THE EFFECT OF ABSTINENCE ON ACROSOME CHARACTERISTICS OF SPERMATOZOA OF HEREFORD AND ANGUS BULLS

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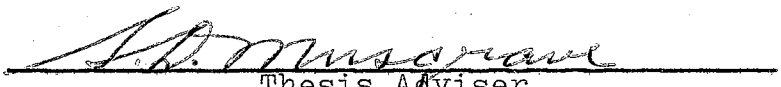

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CHAPTER I

INTRODUCTION

The demand for better bulls. The rapid growth and development of artificial insemination has demanded superior genetics and efficient bulls which can produce normal and fertile spermatozoa in large quantities, that can withstand freezing to and storage at - 196° C.

Under the best conditions some deterioration in live, normal cells is to be expected. Therefore, it is not advisable to start with marginal quality semen.

Bulls in artificial insemination centers are usually brought into sperm cell production at an early age and are maintained in use during their mature life for as long as desired sperm cell production continues and their performance is consistent with genetic goals.

The health, condition and size of testes are determining factors for the optimum production of normal, healthy spermatozoa. Maintaining the bulls under normal conditions will help keep the testes under optimum condition.

Semen evaluation. After the invention of the light microscope, the development of the ultraviolet, phase-contrast and electron microscope helped by the advancement of histochemical techniques, the structure and function of the sperm-

atozoa is being studied more deeply than before.

The acrosome, which has captured attention lately, has inspired researchers to study and acquire more knowledge. It is known that the acrosome has close relationship with respect to the fertilizing ability of the spermatozoa. The development of the Wells-Awa (5) stain and the improved laboratory technique has facilitated the study on the acrosome characteristics. The study of the relationship of the state of the acrosome to the potential fertility of the animal will be more possible than before. Determination of the influence of various animal oriented and semen care oriented factors on acrosomal characteristics should be established.

The objective of this study is to investigate the state of acrosomal characteristics of young bulls of breeding age which have not been used before and older bulls which have been used in the previous breeding season and then subjected to abstinence.

The usual evaluation of ejaculates includes the estimate of percent spermatozoa alive, their rate of movement, percent and kinds of abnormalities of structure, and concentration measurements. These are positively correlated with fertility. However, no single semen quality assay is effective in explaining more than 30 percent of the variation in fertility among bulls. The inclusion of acrosomal characteristics may decrease the variation in fertility among bulls. Therefore, the definition of the acrosomal characteristics can not be over emphasized. This study is among the basic

work in the development of knowledge for new criteria in semen evaluation.

CHAPTER II

LITERATURE REVIEW

Most of the research on factors affecting sperm cell production by bulls has been done since 1950.

VanDemark (69) stated that in properly progeny tested dairy bulls, the minimum age of the bulls would be five years with the average being six to seven years. Bulls proven at five years of age in natural service have an expected remaining life of about five and one-half years. A decrease in life expectancy for each added year of age was observed. However, he pointed that under artificial insemination the life expectancy wasless than in natural service. This, he stated, was mainly due to greater selective pressure against low fertility in artificial insemination centers.

Age, Growth, Puberty and Breeding Potential

Baker (6, 7, 8) attempted to study the relationship of age to growth rate. Up to a certain age, two years, there was a significant linear relationship between weight and age of bulls. He found significant correlationsof 0.989 for all observations on bulls and 0.996 within bulls between weight and age. Furthermore, for the first two years linear regression on the range and mean weight to age was found to be

significant. However, after two years the correlation between weight and age was less but still highly significant (all bulls 0.642, within bulls 0.956). Regressions of weight on age beyond two years were also lower (5.42 to 8.90 pounds of growth per week; 0.4 to 0.5 pounds per 100 pounds body weight).

Sexual development and level of nutrition. VanDemark (69) reported that evidence indicated that sperm cell production was dependent on the amount of actively functioning testicular tissue. Testis size, however, was directly dependent on the size of the bull. His recommendation indicated that dairy bulls should be grown out early and well, insofar as possible, avoiding damages to the testicular tissue from any cause. Adequate feeding of energy, protein, minerals and vitamins is considered mandatory to promote growth and development of the young bull to an optimum level. Younger bulls were affected to a greater degree than older bulls by nutritional deficiencies. Deficiency of energy or protein in the ration markedly delayed the onset of puberty. Among the primary vitamins necessary for proper reproductive function, the most necessary and most studied is vitamin A. In younger bulls, if vitamin A deficiency is prolonged permanent damage may result. Though less is known about most of the minerals, the growth of young dairy bulls was severely retarded when phosphorus intake was 44% of the amount recommended by the National Research Council (70).

It is known that the reproductive function has a higher

physiological priority than does body maintenance in young males. Thus, nutritional deficiency at younger age may produce permanent reproductive trouble if not corrected before maturity is reached.

Wells (70) fed 0.4 to 0.5 pounds of concentrates with two pounds low quality roughage per 100 pounds body weight and found a mean growth rate of 1.43 pounds per day. For growing bulls Morrison states that 1.96 pounds per day is acceptable and found average age of puberty to be 40 weeks, indicating 1.43 pounds is not too subnormal.

Bratton et al. (23) fed Holstein bulls at 160 percent of Morrison's recommended TDN allowance for growing dairy heifers and reported an age of 37 weeks for the onset of puberty with an average weight of 644 pounds. Bulls at 100 and 60 percent of Morrison's recommended TDN allowance for growing dairy heifers resulted in slower sexual development and delayed the onset of puberty by approximately four months.

From the results of his study, Wells (70) indicated that 0.4 to 0.5 pounds concentrate and 1 to 2 pounds of hay per 100 pounds body weight, depending on hay quality, would be a satisfactory feeding program for growing dairy bulls.

Williams (72) in his study on phosphorus found that feeding 44 percent of the amount recommended by the National Research Council retarded the growth of young dairy bulls severely. Though semen of acceptable quality was collected from all bulls in his experiment, puberty was delayed significantly. The semen volume, concentration of spermatozoa,

the percentage of live spermatozoa, and the total number of live spermatozoa were lowered. Rations slightly deficient in phosphorus may not create serious reproduction difficulties, but will retard growth and puberty. He recommended that growing dairy bulls be maintained at higher nutritive levels than heifers of equivalent weight.

Phillips (57) and Hooker (44) both indicated the presence of mature spermatozoa in the testes, at 7½ to 8½ months of age which was the beginning of active spermatozoa production. This coincided with the period of first sexual interest which was indicated by willingness of the bull to mount.

Baker and VanDemark (6) reported first mounting age average to be 29 weeks, with 25 to 45 weeks range (about 6 to 11 months). First ejaculation occurred from 35 to 46 weeks (8½ to 11 months). A rapid penis growth during the period from first sexual interest to first ejaculation was observed.

Christian and Wolf (25) electroejaculated 20 Hereford, five Shorthorn and five Angus bulls to determine the age at which these bulls were able to produce the first satisfactory semen. Collections were done twice weekly, commencing when the bulls were at eight months of age. The age of first erection was also observed, and on the average, was 271.4 days for Hereford, 245.8 days for Shorthorn and 259.0 days for Angus bulls.

They characterized a satisfactory ejaculate as semen with more than 100 million sperm cells per cubic centimeter and greater than 50 percent mobility. The average age of

producing such semen was found to be 340.4 days for Hereford, 332.8 days for Shorthorn and 379.8 days for Angus bulls. The correlation of age at first erection with age at first successful ejaculate was +0.19, not significantly high. Also, a correlation of within breed for age at first sperm production with first satisfactory ejaculate was -0.13. Both the adjusted 200 day weaning weight and the average daily gain correlation with age at first erection or with age at which the first satisfactory semen was produced were not significant.

Baker (8) found a significant correlation ($P<.05$) between age and weight and a highly significant correlation ($P<0.01$) of sperm production with weight.

Phillips and Andrews, Hooker, Musgrave, Dunn, and Swanson (57, 44, 23, 24) showed that larger bulls generally gave greater volumes of semen than did smaller bulls. However, none of them gave any statistical relationship.

VanDemark (69) found high correlation in the bull between testes-epididymides weight and sperm production and indicated that the rate of sperm production in some bulls may be greater than previously recognized. For four consecutive years beginning at puberty, semen was collected three times weekly, and 523, 898, 1093 and 1197 x 10^9 spermatozoa for years 1, 2, 3 and 4 were produced, respectively. The minimum mean sperm production rate per day increased by (1,437 x 10^6 for first year and 3,280 x 10^6 during fourth year) 1,843 x 10^6. From this it was concluded that sperm producing ca-

pacity became greater in conjunction with the increase in the size of the testes. He concluded that size of the testes was a good indicater of sperm cell producing capacity in young bulls, but not necessarily in older bulls.

Boyd (21) calculated that a minimum of 2,880,000 sperm cells was produced each day (20 x 10^6 per week) by each gram of testes in six two year old Holstein bulls. He found a correlation of 0.94 between testis weight and the measurement of length x width of testis.

Breed difference. In studying age and breed factors on sperm production and breeding ability, Bellows et al. (12) subjected young straight and cross breed bulls to electro-ejaculation at intervals of 28 days. They found that cross-bred bulls reached all electroejaculation classification criteria earlier than straight breeds. A summary of their data is presented in Table I.

TABLE I

AGE, BREED FACTORS ON SPERM PRODUCTION AND BREEDING ABILITY

Criteria	Crossbreds	Straightbreds
Age, appearance of sperm	228	243 days
Sperm number sufficient for detailed classification	260	276 days
Age at lst ejaculation	288	313 days
Age at satisfactory breeding ability	355	397 days

Slightly greater libido was exhibited in cross breeds. Bulls by Hereford sires reached most electroejaculation criteria at a later age than did Angus or Charolais sired offspring. But, Charolais sired offspring were latest in attaining satisfactory breeding ability.

Service and disposal. Bearden, Hansel and Bratton (10) in their work concluded that instances of repeat breedings with high fertility bulls were nearly always embryonic mortality, and both nonfertilization and early embryonic mortality occur with bulls of low fertility.

Foote, Henderson and Branton (31) reported a number of factors other than genetic reasons responsible for elimination of bulls from active use. Becker et al. (11) showed that only 10 percent of the usable bulls in natural service were discarded because of low production by their daughters. Accidents and disease played a large part. He reported that 46.88 percent of 556 bulls with compiled records were removed due to low breeding efficiency and about 14.25 percent removed due to refusal to work. Roman et al. (63) made a similar study on beef bulls and reported disposal of 47 percent of the beef sires in artifical insemination due to lowered reproductive inefficiency. Sixteen percent were disposed of because of inability or lack of desire to serve, and poor temperament. The above researchers agree on about a 60 percent disposal rate of beef bulls in artificial insemination due to the three factors stated above.

Becker et al. (11) in two separate studies reported an

average tenure of about 2.72 years, and 2.79 years for beef bulls in bull studs. The average age at which the bulls entered into service was 3.20 years. During 1960 to 1964 the useful life span increased to an average of 3.15 years.

Characteristics of Sperm Cell

The sperm cell. The study of sperm cell dates back more than 300 years to the time when Leeuwenhock discovered the microscope.

Salisbury and Hafez (66, 38) defined the sperm cell as a condensed highly specialized cell, that does not grow or divide. The sperm cell has been further described as having an over-all length of about 70 μ with two main parts. The head portion which contains the paternal hereditary material, is 8 to 10 μ in length, about 4 μ at its widest, and about one micron thick. Each sperm carries half the amount of DNA of the somatic cell. This specialized cell is responsible for determination of the sex of the individual produced by combination with a fertile egg.

Morphology and fertility. A bull produces semen in a limited but constant amount with few changes in semen morphology, unless the bull is subjected to some stress condition (42, 60). Much research has been conducted to reveal the effect of various environments, treatments of stresses on the character of the ejaculate.

A high correlation between the breeding record of bulls and morphology of their spermatozoa was reported by Williams

and Savage (68). Moench (53) also reported more specially that sterility occurred when the number of abnormal forms was above 25 percent in human sperm.

Pollak (58) studied the relationship between head abnormalities and sperm motility and found that general sluggishness was exhibited by giant headed sperm cells and that sperm cells were immotile when the centrosome was absent or displaced.

Folk and Kaufman (30) stated that the 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 one important factor which could compensate for any deficiencies present was good morphology. Hence, morphology has a higher stabilizing influence for fertility than any other aspect of semen quality. However, Salisbury (67) stated that the proportion of the abnormal spermatozoa to normal sperm cells in ejaculates determines the fertility of the bull producing them. Also, some reduced fertility was indicated by morphologically normal spermatozoa that were deficient in DNA.

Salisbury et al. (66), Mercier (52), and Rao et al. (61) 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 anomal-

ies and they concluded that true abnormalities were generally lower than that reported.

Salisbury et al. (66) showed that, at least in some cases, the semen of individual males may vary 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 over-emphasized.

Rao et al (61) pointed that semen with protoplasmic droplets present in relatively low numbers (less than one percent) should be considered normal. Bioly et al (13) showed the distribution of spermatozoa with protoplasmic droplets, from slaughter house bulls in 11 paired and nine single epididymides, to be 62, 42 and 84 percent, respectively, in the caput, corpus and cauda epididymis. The variation between the right and left epididymides of the same bulls was less than among bulls. From the ampullae, about 6 percent of the spermatozoa had droplets. Bioley et al (13) 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 ($P<0.01$) when seminal fluid was added.

Branton and Salisbury (22) studied the morphology of spermatozoa from different levels of the tract and reported

no significant difference 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.

Kirillov and Morozov (48) studied duration of survival of bull spermatozoa in an epididymis isolated from the testis and concluded that the epididymis was highly adapted for storage of spermatozoa. They ligatured the left epididymis of a two year old bull in the region of the deferent ducts and removed the right epididymis and testis. The result was that the bull yield 6 c.c. of semen containing 300,000,000 spermatozoa showing an activity of 0.5 on the fifth day following the operation. The corresponding figures were 2 c.c., 100,000,000 and 0.7 on the thirty-second post-operation day. The percent of spermatozoa with progressive motion decreased, and for over two months oscillatory motion was observed. Though spermatozoa did not show any morphological anomalies, all spermatozoa were immotile.

Semen quality and male reproductive function was studied by Glover (34). He studied thermal sterilty and suggested that degeneration took the course of passing from the most highly differentiated cells to the simplest forms. The spermatid degenerated before the spermatocytes did. An adverse effect could result even with short time exposure to high ambient temperature. Prosser et al. (60) and other researchers have indicated the abnormalities that occur as a result of faulty spermatocytogenesis, abnormalities that occur in the tubular genitalia as a result of incomplete and/or irregular ejaculation or conditions which were adverse to the sperm cells at the time of ejaculation. Colorado researchers (42, 60) proposed and used the following system presented in Table II for ascertaining morphological characteristics of an ejaculate.

TABLE II

ESTIMATING MORPHOLOGY

Letter Grade	Abnormalities	
	Primary	Secondary
VG - Very Good	Less than 10%	Less than 20%
G - Good	Less than 20%	Less than 35%
F - Fair	Less than 30%	Less than 50%
P - Poor	More than 30%	More than 50%

Morphology is one criterion among many others used in assessing the quality of semen.

Primary and secondary abnormalities. In estimating potential breeding capacity, the primary abnormalities were more important than secondary abnormalities and were of extreme importance. Primary abnormalities were reported to appear in a rather constant pattern in successive ejaculates and were a gross measure of histo-pathological change occuring in the seminiferous tubules. The following types of abnormalities were included in the primary category:

1. All head abnormalities
2. Double forms
3. Coiled tails
4. Abaxial middle piece
5. Medusa formations
6. Abortive tails and other abortive forms
7. Spheroids.

Though the primary abnormalities were of extreme importance, the secondary abnormalities should not be under estimated or overlooked. Chiefly, the following were considered as secondary abnormalities:

1. Loose heads
2. Proximal protoplasmic droplets
3. Distal protoplasmic droplets
4. Bent or reversed tail
5. Detachment of the galea capitis
6. Coiling at the distal end of the tail.

Among these points the detachment of the galea capitis and loose heads were primary in the present study.

Blom (14, 42) found an average frequency of 2.1% loose heads with a maximum of 8.6% found in routinely used bulls.

Origin and structure of the acrosome. Salisbury (67), Hafez (38), Hartree and Srivastova (41) reported that the acrosome covered about two-thirds of the anterior part of the sperm cell head and was a protein bound polysaccharide, composed of manose, galactose, fucose and hexosamine. The acrosome was believed to originate from the idiosome in spermatogenesis.

Bowen (19, 20) indicated that the idiosome, Golgi apparatus, and acrosome were cytoplasmic components not related to mitochondria, chromidia, or other formed elements of the cytoplasm. He reported that the exact source of the acrosome was not understood. It appeared to be produced by a differentiation of the acroblast material. Whether both the idiosome and Golgi apparatus or the idiosome alone was produced by the differentiation of the acroblast material was not known. Vasicular type and the granular type of construction during its period of formation seemed to characterize the acrosome. Bowen (20) in his latter work found that at some point on the nuclear membrane, usually before the nucleus changed from its original spherical shape, the acrosomal material was deposited by the acroblast.

Waldeyer (20) postulated earlier that the acrosome, because of structure and position at the anterior end of the

head acts as a cutting or boring apparatus would help the sperm to penetrate into the egg. Bowen (20) disproved the mechanical act of the sperm and stated that the egg responded actively to the sperm at the point of periphery. The sperm cell appeared motionless and seemed to be swallowed by the egg. But, he said that the acrosome might sometimes play a mechanical role. Yet, he did not state the real significance of the acrosome.

Nassonov's (20) extensive research on the origin of secretory granules lead to the idea that the acrosome invariably developed in association with the Golgi apparatus. The granules which appeared as small droplets became detached and cast out of the cell in the manner of secretory phenomena. Bowen (20) supported Nassonov's finding after making studies in various animals. Thus, in interpreting the acrosome in brief, it would be regarded as a secretory product, constructed from materials related essentially to enzymes. Henceforth, the acrosome would essentially be regarded as a secretory product with principal function of initiating the physicochemical relations of fertilization (Lillie - fertilization theory).

Hancock (40) showed in a living spermatozoa that the acrosome was a compact cap on the sperm head above which the galea capitis overlaps as a loose cap covering the anterior half of the head with the equatorial segment marked posteriorily by the posterior margin of the acrosome. Dead spermatzoa could be characterized by their affinity for eosin

stain and by the acrosome cap which showed irregular outline, loosely enveloping the anterior part of the sperm.

Saacke and Almquist (64) studied the acrosome by electron microscopy and reported that it had three major layers. They were the inner membrane, and outer membrane, which are continuous with one another at the posterior region of the head cap, enveloping the third part which was a homogeneous, moderately electron dense layer. During spermatogenesis the inner and outer membranes of the head cap represented the wall of the acrosome vesicle and the layer between was acrosomal origin.

Hancock (40) summarized some observations on the incidence of capless spermatozoa in freshly ejaculated semen. This was determined by counting 200 spermatozoa in each of two Giemsa-stained smears of whale semen. The relationship between the proportion of capless cells and dead cells has been studied. The evidence clearly indicates that denudation of the sperm head was associated with dead spermatozoa.

Functions of the acrosome. Blom (15) proposed the following function of the acrosome in the fertilization process. The head of the sperm cell was intact, the galea capitis or acrosome was in situ and the apical body was seen in closed form.

The loosening of the galea capitis started after the sperm travelled through the female genital tract and had almost reached the egg. This was hypothesized to happen when the enzyme or similar agent in the apical vacuole interacted

with some egg secreted agent or its coverings. This broke the cell membrane and allowed the apical body to be opened and the galea capitis to move forward and become loosened from its close contact with the nucleus.

After the double membrane broke into two halves, the hyduronidase was released and the sharp edge of the nucleus was free and ready to penetrate the ovum.

Though Blom's explanation was theoretical, the fact remained that the galea capitis played a double role, as a carrier of enzyme and protected the sharp edge of the nucleus. Where the typical morphology of the acrosome was disturbed, either knobbed, elevated or loosened in the bull, no fertilization was possible.

Acrosomal abnormalities. Saacke and Amann (65) studied inherited acrosomal abnormalities of bull sperm and classified them as follows:

> the knobbed acrosome with an intensely stained region at or near the apex of the sperm head;
> the ruffled acrosome with irregular staining intensity; and
> the incomplete acrosome with a portion of the cap removed.

They also studied the fertility of bulls with these characteristics. They used a subfertile Holstein bull (39% non returns for 89 first services) and three of four sons tested. From the subfertile bull and two affected sons, two ejaculates were collected weekly for 8 weeks. Ten unrelated Holstein bulls were studied concurrently as controls. The average frequencies of acrosomal abnormalities in ejaculates

TABLE III

THE AVERAGE FREQUENCIES OF ACROSOMAL ABNORMALITIES IN EJACULATES OF A SUBFERTILE BULL AND TWO OF HIS AFFECTED SONS AND 10 UNRELATED HOLSTEIN BULLS AS CONTROL

	Subfertile %	Each of two sons %	Control bulls %
Knobbed	6.2	3.6, 5.9	0.3
Ruffled	7.9	7.8, 9.1	1.7
Incomplete	19.0	19.0, 21.9	2.1

were as given in Table III.

Their report concluded that asymmetry of the equatorial segment was associated with many abnormal acrosomes. Irregular acrosomic granule spreading within the young spermatid was the most prevalent abnormality in the testis of all the three related bulls.

Effect of Frequency on Ejaculate Characteristic of Spermatozoa

It has been confirmed by several researchers (1, 2, 6, 7, 9, 12, 26, 42) that the collection interval is one of the most important factors influencing the production of satisfactory semen. However, it is also known that the production of semen by a bull is limited but constant through the years. Hence, it is apparent that neither too often nor too infrequent collection should result in good quality semen.

Too frequent collections resulted in lower concentration and quality. Too infrequent collections resulted in large numbers of secondary abnormalities and dead cells. Thus, a regular collection schedule to prevent the accumulation of cells in the epididymis for long periods of time resulted in getting better quality semen.

Age and frequency of ejaculation. Colorado researchers (42, 60) suggested a weekly collection schedule for average bulls. But, they have found wide variations, with a four to five day schedule suggested for animals that produced large quantities of highly concentrated semen. On the contrary, a ten day schedule was suggested for animals that produced less than an average amount of semen, especially young bulls.

Baker and VanDemark (6, 7, 8) studied the effect of frequency of ejaculation on semen production, characteristics and libido of both young and postpuberal bulls. Their first study was on nine Holstein bull calves. They assigned the calves at random for schedules of once, twice and thrice weekly collections. At 25 to 45 weeks of age, the calves showed sexual interest, but could not accomplish erection. At 36 to 46 weeks of age, first ejaculates of acceptable quality were obtained. The study showed that high variation existed in semen characteristics. During the first eight weeks of collection, an increase in the total percent motile, percent normal and rate of motility of spermatozoa in all the groups was observed. However, two of the bulls in the three times a week collection group showed a reduction in

semen quality, and showed a reduction in semen quality and libido by the 16th week of collection.

In their later study (7) the semen volume, total motile sperm per ejaculation, sperm concentration, percent motile, semen pH and percent atypical sperm were found attributable to frequency of ejaculation. When frequencies of once, twice, and three times per week were compared, no significant differences were found. In the bulls scheduled to ejaculate three times per week, reduction of libido as measured by proportion of failures to mount and ejaculate was highly significant. Baker and VanDemark (7) drew the conclusion that no harmful effect on seminal characteristics nor to spermatozoan production of young bulls except the adverse effect on libido was possible in ejaculation frequencies scheduled up to three times per week.

Breed difference. Almquist and Cunningham (1, 2) studied semen traits of beef bulls ejaculated frequently. They collected semen from seven Angus and Hereford bulls by artificial vagina from puberty to one year, at bi-weekly intervals, and from one to two years of age at weekly intervals; semen from five bulls was collected from puberty to one year and from one to two years of age six times weekly. An increase of motility from 35% for weeks 17 to 20 with a little change thereafter was found as the bulls aged and were collected six times per week.

Bull variation within breed and frequency groups for all semen traits was significant. Except the 14 bulls which

showed no decline, the semen traits for thrice weekly collection in Hereford showed a drastic decline. In the two year old group, the total motile sperm output from puberty was 2.1 and 3.0 times greater for bulls collected twice and six times weekly than for the once a week group.

Almquist and Cunningham (1, 2) studied reproductive capacity of beef bulls and post puberal changes in semen production at seven ejaculation bi-weekly from puberty to one year and weekly from one to two years of age. The ejaculate volume and weekly total and total motile sperm output was increased significantly for one to two year age group. Also, as the bulls aged, significant improvement in sperm concentration was found. The first 20 weeks following puberty, there was a rapid increase in sperm motility ratings, with very little change thereafter. Though the total sperm and total motile sperm per week were significantly greater, the ejaculate volume was highly significantly smaller as ejaculation frequency increased. No significant difference in sperm concentration between frequencies was observed.

Hereford bulls were significantly higher in sperm concentration and weekly sperm output than Angus bulls. Ejaculate volume in three Hereford bulls collected three times weekly showed no decline in summer while sperm motility and concentration declined during July, August and September. They proposed this decline to be caused by the interaction of bull and season. They suggested that there was no harmful effect on semen characteristic or weekly sperm output in

ejaculation frequencies up to six times per week on young beef bulls.

Frequency and level of nutrition. Graham and Frederick (35) studied the effect of plane of nutrition and frequency of collection on bovine semen production, and found no significant difference in semen quality. However, highly significant differences occurred for volume of semen produced and total spermatozoa per unit time, on bulls fed either 73% or 130% of Morrison's standard requirements and collected either two or four times per week.

Frequency and freezability. Martig, Almquist and Amann (50) studied bull sperm freezability after varying ejaculation frequencies and from their analysis suggested that after prolonged sexual rest, satisfactory freezability of semen collected from first ejaculate of a bull would be obtained. In conclusion they stated that neither continuous semen collection at high frequency (six times per week) for up to four years nor sexual rest of 44 weeks period exerted a detrimental effect on freezability of sperm cells.

In VanDemark, Boyd and Baker's (69) work, there was no critical change in fertility level due to frequency of collection. After exhaustive semen collection, sperm numbers in the bulls studied was restored to normal in 24 hours. Their data on three times per week collection suggested that some bulls were still functioning at less than their potential capacity to produce sperm. Very low numbers of abnormal spermatozoa were reported and sperm cells with protoplasmic

droplets were negligable and did not increase over a four year collection period. They stated that more than three times per week collections would be possible but proper training and care and caution should be used.

Hafs, Hoyt and Branton (39) ran an experiment on libido, sperm characteristics, sperm output and fertility of mature dairy bulls ejaculated daily or weekly for thirty-two weeks. Their results showed that aged bulls could be ejaculated daily for as long as eight months with no harmful effects on the bulls or their semen quality. Two to three ejaculations on two or three days a week yielded satisfactory fertility for most stud bulls.

Periodic Sexual Rest

Salisbury (67), stating that although objective data, free of the effects of season was lacking, suggested the practice of periodic rest of artificial insemination bulls from semen collection. He stated that there was strong opinion that periodic sexual rest would help in recuperating the sexual interest and fertility of bulls.

Colorado researchers (42, 60) found that bulls that have been used in pasture mating should be given at least a seven to ten day rest period before collection of semen for evaluation or freezing.

Summary

It appears that more evidence is needed to elucidate

acrosome characteristics and fertility levels of bulls. The acrosome undergoes quick and complete changes after abstinance, maltreated or incapacitated and upon the death of the spermatozoa. Very little is known for certain about the acrosome of the sperm. Abnormality of the acrosome, such as knobbed acrosomes, are found to be hereditary and may result in lowering the fertility and even in complete sterility. There are indications that much of the variations in fertility among bulls may be answered through further studies in clarifying the relationship of the state of the acrosome to the fertility of the bulls.

CHAPTER III

EXPERIMENTAL PROCEDURE

Experimental bulls and semen collection. Bulls from the dairy and beef units at Oklahoma State University and the Ft. Reno Experiment Station were used in this study. The age of the bulls ranged from 13 months to two and one-half years (30 months) with most of the younger bulls being at the Ft. Reno Station. With the exception of one questionable fertility individual, all the bulls were to be used as normal breeders with no known stresses imposed either in nutritional or environmental regimes. The bulls had not been in reproductive use prior to sampling. Some had been used in the previous breeding season but had not been collected or served a cow for several months.

Semen was collected by electroejaculation with either a Plectron or a Transjector ejaculater. Rectal massage was used to secure a sample from three Ft. Reno bulls where electroejaculation failed to give the desired result.

Laboratory procedure. A differential staining technique which distinguished the acrosome clearly, developed by Wells and Awa (5), was used in this study. The stain for this technique is made by mixing one volume of a one percent solution of water soluble eosin B, two volumes of a one per-

cent solution of water soluble fast green FCF with one and seven-tenths (1.7) volumes of ethyl alcohol. Freshly mixed stain was used in all cases, with no older than five days being used.

All of the acrosome smears were prepared within a few minutes after collection. The smears were made as follows:

The semen was diluted with 2.9% sodium citrate solution, with the rate of dilution ranging from 1:5 to 1:10, dependent on sperm cell concentration. A small drop of the diluted semen was mixed with a small drop of the stain and then smeared in a thin layer on a glass slide. After drying, a cover slip was attached with Diophane. At the same time when the acrosome smears were made, live-dead smears for computing the percentage of live cells were also made using the nigrosin-eosin stain. This stain was made by preparing a 10 percent solution of nigrosin and to 300 ml. of this solution, adding five grams of eosin.

Two slides per bull for both acrosome and live-dead stain were made making a total of four slides per bull.

Abnormal acrosome count. Three hundred sperm cells per slide were counted to determine acrosomal characteristics of each ejaculate. The cells per microscopic field were divided into the following categories: Morphologically normal cells with normal acrosomes, morphologically normal cells with abnormal acrosomes, morphologically abnormal cells with normal acrosomes and morphologically abnormal cells with abnormal acrosomes. A sperm cell with abnormal acrosomes was

defined as one where the acrosome was either absent, elevated, knobbed or disintegrating.

Additional examinations were also made to determine the percentage of tailless spermatozoa with abnormal acrosomes and total percentage of morphologically abnormal spermatozoa.

The percentage of live spermatozoa in each ejaculate was computed by counting from 250 to 300 cells on the nigrosin-eosin differential stained slides. With this stain, dead cells stain red while the live cells do not stain at all. The ratio of these cells in several fields gives a good estimate of the percent of live cells.

Association between acrosomal defects and the various abnormal spermatozoa and the percent of live cells were computed.

All examinations were made with a trinocular fluorestar light microscope, model L 16 TG -FW at magnifications of either 430X or 970X. Live-dead evaluations were typically made at 430X magnification while acrosomal evaluations were usually made at 970X magnificantion.

All statistical evaluations were based on standard analysis of variance procedures.

CHAPTER IV

RESULTS AND DISCUSSION

Abnormal, live and tailless spermatozoa. The study consists of 17 Angus and 23 Hereford bulls. All the bulls were selected, previous to this study, for breeding use. Semen was collected about two weeks before the bulls were placed in the breeding pastures. On these 40 bulls percentage alive cells, abnormal cells (primary and secondary abnormalities grouped together), and tailless spermatozoa were computed. The mean value and standard deviations were computed for these observations and are presented in Table IV.

TABLE IV

GENERAL CHARACTERISTICS FOR ALL BULLS PER EJACULATE

Breed	Number of Bulls	Number of Ejaculates	Abnormality $\bar{X}$ %	Abnormality S %	Viability $\bar{X}$ %	Viability S %	Tailless $\bar{X}$ %	Tailless S %
Angus	17	17	22.0	10.9	55.0	18.6	5.9	5.9
Hereford	23	23	26.7	13.2	52.0	5.7	11.5	9.9
Total	40	40	24.7	12.4	53.2	18.2	9.2	8.8

In normal semen evaluation, most normal bulls averaged 2.1%, maximum of 8.6%, tailless cells and not more than 18% morphological abnormalities. Enough data has been presented by other researchers that above these maximums impaired fertility or complete sterility would result. Also, acrosomal abnormality of more than 10% has resulted in very low fertility and complete sterility. As low as 1 - 5% of certain type acrosomal abnormalities would result in low fertility and/or complete sterility.

The data from these 40 bulls should give a good indication of the characteristic ejaculate produced by beef bulls less than two years old and in a state of sexual rest. As can be seen from Table IV, ejaculate quality is characteristically low. The average percent of abnormals was 24.7, the average percentage live cells was 53.2 and the percent of tailless cells was 9.2, all indicating a low quality ejaculate. Some differences between breeds can be seen. In general, Angus bulls had a somewhat lower percentage of abnormal cells and tailless cells per ejaculate than did the Hereford bulls as shown in Table V.

TABLE V

GENERAL CHARACTERISTICS FOR 22 BULLS PER EJACULATE

Breed	Number of Bulls	Number of Ejaculates	Abnormality $\bar{X}$ %	Abnormality S %	Viability $\bar{X}$ %	Viability S %	Tailless $\bar{X}$ %	Tailless S %
Angus	11	11	16.7	17.3	51.6	17.4	4.2	6.3
Hereford	11	11	25.8	16.4	50.8	16.1	12.0	12.6
Total	22	22	21.3	--	51.2	--	8.1	--

From the above described 40 bulls, complete data for general characteristics and acrosomal characteristics was available for 22 bulls, 11 Angus and 11 Hereford. These data were analyzed statistically to determine any breed differences in ejaculate morphological characteristics and degree of association of acrosomal abnormalities with the various cell types.

As mentioned previously, and as can be seen in Table V, Angus bulls tended to have a lower percentage of abnormal cells in the ejaculate than did Hereford bulls. As can be seen from Appendix Table VII, this difference in percent of abnormal cells approached significance ($P<0.10$). There was no significant difference between breeds in the percent of live cells per ejaculate, Appendix Table VIII. The breeds were essentially equal in this respect with 51.6% for Angus and 50.8% for Hereford bulls. Angus bulls again appeared to have an advantage over Hereford bulls in having a lower percentage of tailless cells per ejaculate. This advantage, 4.2% versus 12.0% approached significance at the 5% level of probability, Appendix Table IX. These percentages of tailless cells per ejaculate are far greater than desirable. Good quality ejaculate will usually have less than 10% tailless cells.

Distribution of the abnormal acrosomes. The percentage of acrosomal abnormalities for normal spermatozoa, abnormal spermatozoa and tailless cells (a form of abnormality) were determined and are presented in Table VI. As can be seen

TABLE VI

DISTRIBUTION OF ACROSOME ABNORMALITIES

Breed	Number of Bulls	Number of Ejaculates	Normal Cells $\bar{X}$ %	Normal Cells S %	Abnormal Cells $\bar{X}$ %	Abnormal Cells S %	Tailless Cells $\bar{X}$ %	Tailless Cells S %	Abnormal Acrosomes* $\bar{X}$ %	Abnormal Acrosomes* S %
Angus	11	11	13.6	6.7	40.4	19.6	76.9	16.4	16.7	2.0
Hereford	11	11	19.3	9.0	48.5	18.9	76.5	15.7	25.7	12.7
Total	22	22	16.5	8.2	44.5	14.9	77.0	9.6	21.2	10.8

* As percent of normal cells using the presently accepted method for evaluation.

from the totals of all cells, 16.5% of the normal cells, 44.5% of the abnormal cells and 77.0% of the tailless cells had abnormal acrosomes. The analysis of variance in Appendix Table X shows that there were no breed differences in the distribution of abnormal acrosomes while the percentage associated with each cell type was highly significant ($P<0.01$). Duncan's multiple range test showed that the percentage of acrosomal abnormalities in each cell type was significantly different ($P<0.01$) from other cell types. Breed differences within each cell type were not statistically significant.

The total percentage of abnormal acrosomes per ejaculate was computed and the results are presented in Table VI. The average was 21.2% abnormal acrosomes for all bulls. Breed analysis, Appendix Table XI, revealed that Angus bulls were significantly lower ($P<0.05$) in percent of abnormal acrosomes than were Hereford bulls. It should be pointed out that the Hereford bulls were older, averaging 22.0 months old while the Angus bulls averaged 16.3 months of age. The Hereford bulls theoretically had an age advantage, yet, semen quality measures fail to equal that of the younger Angus bulls in the percent of abnormal cells and abnormal acrosomes per ejaculate. Popular opinion holds that Angus bulls can be used at an earlier age with better results than can Hereford bulls. These data on young bulls just prior to the breeding season support this opinion. The data on abnormal acrosomes in particular lend strong support to this opinion. As can be seen in Table VI, 19.3% of the normal cells in

Hereford bulls had abnormal acrosomes as compared to 13.6% for Angus bulls. This difference was not statistically significant, but when these figures are combined with the percentages of abnormal cells (Table V), 16.7% for Angus and 25.8% for Hereford, the cummulative effect could well result in a lowered fertility level for Herefords.

The percentage of abnormal acrosomes per ejaculate for both breeds was far in excess of what is found in bulls collected routinely (at least weekly). Awa (5) examined several ampules of frozen semen secured from a large bull stud and found that the usual percentage of abnormal acrosomes ranged from 1.0 - 4.5%.

As can be seen in Tables IV, V, and VI, the standard deviations of the various measurements are routinely large. This is to be expected as bulls in sexual rest will show wide ranges of variation in semen quality. A few, due to self-stimulation or other-bull stimulation, may ejaculate frequently enough to maintain a fair level of semen quality. Most bulls, as illustrated in this study, will have a lower level of quality which will usually improve with use of the bulls in the breeding season.

This study needs to be extended to ascertain how and when the characteristics of the ejaculate change as the bull is subjected to frequent use. Acrosomal characteristics in particular should be carefully studied to more fully reveal their relationship to age, frequency of use and ultimate fertility of the bull.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study was conducted to determine the effect of abstinence on acrosome characteristics of spermatozoa of Hereford and Angus bulls. General characteristics as percent abnormality, percent alive and percent tailless were used primarily in evaluation of ejaculates. Significant differences were ($P<0.10$) between breeds in percent abnormal cells and tailless cells per ejaculate were found. Hereford bulls showed a lower level of semen quality than the Angus bulls.

Acrosomal characteristics such as knobbed, detached, elevated and degenerated were all considered as abnormal acrosomes. The percent of abnormal acrosomes in the normal, abnormal and tailless spermatozoa were investigated. Acrosomal abnormalities were associated to the greatest degree with tailless and less with abnormal, and to the least degree with normal spermatozoa. Percent abnormal acrosomes for each cell type were higher in Hereford than in Angus bulls. It was also learned that larger number of abnormal acrosomes were found in Hereford than in Angus. The difference between the breeds was significant ($P<0.05$). On the whole, the effect of abstinence was responded by a high percentage of abnormal acrosomes in ejaculated sperm cells.

The percentage of abnormal acrosomes in the normal cells is a key finding and should be carefully evaluated for potential in improving semen evaluation technique.

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APPENDIX

TABLE VII

ANALYSIS OF VARIANCE ON PERCENT ABNORMAL CELLS PER EJACULATE

Source	df	SS	Mean Square	F (P<0.10)
Between breeds	1	652.3	652.3	3.25*
Within breeds	20	4010.4	200.5	
Total	21	4662.7		

* Significant

TABLE VIII

ANALYSIS OF VARIANCE ON PERCENT LIVE CELLS PER EJACULATE

Source	df	SS	Mean Square	F (P<0.10)
Between breeds	1	3.9	3.9	0.014
Within breeds	20	5601.6	280.1	Nonsignificant
Total	21	5605.5		

TABLE IX

ANALYSIS OF VARIANCE ON PERCENT TAILLESS CELLS PER EJACULATE

Source	df	SS	Mean Square	F (P<0.10)
Between breeds	1	302.6	302.6	3.69*
Within breeds	20	1641.1	82.1	
Total	21	1943.7		

* Significant.

TABLE X

ANALYSIS OF VARIANCE ON PERCENT CELL TYPE PER EJACULATE

Source	df	SS	Mean Square	F (P<0.01)
Cell type	2	40,081.9	20,040.9	86.8**
Breed	1	351.9	331.9	1.4
Cell type x breed	2	213.3	106.7	
Error	60	13,855.7	230.9	
Total	65	54,482.8	8,382.0	

** Highly significant.

TABLE XI

ANALYSIS OF VARIANCE ON PERCENT OF ABNORMAL ACROSOME PER EJACULATE

Source	df	SS	Mean Square	F ($P<0.05$)
Between breeds	1	448.2	448.2	4.47*
Within breeds	20	2005.6	100.3	
Total	21	2453.8		

* Significant

VITA

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