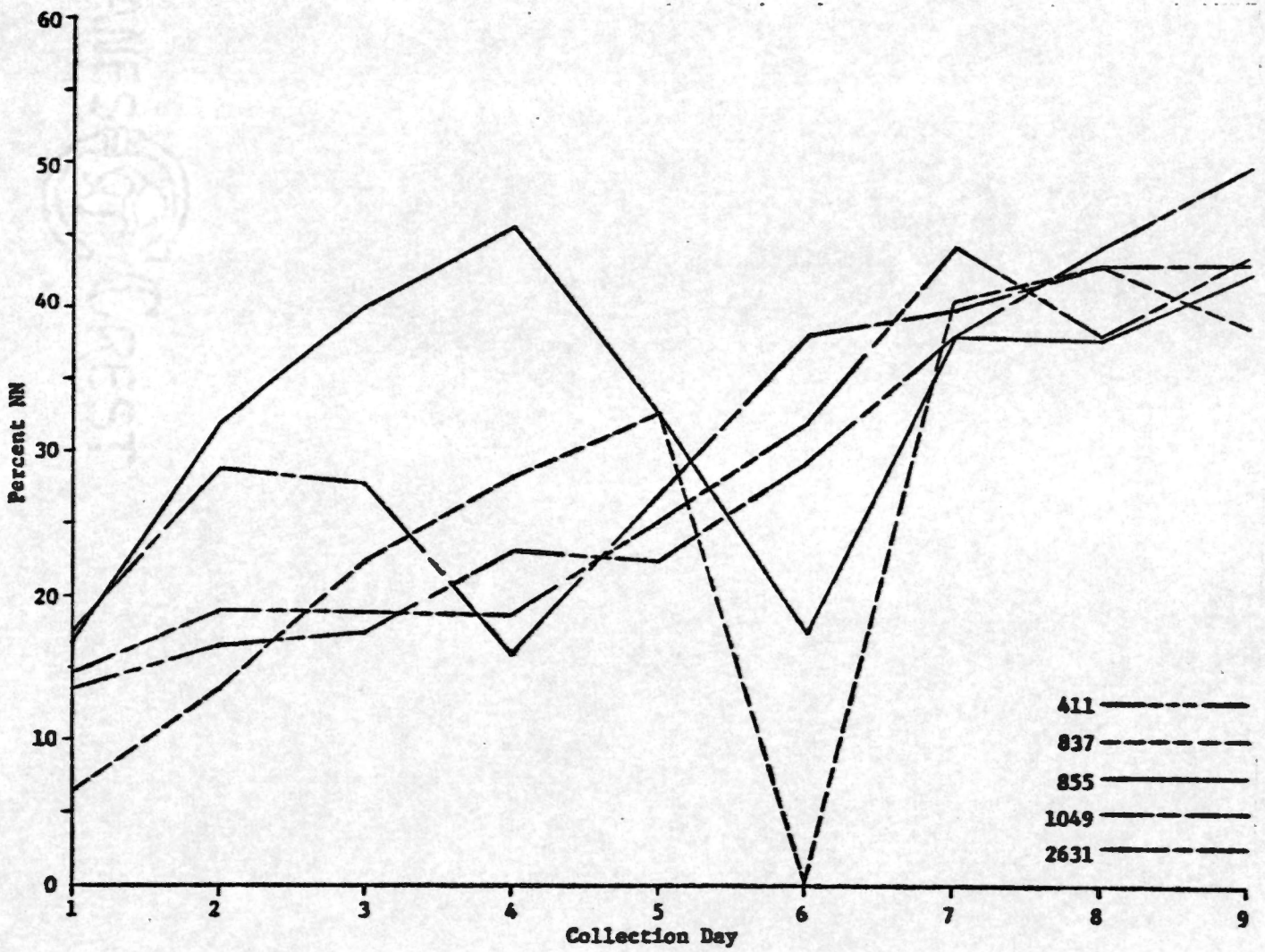


Figure 7. Daily variations of the NN classification for Group I (Hour 8).

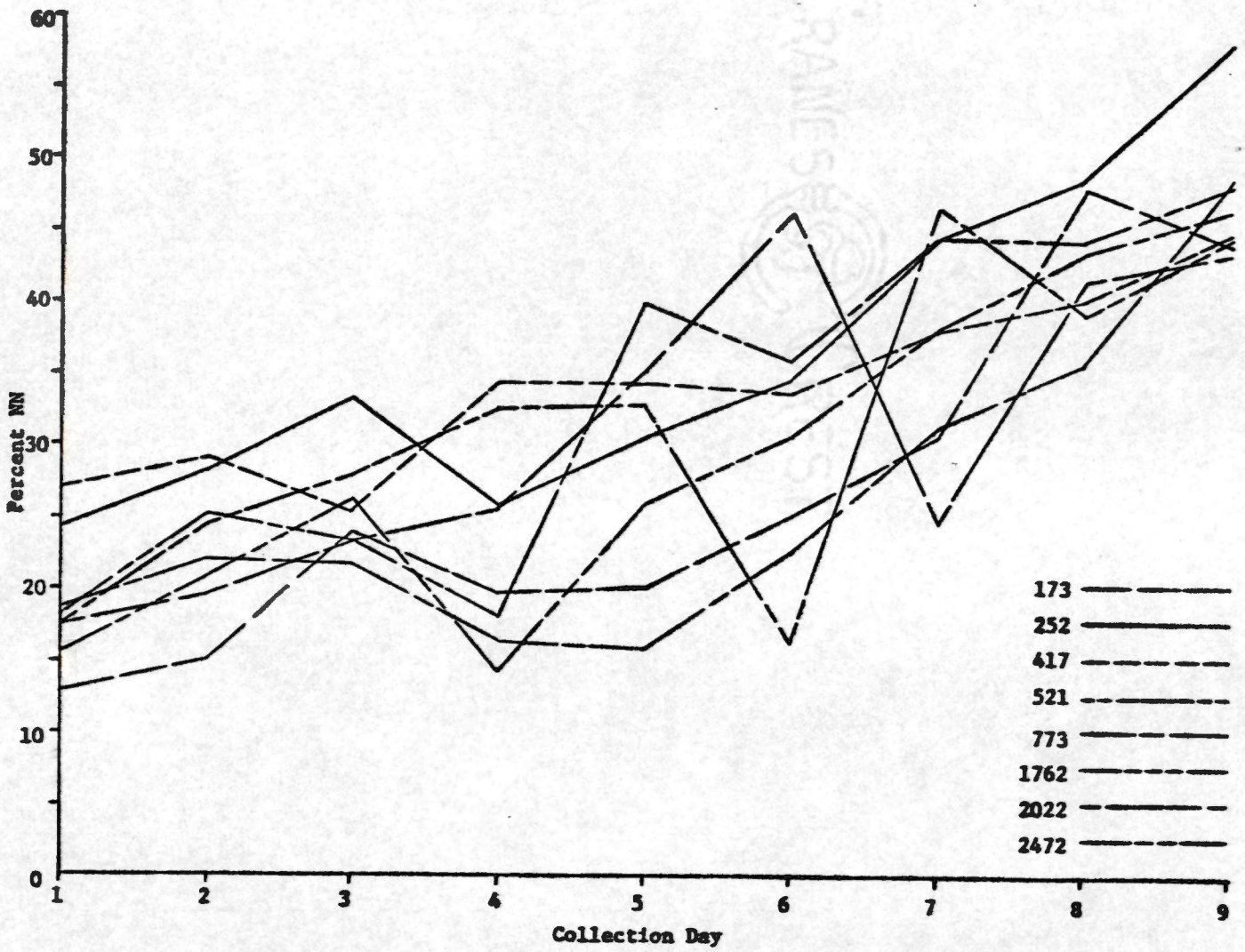


Figure 8. Daily variations of the NN classification for Group II (Hour 1).

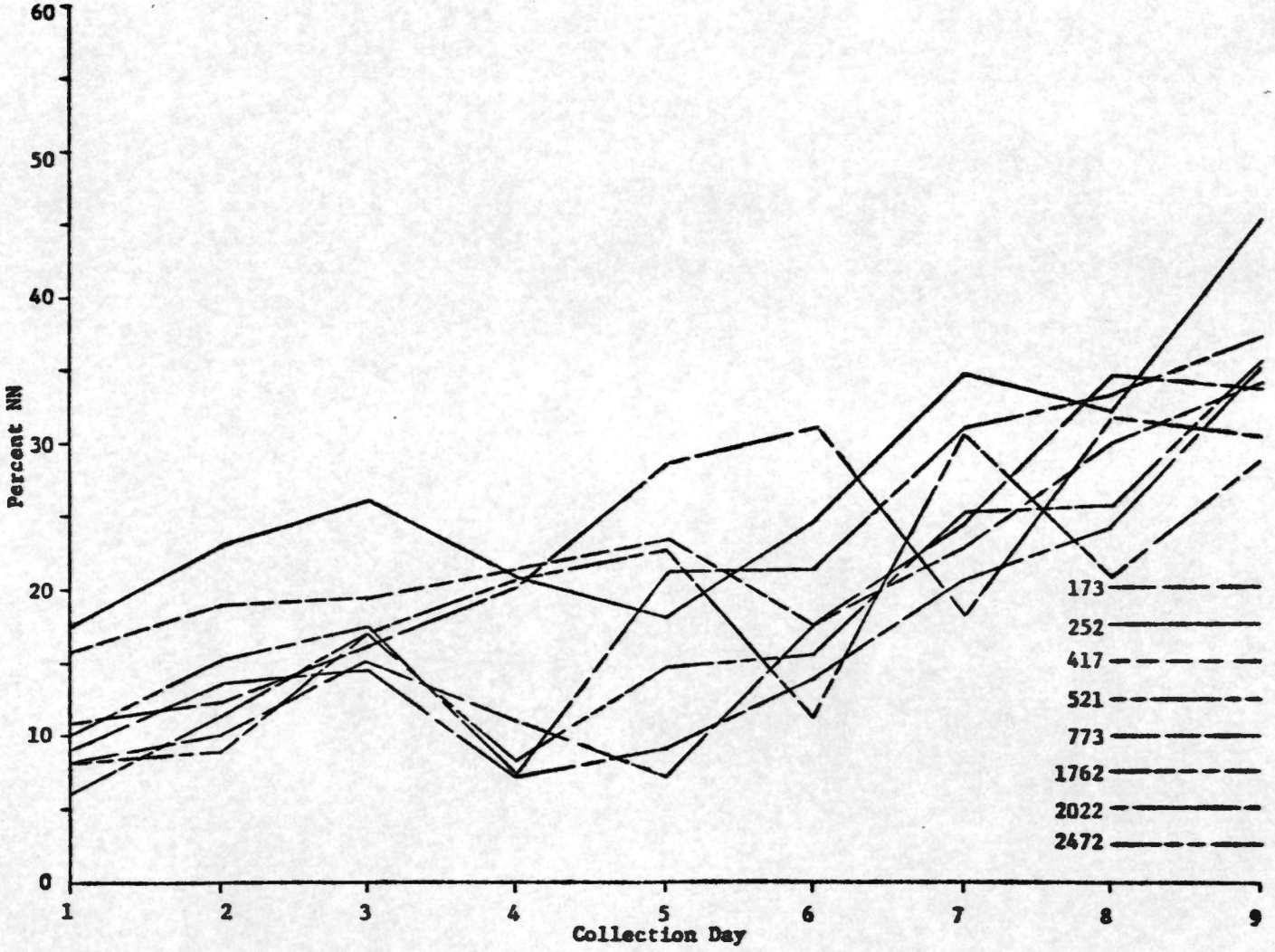


Figure 9. Daily variations of the NN classification for Group II (Hour 8).

Table V presents the correlation coefficients that were used as the basis to set up the initial regression model.

Appendix A, Tables A-I, A-II, and A-III contain the results of the Student-Newman-Keuls Tests.

Appendix B, Tables B-I through B-X show the results of the regression analysis of the semen characteristics and the change in R^2 (ΔR^2).

The results of the regression of the percentages of normal sperm cells with normal and abnormal acrosomes on the calculated fertility variables are presented in Appendix C, Tables C-I and C-II.

Analysis of the Percent Normal Sperm Cells with Normal Acrosomes

As shown in Appendix B, Tables B-I and B-II, collection day was highly significant ($P < .01$) in accounting for most of the variation in the percent of normal sperm cells with normal acrosomes ($\Delta R^2 = .4257$). Figures 6 and 8 on pages 62 and 64 show the daily variation for the bulls, by group, in the NN classification. This coincides with the observations of Wells et al. (1970) who found the weekly changes in acrosomes to be more variable than the numbers of normal cells. It is evident from the graphs that the initial quality of the semen, as measured by the percent of the NN classification, was very low. However, this is still in agreement with the findings of Awa (1968), Wondafrash (1968), Wells et al. (1970), and Wells et al. (1971) who found that the initial percent of normal sperm cells with normal acrosomes was low in first ejaculates from beef fulls in a state of sexual rest. Regardless of the fact that many of the bulls in this study produced semen with very low initial ejaculate quality (percent NN), and despite some fluctuations

TABLE V

SIMPLE CORRELATION COEFFICIENTS BETWEEN SEMEN CHARACTERISTICS AND SELECTED VARIABLES

	Semen Characteristics and Variables														
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
(1) MM Σ																
(2) Mm Σ	-.467***															
(3) AM Σ	.432***	-.338***														
(4) Aa Σ	.024**	.116**	.232***													
(5) Live Σ	.885***	-.141***	.371***	.085*												
(6) Dead Σ	-.384***	.704***	-.293***	.196***	-.616***											
(7) Volume (ml)	.883	.385***	-.016	.188**	.836	.151***										
(8) Subjective Concentration	.844	.187***	-.050	.014	.291***	-.093*	-.084*									
(9) Motility Σ	.488***	-.042	.877	.085*	.343***	-.263***	.836	.286***								
(10) Vigor	.838***	-.052	.894*	.113**	.538***	-.268***	.832	.472***	.870***							
(11) Ball	.836	-.063	.888*	.238***	-.839	.838	-.187**	-.182**	-.185**	-.111**						
(12) Age	.182***	-.185***	.881	-.217***	.857	-.188**	-.265***	.185***	.818**	.123**	-.243***					
(13) Collection Day	.829***	-.315***	.252***	.879	.166***	-.172**	.826	-.238***	.188***	.193***	.888	.889				
(14) Sample Number	-.826	.825	.815	-.661	-.836	.857	-.886	-.234***	-.173***	-.178***	.888	.889	.886			
(15) Hours	-.384***	.343***	-.499***	.821	-.384***	.387***	-.888	.888	-.888	.888	.888	.888	.888	.888		
(16) Temperature at Spermatogenesis	-.381***	.384***	-.199***	-.848	-.254***	.211***	-.856	.138**	-.385***	-.385***	-.822	.823	-.578***	.859	.888	
(17) Temperature at Collection	-.287***	.211***	-.115**	-.861	-.186***	.162***	-.184**	.812	-.272***	-.248***	.888	-.887	-.365***	.886	.888	.219***

* $p < .05$

** $p < .01$

*** $p < .001$

**** $p < .0001$.

(percent NN) between collection days, the overall trend in the production of normal sperm cells with normal acrosomes increased for all bulls. This increase in the NN category followed more frequent sexual stimulation and collection of sperm cells and resulted in fewer sperm cells with aged (in vivo) acrosomes being obtained from the bulls.

As shown by the graphs, the bulls of Group I established an improved trend in the production of higher quality semen, a trend above that of the bulls in Group II. However, the rates of improvement were not of the magnitude of the changes reported by Wells et al. (1970). Admittedly, some technique-induced acrosome damage (Johnson et al., 1976) could have been produced, but the 2.9 percent sodium citrate, having been widely used as a sperm cell suspension medium (Awa, 1968; Wondafrash, 1968; Wells et al., 1971), should not have caused environmental stress so great as to have unduly damaged the acrosomes evaluated, especially on the slides made immediately after collection and dilution of the semen.

The effect of collection day is also reflected by the correlation presented in Table V, page 67, where the correlation between collection day and the percent normal sperm cells with normal acrosomes was .619 and highly significant ($P < .0001$). This effect also corresponds with the reports of Wells et al. (1970, 1971), who found that regular semen collection improved the percent of normal sperm cells with normal acrosomes produced by beef bulls, with the subsequent reduction of the percent of normal sperm cells with aged acrosomes.

Appendix A, Table A-I, shows that bulls 855 and 1049, both in Group I, were significantly higher ($P < .05$) in their ability to produce semen with increased percentages of sperm cells in the NN classification

and to maintain the integrity of the acrosome through the incubation period. It is also shown that bull 173 of Group II was significantly lower ($P < .05$) in the production of sperm cells in the NN classification.

Incubation time was highly significant ($P < .0001$) in explaining 15.6 percent of the variation in the percent of normal sperm cells with normal acrosomes (Appendix B, Tables B-I and B-II). The effect of incubation was expected (Saacke and White, 1972), as this procedure was to be a direct survivability test of the spermatozoa. Referring to Appendix A, Table A-II, it is evident that some bulls of Group I (855, 1049, and 2631) were not significantly different ($P < .05$) in the ability of their spermatozoa in maintaining the integrity of the acrosome in normal sperm cells through 8 hours of incubation, as compared to spermatozoa (NN) that some of the Group II bulls (417, 521, and 2472) were producing at the initial collection, prior to incubation. The improvement in the ability of the spermatozoa to maintain the integrity of the acrosome (in vitro) is an indication of the qualitative changes exhibited by the bulls. This is also graphically illustrated in Figures 6 through 9, pages 62 through 65.

Bull was also a highly significant ($P < .0001$) source of variation in the NN category, explaining 15.1 percent of the variation. Appendix A, Tables A-I and A-II point out the inherent ability some of the Group I bulls exhibited in producing semen of higher quality, as compared to some of the bulls in Group II. Adding motility to the model increased the amount of variation explained by 4.6 percent ($P < .0001$). As noted in Table V, page 67, motility possessed a correlation of .458 ($P < .0001$) with the percent normal sperm cells with normal acrosomes, and a

correlation of .543 (P <.0001) with the percent live cells. Percent live cells were found to have a highly significant, positive correlation with the NN classification (r = .685, P <.0001). One should also note the highly significant (P <.0001) correlation of .870 between motility and vigor.

By examining these correlations, it appears that subjective motility and vigor scores, estimated over a period of time, are reasonably good indicators of ejaculate quality, in terms of the percent normal sperm cells with normal acrosomes. This concurs somewhat with the conclusions of Wiltbank (1965) and Onks (1972), who stated that motility was a good indicator of ejaculate quality, in predicting fertility from semen characteristics.

Motility and vigor are useful as indicators of sperm cell longevity, since it is known that, through uterine contractions, spermatozoa reach the site of fertilization in the oviduct shortly after coitus. This coupled with the facts of sperm capacitation and the timing of estrus and ovulation in the bovine female, lends support to the conclusion that the increased in vitro survivability and maintenance of acrosomal integrity of the spermatozoa of bulls is an acceptable indicator of the ability of the spermatozoa to survive in the tract of estrus females. Thus, in vitro survival tests of spermatozoa could aid in the selection of bulls whose semen maintains a higher capability of effecting fertilization.

The inclusion of the percent dead sperm cells in the model accounted for only 2.3 percent (P <.0001) of the variation (Appendix B, Tables B-I and B-II). Table V, page 67, shows a negative correlation of

-.364 ($P < .0001$) between the percent dead sperm cells and the NN classification.

Little of the variation was explained when the following were added to the model:

1. The number of days each collection day was subsequent to the first collection day.

2. The ambient temperature at the approximate time of spermatogenesis.

3. The ambient temperature at the time of collection.

Analysis of the Percent Normal Sperm Cells with Abnormal Acrosomes

Appendix B, Tables B-III and B-IV show that collection day was significant ($P < .05$) in accounting for most of the variation in the percent normal sperm cells with abnormal acrosomes ($\Delta R^2 = .2838$). As evidenced by the overall increase in the percent normal sperm cells with normal acrosomes during the semen collection period and by the correlation between the NN and NA classifications ($r = -.467$, $P < .0001$) presented in Table V, page 67, the percent of normal sperm cells with abnormal acrosomes should decrease during the same period (Wells et al., 1970; Wells et al., 1971). It should be noted that the term "abnormal" used in describing the state of the acrosome is actually a misnomer, since this particular phenomenon is not considered to be strictly a physical or structural abnormality, in the same sense as sperm with giant heads or broken tails. The abnormality observed in this project is mainly a result of the *in vivo* and *in vitro* aging of sperm cells, which leads to the disruption and breakdown of the acrosome at the molecular level. This represents the false acrosome reaction described by Bedford (1970).

In a sense, ejaculates from beef bulls containing high percentages of cells in the NA classification would be a factor detrimental to reproductive efficiency, especially if the condition persisted through an extended period of the breeding season, or one of stimulation with semen collection and evaluation in a research project. During extended semen evaluation periods, unequal rates of change between bulls in the percent of normal acrosomes (NN) and aged acrosomes (NA) might provide a ratio or proportion that would be more indicative of a bull's potential performance in the pasture than a brief semen evaluation period might yield.

The percent dead sperm cells were highly significant in explaining 24.2 percent of the variation in the NA classification (Appendix B, Tables B-III and B-IV). As shown in Table V, page 67, the correlation between the percent of dead sperm cells and the NA classification was .704 ($P < .0001$). The acrosome reaction may take place at or near the death of the sperm cell. However, the percent of dead sperm cells present at the initial collection would be due to <u>in vivo</u> aging, whereas the percent of dead sperm cells after incubation would be influenced by the initial percent of dead sperm cells and the <u>in vitro</u> survivability of the sperm cells.

As with the NN classification, bull was highly significant ($P < .001$) in accounting for 15.3 percent of the variation in the percent of normal sperm cells with abnormal acrosomes. Appendix A, Tables A-I and A-II, demonstrate the ability of bulls 855 and 837 of Group I to be significantly lower ($P < .05$) in the overall production and maintenance of sperm cells without this undesirable defect. Again, this is evidence of the improved ability of their spermatozoa to maintain acrosomal integrity during incubation.

Incubation time was highly significant ($P < .001$) in the explanation of the 11.7 percent of the variation in the NA category. This is also indicative of the different rates of survival of the spermatozoa. Appendix A, Table A-II, shows that the sperm cells of Group I bulls 1049 and 2631 were not significantly different ($P < .05$) in the NA classification after 8 hours of incubation, from the spermatozoa of some Group II bulls (173 and 773) at time of initial collection.

The in vitro aging effect corresponds with the observations of many researchers (Lovell and Getty, 1960; Iype et al., 1963; Onuma, 1964; Pursel et al., 1972; Saacke and White, 1972; and Pursel et al., 1974), who observed increased deterioration of the acrosome during various sperm cell storage and aging conditions designed to place stress on the spermatozoa.

The inclusion of motility in the model was highly significant ($P < .0001$), but explained only 0.9 percent of the variation (Appendix B, Tables B-III and B-IV). The correlation between motility and the NA classification was negative ($r = -.062$) and was not significant. If the acrosome reaction observed occurs near the death of the sperm cell, then the motility in the sperm cells of this classification would probably be slight or almost non-existent.

The effect of the remaining variables added little to the model.

Analysis of the Percent Abnormal Sperm Cells with Normal Acrosomes

Small percentages of sperm cells in the AN classification were produced by all bulls. Tables III and IV, pages 60 and 61, show the arithmetic means for this variable. The overall mean was 1.1 percent at the time of collection. This is a much smaller percentage than the

results obtained by Wells et al. (1970), who found approximately 8.5 percent in the AN category for the first 6 collection days of five sexually rested dairy bulls. Of the 1.1 percent observed, almost all of these cells exhibited secondary abnormalities. Few cells were present with primary abnormalities, at the initial collection, that possessed a normal acrosome.

Incubation time explained the largest amount of variation in the AN category (ΔR^2 = .2488). The survivability of these cells apparently was not good, and, as such, the disintegration of the acrosome would have placed these cells in the classification of abnormal sperm cells with abnormal acrosomes, if they were observed a second time on the slides evaluating incubated spermatozoa. This is evident from the arithmetic means by hour (Tables III and IV, pages 60 and 61).

Bull was a highly significant ($P < .001$) source of variation in the AN category, while accounting for 7.54 percent of the variation. As shown in Appendix A, Table A-I, bulls 2472, 1029, and 855 had the highest overall least-squares means in this classification, but the percentages for all bulls were not large enough to have been detrimental to fertility.

The number of days subsequent to the first collection day was significant ($P < .05$) but only explained 0.5 percent of the variation. During the random evaluation of the slides, more cells in the small percentages of this category might have been observed for several consecutive days. Similarly, the percent of dead sperm cells was significant, while accounting for only 0.5 percent of the variation. As previously stated, this could be due in part to some cross-classification that might have occurred as a result of the effects of incubation.

The other factors in the model contributed little to the equation.

Analysis of Percent Abnormal Sperm Cells with Abnormal Acrosomes

As outlined in Appendix A, Table A-I, bull 2472 of Group II was significantly higher ($P < .05$) than all bulls in the production of sperm cells in the AA category. With the exclusion of bull 2472, bulls 1029 (Group III) and 173 (Group II) were also significantly higher ($P < .05$) in the percentage of abnormal sperm cells with abnormal acrosomes. Tables B-VII and B-VIII of Appendix B indicate that bull was a highly significant ($P < .0001$) source of variation for sperm cells in the AA classification ($\Delta R^2 = .4386$). According to Wiltbank (1963), bull 2472 (Group II), with approximately 9 percent total abnormal sperm cells, would have had a conception rate of 70 percent. Munro (1961) felt that bulls with total abnormalities of 18 percent or higher would affect fertility. Wells et al. (1970) found approximately 7 percent of sperm cells in the AA classification for 6 consecutive collection days. From the data of this study, it was thought that none of the bulls evaluated had primary or secondary abnormality percentages high enough to affect fertility.

The percent of dead sperm cells was responsible for 4.1 percent of the variation in the AA category ($P < .0001$). Table V, page 67, indicates the percent abnormal sperm cells with abnormal acrosomes was positively correlated with the percent of dead sperm cells ($r = .196$, $P < .0001$). Cells in the AA category were also positively correlated with the sperm cells of the NA category ($r = .114$, $P < .01$) and with sperm cells in the AN classification ($r = .232$, $P < .0001$). The positive correlation with the percent of dead sperm cells coincides with the supposition that the acrosome reaction may take place at, or near, death of the sperm cell.

This could be especially true in a survivability test which might cause a faster rate of the false acrosome reaction (Jones, 1973a).

Motility was highly significant ($P < .0001$) in explaining 1.2 percent of the variation (Appendix B, Tables B-VII and B-VIII). However, it is doubtful that these cells could have retained motility for extended periods of time. This is not to imply that more of these cells could not have been observed on days of increased or decreased motility in the ejaculate, especially since collection day was highly significant ($P < .01$) in explaining 2.8 percent of the variation in the cells of the AA category. Also, the days from first collection were highly significant ($P < .01$) but only accounted for 0.4 percent of the variation.

Incubation time was highly significant ($P < .0001$) but explained little of the variation ($\Delta R^2 = .0004$). This is probably due to the small, but constant, percent of this classification produced throughout the study. Ambient temperature at the time of collection was significant ($P < .05$) while accounting for 4.1 percent of the variation, possibly being due to some temperature fluctuation causing coiled or looped tails in some spermatozoa.

Other factors in the model added nothing significant to the amount of variation explained.

Analysis of the Percent Live Sperm Cells

In any semen evaluation, the percent of live sperm cells should be included as a qualitative measure of sperm cell viability.

Appendix B, Tables B-IX and B-X, show that incubation time was highly significant ($P < .0001$) in explaining the greatest amount of variation in the live classification ($\Delta R^2 = .3408$). As mentioned previously,

the incubation was a direct test of survivability and maintenance of the acrosome. Table V, page 67, shows that the percent live cells had a positive correlation with the sperm cells of the NN category ($r = .685$, $P < .0001$), a negative correlation of $-.616$ ($P < .0001$) with the percent dead sperm cells, and a negative correlation of $-.584$ ($P < .0001$) with hours of incubation.

Tables B-IX and B-X (Appendix B) also reveal that collection day was highly significant in accounting for 13.6 percent of the variation in the percent live sperm cells. This was due to daily fluctuations within a bull and between bulls in the percent live spermatozoa produced.

Motility was highly significant ($P < .0001$) in explaining 12.7 percent of the variation. Despite daily fluctuations in motility, it was positively correlated with percent live sperm cells ($r = .543$, $P < .0001$). With the correlations between motility and the evaluated semen characteristics in mind, the evidence becomes stronger that motility is a good indicator of semen quality (Wiltbank, 1965; Onks, 1972). Thus, in a study of this nature, motility, vigor, and percent live sperm cells might be positive indicators of semen quality in terms of cell longevity and viability.

Bull was also a highly significant ($P < .0001$) source of variation in the percent live sperm cells, accounting for 11.7 percent of the variation explained (Appendix B, Tables B-IX and B-X). As shown in Appendix A, Table A-I, bull 252 (Group II) was significantly higher ($P < .05$) in the production and maintenance of live spermatozoa. Table A-II shows that spermatozoa of bull 252 were not significantly different after 8 hours of incubation than the spermatozoa of bulls 2472, 837, and

173 at the initial time of collection. Figure 8, page 64, also reveals that 252 was the only Group II bull to reach the 50 percent level in the NN classification but only on the last collection day.

Tables III and IV, pages 60 and 61, present the arithmetic means, the overall mean of the percent live sperm being 60.9 percent at the initial collection. This is slightly lower than the percent live sperm observed by Wells et al. (1970).

The percent dead cells accounted for 0.7 ($P < .001$) percent of the variation in the percent live category. The correlation between percent live sperm cells and the percent dead cells was -.616 ($P < .0001$).

The other factors in the model contributed little to the amount of variation explained.

Effects of the NN and NA Classifications on Fertility

The percent of sperm cells in the NN and NA categories are the most important acrosome factors of this study to be analyzed for their effect on fertility, because of the low percent of sperm cells observed in the AN and AA classifications.

The regression analysis revealed significance ($P < .05$) only for the Group I bulls when the percent of sperm cells in both the NN and NA categories for the 8th hour of incubation or the hours combined category were included in the model (Appendix C, Tables C-I and C-II). These acrosome classifications accounted for no significant amount of the variation in fertility explained for the bulls of Group II or for all bulls (Groups I and II combined).

Appendix C, Table C-I, shows the combined effect the acrosome classifications of Group I had on the calculated number of estrous cycles

of the females calving. The table reveals that, for the 8th hour of incubation, the NN category was significant ($P < .05$) and the NA category was highly significant ($P < .01$) in accounting for 98.2 percent of the variation in the number of estrous cycles of the females that calved, while the hours combined category accounted for 97.4 percent of the variation.

This is a reflection of the improved semen quality exhibited by the Group I bulls and indicates the value of testing not only the rate of improvement of sperm cells in the NN category during an extended period of semen evaluation but also the rate of improvement of spermatozoa in their ability to maintain the integrity of the acrosome under stressful conditions. These results agree with Saacke and White (1972) who found that the degree of acrosomal maintenance during 10 hours of incubation explained 65 percent of the variation in fertility between bulls, and Wells et al. (1970) and Wells et al. (1971) who stated that the inclusion of the percent of normal sperm cells with abnormal acrosomes would add to the effectiveness of semen evaluation.

The results are also supported by Table II of Appendix C (Group I) which shows that for the 8th hour of incubation the effect of the percent of sperm cells in the NN category approached significance ($P < .06$), while the effect of the percent of sperm cells in the NA category was significant ($P < .05$). Together, these factors explained 96.7 percent of the variation in the number of estrous cycles completed by all females exposed to the bulls. For the hours combined category, both acrosome classifications were significant ($P < .05$) in explaining 97.4 percent of the variation in the fertility variable.

CHAPTER V

SUMMARY

This study was an attempt to determine the effects of sperm cell morphology and changing state of the acrosome on the fertility of beef bulls in natural mating systems.

The semen characteristics evaluated were as follows:

1. The percent of morphologically normal sperm cells with normal acrosomes (NN).

2. The percent of morphologically normal sperm cells with abnormal acrosomes (NA).

3. The percent of morphologically abnormal sperm cells with normal acrosomes (AN).

4. The percent of morphologically abnormal sperm cells with abnormal acrosomes (AA).

5. The percent of live sperm cells.

The fertility variables calculated were:

1. The possible number of estrous cycles each female that calved as a result of exposure to one of the herd sires completed to achieve conception.

2. The possible number of estrous cycles completed by all females in the presence of a herd sire, regardless of conception.

The parity status of the females varied, and all lactating cows were computationally allowed adequate involution time (42 days) when calculating the number of estrous cycles. All estrous cycles were calculated in a manner to avoid penalizing a bull for a female's lack of conception in

an estrous cycle that could have started prior to exposure to a bull and which terminated in his presence.

Simple correlations between the semen characteristics and variables that could influence the results obtained during the semen collection period were calculated. Highly significant ($P < .0001$) positive correlations were found for the NN classification with collection day, percent live sperm cells, motility, vigor, and the AN classification. Highly significant ($P < .0001$) negative correlations were found for the NN classification with the NA classification, percent dead sperm cells, hours of incubation, ambient temperature at the approximate time of spermatogenesis, and the ambient temperature at collection.

Highly significant ($P < .0001$) positive correlations for the NA classification were found with the percent dead sperm cells, ambient temperature at the approximate time of spermatogenesis, hours of incubation, and ambient temperature at collection, as well as with the AA classification ($P < .01$). Highly significant ($P < .0001$) negative correlations for the NA classification were found with collection day, the NN classification, the AN classification, and the percent live sperm cells.

The initial regression analysis revealed that the factors which exerted the greatest influence on spermatozoa of the NN category were collection day, hours of incubation, bull, motility, and the percent dead sperm cells. Factors exerting the greatest influence on sperm cells of the NA category were collection day, percent dead sperm cells, bull, and the hours of incubation.

Regression analysis also revealed that the semen characteristics exhibited by the Group I bulls in the NN and NA categories for the

overall mean of the incubation period, and after 8 hours of incubation, exerted the greatest influence on the reproductive efficiency when regressed on the calculated fertility variables.

The results of this study indicate the following:

1. Bulls can be characterized according to the qualitative aspects of sperm cell morphology and changing state of the acrosome.

2. Rates of improvement in acrosome characteristics and the degree of improvement in acrosome maintenance over an extended period of semen collection and evaluation could be used to separate bulls on their potential fertility.

3. A range of semen characteristics may exist in which it would be difficult to separate possible subtle differences between bulls of average fertility.

4. Improved techniques are available to identify bulls that would exceed either the upper or lower limits of this range of semen characteristics.

5. An extended study is needed to clarify, if possible, the limits that delineate this range.

6. More improved methods of sperm fixation and better control of management factors could greatly improve the results obtained.

In the analysis and interpretation of a data set of this nature, consideration must be given to the fact that the beef cattle production system has taken a species of continuous breeders and, influenced by management and marketing decisions, have defined time limits in which various reproductive phenomena should occur. This is an important consideration, since the phenomena of the estrous cycle is related to time

intervals and to neuroendocrine functions within these time intervals. By using limited breeding seasons, beef cattle that cannot conform to the expected standards of production and reproductive efficiency should eliminate themselves from their respective herds.

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APPENDIXES

APPENDIX A

STUDENT-NEWMAN-KEULS TESTS

TABLE A-I

EFFECT OF BULL ON SEMEN CHARACTERISTICS CLASSIFYING SPERM CELL MORPHOLOGY, VIABILITY, AND STATE OF THE ACROSOME (HOURS COMBINED)

Bull	Percent				
	NN	NA	AN	AA	Live
Group I					
411	33.97^{b}	63.13^{cd}	0.41^{efg}	2.53^{ef}	51.88^{bcde}
837	30.33^{bcde}	50.18^{e}	0.57^{cdefg}	2.31^{f}	42.52^{fgh}
855	40.04^{a}	50.56^{e}	0.89^{abc}	3.13^{cd}	52.74^{bcd}
1049	38.25^{a}	58.40^{d}	0.65^{bcdef}	2.78^{def}	54.68^{bc}
2631	32.87^{bc}	63.28^{cd}	0.75^{bcde}	3.38^{c}	57.03^{b}
Group II					
173	22.33^{hi}	73.19^{ab}	0.47^{efg}	4.04^{b}	48.14^{cdef}
252	31.64^{bcd}	65.33^{c}	0.23^{g}	2.83^{de}	65.64^{a}
417	28.28^{cdef}	68.28^{bc}	0.38^{efg}	2.99^{cde}	50.18^{bcde}
521	24.98^{fghi}	66.24^{c}	0.49^{defg}	2.78^{def}	46.07^{defg}
773	21.36^{i}	75.13^{a}	0.50^{defg}	3.15^{cd}	37.38^{h}
1762	23.42^{ghi}	67.89^{bc}	0.50^{defg}	2.69^{def}	44.95^{efg}
2022	26.43^{efgh}	63.82^{cd}	0.86^{abcd}	3.38^{c}	49.53^{cde}
2472	27.25^{defg}	58.59^{d}	1.21^{a}	7.43^{a}	41.16^{gh}
Group III					
1029	27.04^{defgh}	67.77^{bc}	2.96^{ab}	4.26^{b}	51.35^{bcde}
3191	32.69^{bc}	62.44^{cd}	0.29^{fg}	3.11^{cd}	49.63^{cde}

^{abcdefghi}Means in a column, followed by the same letter, are not significantly different at the .05 percent level of probability to Student-Newman-Kuels Test.

TABLE A-II

EFFECT OF BULL AND INCUBATION TIME ON SELECTED SEMEN CHARACTERISTICS CLASSIFYING SPERM CELL MORPHOLOGY, VIABILITY, AND STATE OF THE ACROSOME

	Percent					
	NN		NA		Live	
Bull	Hour 1	Hour 8	Hour 1	Hour 8	Hour 1	Hour 8
Group I						
411	39.94^{bc}	28.00^{ghijkl}	57.00^{ijkl}	69.25^{cdef}	61.56^{bcd}	42.19^{hijk}
837	35.56^{cdef}	25.09^{ijkl}	44.40^{m}	55.95^{jkl}	52.48^{fg}	32.56^{lmn}
855	46.53^{a}	35.56^{defg}	43.61^{m}	57.50^{ijkl}	63.50^{bc}	41.98^{hijk}
1049	45.28^{ab}	31.22^{efgh}	50.64^{lm}	66.17^{cdefgh}	66.28^{b}	43.08^{hij}
2631	37.53^{cd3}	28.22^{ghijk}	58.11^{hijkl}	68.44^{cdefg}	67.06^{b}	47.00^{ghi}
Group II						
173	26.72^{hijkl}	17.94^{m}	68.42^{cdefg}	77.97^{ab}	61.69^{bcd}	34.58^{klm}
252	36.42^{cdef}	26.86^{hijkl}	60.33^{ghij}	70.33^{bcde}	76.78^{a}	54.50^{defg}
417	30.06^{cdefg}	22.50^{klm}	62.14^{efghij}	74.42^{abc}	59.08^{bcdef}	41.28^{hijk}
521	31.34^{efghi}	18.61^{m}	59.23^{hijk}	73.25^{abcd}	62.11^{bcd}	30.03^{mn}
773	25.83^{ijkl}	16.89^{m}	70.42^{bcde}	79.83^{a}	48.22^{gh}	26.53^{n}
1762	29.06^{ghij}	17.78^{m}	61.56^{fghij}	74.23^{abc}	57.23^{cdef}	32.67^{lmn}
2022	30.75^{fghi}	22.11^{klm}	58.78^{hijkl}	68.86^{cdef}	59.22^{bcdef}	39.84^{ijkl}
2472	34.92^{efgh}	21.59^{lm}	51.86^{kl}	65.31^{defghi}	53.36^{efg}	28.95^{mn}
Group III						
1029	31.22^{efghi}	22.86^{jklm}	63.00^{efghij}	72.53^{abcd}	63.42^{bc}	39.28^{ijkl}
3191	39.53^{cd}	25.86^{ijkl}	56.89^{ijkl}	71.56^{bcd}	61.00^{bcde}	38.25^{jkl}

^{abcdefghijklmn}Means in both columns of incubation time, followed by the same letter, are not significantly different at the .05 percent level of probability to Student-Newman-Kuels Test.

TABLE A-III

EFFECT OF BULL ON THE CALCULATED NUMBER OF ESTROUS CYCLES

Bull	Calculated Number of Estrous Cycles	
	Females Calving	Females Exposed
Group I		
411	0.40^{abc}	0.40^{b}
837	1.06^{abc}	1.42^{ab}
855	0.53^{abc}	0.82^{b}
1049	0.35^{bc}	0.44^{b}
2631	0.32^{bc}	0.64^{b}
Group II		
173	1.60^{ab}	1.60^{ab}
252	1.08^{abc}	1.57^{ab}
417	1.66^{a}	2.14^{a}
521	-0.24^{c}	0.23^{b}
773	0.64^{abc}	0.64^{ab}
1762	0.74^{abc}	0.90^{ab}
2022	0.95^{abc}	1.30^{ab}
2472	1.07^{abc}	1.46^{ab}

^{abc}Means in a column, followed by the same letter are not significantly different at the .05 percent level of probability to Student-Newman-Kuels Test.

APPENDIX B

ANALYSIS OF SEMEN CHARACTERISTICS

TABLE B-I

ANALYSIS OF VARIANCE OF THE EFFECTS OF SELECTED INDEPENDENT VARIABLES ON THE PERCENT OF NORMAL SPERM CELLS WITH NORMAL ACROSOMES

Source	Degrees of Freedom	Mean Square
Regression	28	2938.3925
Bull	14	917.9654****
Collection day	8	100.1044**
Days from first collection	1	92.8623
Motility	1	5651.7037****
Temperature at spermatogenesis	1	22.6465
Incubation time	1	16103.1021****
Percent dead cells	1	2335.3211****
Temperature at collection	1	15.6457
Residual	511	39.7445
R^2		0.8020

*P <.05

**P <.01

***P <.001

****P <.0001

TABLE B-II

CHANGE IN R^2 (ΔR^2) OF THE PERCENT NORMAL SPERM CELLS WITH NORMAL ACROSOMES

Bull	Collection Day	Days From First Collection	Motility	Temperature at Spermatogenesis	Hours of Incubation	Percent Dead Sperm Cells	Temperature at Collection	R^2	ΔR^2
X								0.1514	0.1514
X	X							0.5771	0.4257
X	X	X						0.5772	0.0001
X	X	X	X					0.6227	0.0455
X	X	X	X	X				0.6227	0.0000
X	X	X	X	X	X			0.7792	0.1565
X	X	X	X	X	X	X		0.8019	0.0226
X	X	X	X	X	X	X	X	0.8020	0.0001

TABLE B-III

ANALYSIS OF VARIANCE OF THE EFFECTS OF SELECTED INDEPENDENT VARIABLES ON THE PERCENT OF NORMAL SPERM CELLS WITH ABNORMAL ACROSOMES

Source	Degrees of Freedom	Mean Square
Regression	28	4726.1581
Bull	14	1190.6968****
Collection day	8	132.6101*
Days from first collection	1	89.2158
Motility	1	5046.3697****
Temperature at spermatogenesis	1	75.1272
Incubation time	1	757.6893***
Percent dead cells	1	39311.1841****
Temperature at collection	1	119.8079
Residual	511	62.1354
R^2		0.8065

*P <.05

**P <.01

***P <.001

****P <.0001

TABLE B-IV

CHANGE IN R^2 (ΔR^2) OF THE PERCENT NORMAL SPERM CELLS WITH ABNORMAL ACROSOMES

Bull	Collection Day	Days From First Collection	Motility	Temperature at Spermatogenesis	Hours of Incubation	Percent Dead Sperm Cells	Temperature at Collection	R^2	ΔR^2
X								0.1526	0.1526
X	X							0.4364	0.2838
X	X	X						0.4365	0.0001
X	X	X	X					0.4456	0.0090
X	X	X	X	X				0.4462	0.0006
X	X	X	X	X	X			0.5636	0.1174
X	X	X	X	X	X	X		0.8058	0.2421
X	X	X	X	X	X	X	X	0.8065	0.0007

TABLE B-V

ANALYSIS OF VARIANCE OF THE EFFECTS OF SELECTED INDEPENDENT VARIABLES ON THE PERCENT OF ABNORMAL SPERM CELLS WITH NORMAL ACROSOMES

Source	Degrees of Freedom	Mean Square
Regression	28	8.6068
Bull	14	2.2034***
Collection day	8	0.8879
Days from first collection	1	3.7318*
Motility	1	0.2419
Temperature at spermatogenesis	1	0.6852
Incubation time	1	106.7661****
Percent dead cells	1	3.0242*
Temperature at collection	1	1.5114
Residual	511	0.7113
R^2		0.3987

*P <.05

**P <.01

***P <.001

****P <.0001

TABLE B-VI

CHANGE IN R^2 (ΔR^2) OF THE PERCENT ABNORMAL SPERM CELLS WITH NORMAL ACROSOMES

Bull	Collection Day	Days From First Collection	Motility	Temperature at Spermatogenesis	Hours of Incubation	Percent Dead Sperm Cells	Temperature at Collection	R^2	ΔR^2
X								0.0620	0.0620
X	X							0.1374	0.0754
X	X	X						0.1424	0.0047
X	X	X	X					0.1425	0.0001
X	X	X	X	X				0.1428	0.0003
X	X	X	X	X	X			0.3916	0.2488
X	X	X	X	X	X	X		0.3962	0.0048
X	X	X	X	X	X	X	X	0.3987	0.0025

TABLE B-VII

ANALYSIS OF VARIANCE OF THE EFFECTS OF SELECTED INDEPENDENT VARIABLES ON THE PERCENT OF ABNORMAL SPERM CELLS WITH ABNORMAL ACROSOMES

Source	Degrees of Freedom	Mean Square
Regression	28	33.0350
Bull	14	47.0768****
Collection	8	5.0178**
Days from first collection	1	11.3622**
Motility	1	33.5285****
Temperature at spermatogenesis	1	1.1842
Incubation time	1	25.3832****
Percent dead cells	1	74.0857****
Temperature at collection	1	7.6751*
Residual	511	1.6190
R^2		0.5279

*P <.05

**P <.01

***P <.001

****P <.0001

TABLE B-VIII

CHANGE IN R^2 (ΔR^2) OF THE PERCENT ABNORMAL SPERM CELLS WITH ABNORMAL ACROSOMES

Bull	Collection Day	Days From First Collection	Motility	Temperature at Spermatogenesis	Hours of Incubation	Percent Dead Sperm Cells	Temperature at Collection	R^2	ΔR^2
X								0.4386	0.4386
X	X							0.4664	0.0278
X	X	X						0.4704	0.0040
X	X	X	X					0.4822	0.0118
X	X	X	X	X				0.4823	0.0000
X	X	X	X	X	X			0.4827	0.0004
X	X	X	X	X	X	X		0.5235	0.0408
X	X	X	X	X	X	X	X	0.5279	0.0044

TABLE B-IX

ANALYSIS OF VARIANCE OF THE EFFECTS OF SELECTED INDEPENDENT VARIABLES ON THE PERCENT OF LIVE SPERM CELLS

Source	Degrees of Freedom	Mean Square
Regression	28	5332.8510
Bull	14	718.0243****
Collection day	8	353.7733***
Days from first collection	1	24.9159
Motility	1	23350.8386****
Temperature at spermatogenesis	1	227.5279
Incubation time	1	29635.2018****
Percent deal cells	1	1441.3604***
Temperature at collection	1	7.3934
Residual	511	106.7137
R^2		0.7325

*P <.05

**P <.01

***P <.001

****P <.0001

TABLE B-X

CHANGE IN R^2 (ΔR^2) OF THE PERCENT LIVE SPERM CELLS

Bull	Collection Day	Days From First Collection	Motility	Temperature at Spermatogenesis	Hours of Incubation	Percent Dead Sperm Cells	Temperature at Collection	R^2	ΔR^2
X								0.1172	0.1172
X	X							0.2530	0.1358
X	X	X						0.2554	0.0024
X	X	X	X					0.3827	0.1272
X	X	X	X	X				0.3846	0.0019
X	X	X	X	X	X			0.7254	0.3408
X	X	X	X	X	X	X		0.7324	0.0070
X	X	X	X	X	X	X	X	0.7325	0.0000

APPENDIX C

ANALYSIS OF FERTILITY

TABLE C-I

ANALYSIS OF VARIANCE OF THE EFFECTS OF THE PERCENT NORMAL SPERM CELLS WITH NORMAL AND ABNORMAL ACROSOMES OF GROUP I BULLS ON THE CALCULATED NUMBER OF ESTROUS CYCLES OF THE FEMALES CALVING

Source	Degrees of Freedom	Mean Square		
		Hours Combined	Hour 1	Hour 8
Regression	2	0.1842	0.0173	0.1855
Percent normal sperm cells with normal acrosomes	1	0.1503*	0.0299	0.1185*
Percent normal sperm cells with abnormal acrosomes	1	0.2712*	0.0158	0.2587**
Residual	2	0.0048	0.1717	0.0034
R^2		0.9744	0.0916	0.9818

*P <.05

**P <.01

TABLE C-II

ANALYSIS OF VARIANCE OF THE EFFECTS OF THE PERCENT NORMAL SPERM CELLS WITH NORMAL AND ABNORMAL ACROSOMES OF GROUP I BULLS ON THE CALCULATED NUMBER OF ESTROUS CYCLES OF ALL FEMALES EXPOSED

Source	Degrees of Freedom	Mean Square		
		Hours Combined	Hour 1	Hour 8
Regression	2	0.3328	0.0475	0.3304
Percent normal sperm cells with normal acrosomes	1	0.2514*	0.0715	0.1609†
Percent normal sperm cells with abnormal acrosomes	1	0.5081*	0.0303	0.5101*
Residual	2	0.0088	0.2942	0.0112
R^2		0.9742	0.1390	0.9672

†P <.06

*P <.05

VITA

Charles Michael Irwin was born in Cartersville, Georgia, on November 5, 1945. He is the son of Mr. and Mrs. L. R. Irwin, Jr. He attended both elementary and high school in Cartersville, Georgia, and was graduated from Cartersville High School in 1963.

The following September he entered The University of Tennessee. After working several years, he re-entered The University of Tennessee and received a Bachelor of Science degree in Agriculture on December 8, 1972.

After a year's work on a large commercial cow-calf operation, he began study toward a Master's degree at The University of Tennessee. He received this degree in December, 1976.

He is a member of the American Society of Animal Science and an honorary member of Farmhouse Fraternity.