AN ABSTRACT OF THE THESIS OF

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Title MORPHOLOGY OF EPIDIDYMAL SPERMATOZOA FROM INBRED AND LINECROSS BULLS

Abstract approved

Data were presented on epididymal spermatozoa from 29 inbred and linecross bulls raised at Oregon State University in connection with the Western Regional Beef Cattle Breeding Project, W-1. The inbred bulls came from four inbred lines. Three of these inbred lines were Herefords and the other line was Angus. The 21 linecross bulls were produced from reciprocal crosses of the three Hereford lines.

The bulls were put on test at 450 pounds. They were fed one part concentrate to two parts roughage up to 800 pounds at which time the concentrates were increased. They were slaughtered at 1000 pounds.

The epididymides were stripped from the testes at the time of slaughter and sperm were obtained from the head and tail of the epididymides for making slides. An eosin-fast green differential stain
was used to make slides for characterizing the spermatozoa as dead or alive and for detecting physiological abnormalities. Two slides were made from each location on the epididymis and labeled as head or tail and left or right. This made 16 slides per bull.

The slides were then studied and 200 spermatozoa were counted on each slide. Only ten to 25 spermatozoa were counted in one spot in order to obtain an overall picture of each slide. The number of live, dead, normal, and types of abnormalities were recorded. The different types of abnormalities counted and recorded were neck beads, tail beads, tailless heads, coiled tails, and bent tails.

An analysis of variance was run for breeding types, sides, and locations and all possible interactions between these variables. Statistical significance was determined by use of the F test.

Means were calculated for breeding types and locations. To estimate general combining ability effects, means of lines I, II, or III combined with each of the other two lines are compared. To estimate specific combining ability, means of I x II, I x III, and II x III are compared.

The analysis of variance indicated that there were statistically significant differences between breeding types for live spermatozoa, tail beads, and bent tails. Since a particular breeding type that was superior in one characteristic was also inferior in another, there was no generally superior breeding type.
There were significant differences due to location for live spermatozoa, neck beads, tail beads, tailless heads, coiled tails, and bent tails. Significant differences were also found for type and location interactions for live spermatozoa and tailless heads. A difference was also found for live spermatozoa for breeding type, side, and location interaction.

No one line of inbreds seemed to excel any other line in overall merit for general or specific combining ability.

There were many differences noted between individual bulls in specific categories studied. There were larger differences between bulls than between breeding types.

In all cases, when comparing any two groups of bulls, where there was a higher number of live spermatozoa, there was also a lower number of normals. A higher number of protoplasmic droplets on the neck of the sperm was found from the head of the epididymis and a higher number of midpiece droplets in sperm from the tail of the epididymis.

A higher number of live spermatozoa and normals was found in the tail of the epididymis. More bent tails and tailless heads were found in the head of the epididymis. More coiled tails were found in the tail.

Generally speaking no difference was found between inbred and linecross bulls for spermatozoan morphology. The six inbred and 21 linecross bulls were similar in all categories studied.
MORPHOLOGY OF EPIDIDYMAL SPERMATOZOA FROM INBRED AND LINECROSS BULLS

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MORPHOLOGY OF EPIDIDYMAL SPERMATOZOA FROM INBRED AND LINECROSS BULLS

INTRODUCTION

Bulls differ widely in their effectiveness in settling cows, and this variation in fertility is very important to the success of any cattle breeder. Morphology of semen has been used as one of the tools for determining the estimated breeding soundness of a bull. Although morphology of sperm alone will not accurately determine the fertility of a bull, it may give an indication as to whether the semen is satisfactory or not.

It is the general belief of animal breeders that inbreeding is detrimental to fertility. This has caused some reluctance by breeders to go into an inbreeding program. A larger number of inbred bulls available to commercial cattlemen would probably be of great value to them. These inbred bulls are needed for linecrossing and crossbreeding programs. These breeding systems allow the commercial cattleman to take advantage of the "hybrid vigor" effects of crossing and consequently put added pounds on the calves he has to sell.

Harris, Faulkner, and Stonaker (1960) in a study on 311 Hereford bulls found that inbreds were inferior to linecrosses in estimated breeding soundness. They found that inbreeding adversely affected morphology and percent sperm alive, more than any other semen characteristics. They also found that the lower fertility was
characteristic of a few lines rather than the inbreds as a whole.

Some of these differences found could have been due to the maturity of the bulls. Inbreeding has a retarding effect on sexual maturity. Therefore at one year of age, the inbred bulls may not have been as mature as the linecross bulls and therefore their semen appeared to be inferior.

The object of the present paper is to compare the morphology of epididymal spermatozoa from inbred and linecross bulls of the same weight. Inbred and linecross bulls of the same weight may be more comparable, because of sexual maturity, than young bulls of the same age. Also there has been a great deal of study done on semen morphology and how it is correlated to fertility, but very little on comparing the morphology of inbred and linecross animals.

Fertility of the sire is a fundamental necessity in maintaining inbred lines. Fertility should be one of the factors heavily selected for in an inbreeding program. After selection, if inbred bulls were found to compare favorably with linecross bulls, then more purebred breeders may consider an inbreeding program. It is the purpose of this study to determine if any morphology differences in semen actually exist between linecross and inbred bulls. Also the general and specific combining ability of individual inbred lines have been studied.
LITERATURE REVIEW

Harris, Faulkner, and Stonaker (1960) in a study on 311 yearling Hereford bulls found that inbreds were inferior to linecrosses in estimated breeding soundness. The bulls used were all of about the same age. Twenty three percent of the inbred bulls were classified as unsatisfactory prospective breeders, compared to ten percent of the linecross bulls.

Of the individual semen characteristics, morphology appeared to be the most adversely affected by inbreeding. In an analysis of the sources of variation for individual semen characteristics, highly significant differences were found for morphology and the percent sperm alive from year to year. Morphology was the only semen characteristic where a significant difference was found between mating systems. It was also noted that the inbreeding seemed to be affecting fertility within a few lines rather than there being a general deterioration among all the inbreds.

Stonaker (1954) found that outbred matings exceeded inbred matings 30 percent in number of calves raised to weaning. Most of this difference was due to other factors and not to fertility. Inbreds are more susceptible to disease and adverse conditions than outbreds so more of the inbreds died after birth when compared with the outbreds.
The majority of workers have found inbreeding to have a detrimental effect on fertility. Harris, Faulkner, and Stonaker (1960) in their literature review indicated that many workers had found inbreeding to lower fertility, and in males it tended to raise the frequency of specific structural and spermatic defects. Wiggins, Terrill, and Emik (1953) found that the inbreeding of the ram had a significant depressing effect on the percentage of live lambs and the percentage of lambs weaned, but not on the percentage of ewes lambing. Sharma (1960) working with mice, found that inbreeding did not affect the percentage of live sperm, but did increase abnormals.

Harris, Faulkner, and Stonaker (1960) indicated in their review, that King (1916) working with albino rats, found no adverse effect on fertility after inbreeding as closely as possible for 22 generations and selecting only the best breeding animals in each generation.

Fertility of both the bull and cow has been found to be inherited to a certain degree. Much more work has been done on the inheritance of fertility in the cow than in the bull. Terrill (1938) found that the correlation between the reproductive capacities of rams and their sons was on the borderline of significance, as shown by the correlation coefficient of 0.40. Hancock (1953) found that the pedigrees of sixteen sterile bulls showed that they were all related to two bulls. Many workers, including Beatty and Napier (1960) have found that certain strains or lines of animals tend to be, as a whole, either
high or low in fertility. All of these findings would point to fertility being inherited to some extent.

The literature is in disagreement as to which semen characteristics, or combinations of these semen characteristics, gives the best indication of the fertility level of bulls. The characteristics most often used, either singly or in combination, are as follows: semen volume, concentration, total number of spermatozoa per ejaculate, percentage of live spermatozoa, motility rating, pH value, and percentage of abnormal spermatozoa. Metabolic activities and respiration are also sometimes used as fertility indicators.

The majority of workers have found that the percentage of abnormal spermatozoa is related to fertility (Anderson, 1945; Mercier and Salisbury, 1946; Bratton, et al., 1956). Wiggins, Terrill and Emik (1953), working with rams, found that the percentage of abnormals was significantly correlated with the percentages of ewes lambing. Other workers have found that superiority of ejaculates and lower proportions of abnormals go together, but that abnormals alone are not a good indication of fertility (Terrill, 1937; Lasley and Bogart, 1943).

The percentage of abnormals may not be a good indication of fertility in rams or bulls of good fertility, but an extremely high percentage of abnormals is a good indication of infertility (Comstock et al., 1943; Swanson and Herman, 1944). It has also been suggested
that different types of abnormals may be a better index of fertility than total abnormals (Emik and Sidwell, 1949; Swanson and Herman, 1941).

It seems to be generally agreed that percentage of unstained or live spermatozoa is an indication of fertility in most animals (Bishop et al., 1954; Hulet and Ercanbrack, 1962; Beatty and Sharma, 1960; McKenzie and Berliner, 1937). Erb, et al. (1960) found in bulls that the proportion of living spermatozoa was a good index for detecting semen of poor quality.

Abnormals and percentage of dead spermatozoa increase during the hot seasons. This has been found to be particularly true in rams (McKenzie and Berliner, 1937; Cupps et al., 1960; Hafez, Badreldin and Darwish, 1955). It has also been shown that scrotal insulation will increase percentage of abnormal spermatozoa and percentage of dead spermatozoa, thus lowering the fertility of both the bull and the ram (Maude and Waites, 1963; Glover, 1955; Austin, Hupp and Murphree, 1961; McKenzie and Berliner, 1937). These findings would tend to indicate that as fertility goes down, abnormals and dead spermatozoa increase.

Motility of spermatozoa is considered by many as one of the better indicators of fertility (Swanson and Herman, 1941). Percentage of live spermatozoa has been found to be correlated with motility (Emik and Sidwell, 1947; Lasley and Bogart, 1943). Also semen that
is high in abnormals usually has a poor motility rating (Ely, Herman, and Winchester, 1942; Swanson and Herman; 1944). This indicates that as abnormal and dead spermatozoa increase, motility and fertility is lowered.

Although many conflicting figures have been published, the majority of workers agree that bulls with semen containing 30 percent or more abnormal spermatozoa are of low fertility. Bulls with semen containing 18 percent or less abnormals are generally found to be of high fertility, and bulls containing 50 percent or more abnormals are usually sterile or nearly so. However it has been found that some bulls with 18 percent or lower abnormal spermatozoa are sterile and some containing 50 percent or more, are fertile (Herman and Swanson, 1941; Swanson and Herman, 1941; Dougherty and Ewalt, 1941).

Many publications indicate that semen containing less than 40 percent live spermatozoa should be considered abnormal. Bulls producing semen containing much less than 40 percent live spermatozoa have been found to be fertile and some bulls containing more than 40 percent have been found infertile. These are exceptions, however, to the general rule (Salsbury, Nelms, and Stratton, n.d.; Carroll, Ball and Scott, 1963).

There are many reasons why the different publications vary so much on the number of abnormal and live spermatozoa found in bull semen. Some of the factors that may be responsible for these
differences are differences in staining technique, handling and collection methods, and the judgement of observers on what is abnormal and which ones are dead. Other factors involved are differences between bulls, ejaculates within the same bull, seasons and temperatures, age of bulls, and whether or not the bulls have been ejaculated within a few days prior to the time of collection.

Mayer, Squiers, and Bogart (1947) found that eosin-fast green was an excellent stain for differentiation of live from dead spermatozoa and for observing abnormals. Bodnar (1961) working with human sperm cells found that the eosin stain did not kill any of the cells.

Various papers have reported that the staining technique can increase abnormals and dead spermatozoa. The abnormals most commonly found to be affected were tailless heads and broken tails. The fixing of spermatozoa by heat was reported to be the biggest cause of these increases (Hancock, 1953; Bialy and Smith, 1958). Some workers have found that the tailless, broken tails, and dead spermatozoa were increased by smearing and fixing only if poor semen was used (Emik and Sidwell, 1949; Campbell, Hancock, and Rothschild, 1953). Mercier and Salisbury (1947) reported that the fixing of smears by heat did not influence tailless heads or true abnormals. They also reported that the "pulling" and "drop" methods of making smears produced the same results.

Some variations in abnormals and percent alive may be
attributed to the judgement of different observers. There is also some difference between areas on slides and slides of the same bull (Beatty and Napier, 1960; Campbell, Hancock, and Rothschild, 1953). Handling and collection of semen may also cause some variation (Lake, n. d.).

It has been reported that bulls of different breeds, bulls of the same breed, and bulls of the same line within a breed, vary tremendously in semen morphology. This variance is not only for percentage live sperm and total abnormalities, but for different types of abnormalities and for other semen characteristics as well (Mercier and Salisbury, 1946; Stone, Johnston and Mixner, 1950; Swanson and Herman, 1941). It has also been found that there is a significant difference between ejaculates of the same bull (Campbell, Hancock, and Shaw, 1960; Swanson and Herman, 1941). Age of the bull has also been found to affect the number of live and abnormal spermatozoa. In both young rams and bulls the number of abnormalities have been shown to be higher than in mature males. This is particularly true of protoplasmic droplets. Very old rams and bulls also show more dead and abnormal spermatozoa (Terrill, 1938; Salisbury, Nelms, and Stratton, n. d.).

First ejaculates of sexually rested bulls have been found to differ from subsequent ejaculates. First ejaculates have been found to contain higher sperm concentration, more total sperm, and higher
percentage of dead and abnormal spermatozoa (Amann and Almquist, 1961; 1962). McKenzie, Miller, and Bauguess (1938) working with boars also found a higher percentage of abnormals in first ejaculates following a long sexual rest. Abnormalities indicating that disintegration of spermatozoa had taken place in sexually rested males were the most common. The best morphological indication of disintegration appears to be bent or broken tails, followed by complete loss of the tail (Chang, 1943; Hancock, 1955; Amann and Almquist, 1962). Frequency of ejaculation does not seem to greatly affect morphological characteristics of semen (Hafs, Hoyt, and Bratton, 1959; Salamon, 1962; Hancock, 1959).

The neck and midpiece beads found on spermatozoa are generally known as protoplasmic droplets (Amann and Almquist, 1962; Lasley and Bogart, 1944). Other names for these beads are cytoplasmic droplets (Hancock, 1957; Rao and Berry, 1949) and kinoplasmic droplets (Beatty and Sharma, 1960; White and Wales, 1961).

The protoplasmic droplet is generally believed to be a sign of immaturity. The droplet is usually prominent on the neck of the spermatozoa when collected from the head of the epididymis. It then migrates to the tail or midpiece of the spermatozoa upon reaching the tail of the epididymis. The droplet is usually absent in ejaculated semen. The migration and disappearance of this droplet is believed to be steps in the development and maturation process
of the spermatozoa (Branton and Salisbury, 1947; Hancock, 1955; Rao and Berry, 1949; Lasley and Bogart, 1943). It has also been reported that disintegration of spermatozoa is associated with the migration of the protoplasmic droplet from the neck to midpiece (Hancock, 1955). It has been generally reported that frequent ejaculation does not significantly reduce or increase the number of spermatozoa showing protoplasmic droplets (Hafs, Hoyt, and Bratton, 1959; Amann and Almquist, 1962). Others have reported that frequent ejaculation increases the number of droplets observed (McKenzie, Miller and Bauquess, 1938).

It has been generally reported that ejaculated semen contains a higher percentage of dead spermatozoa than epididymal spermatozoa (Lasley and Bogart, 1944; White and Wales, 1961). However, White, Larsen, and Wales (1959) reported a slightly higher percentage of live spermatozoa in ejaculated semen.

With the exception of protoplasmic droplets, ejaculated spermatozoa have been found to be similar to epididymal spermatozoa from the same bull, particularly in both kinds and numbers of abnormal spermatozoa (Branton and Salisbury, 1947). It has been suggested that since abnormal sperm did not differ greatly in type and number from the epididymis to ejaculated spermatozoa, abnormal sperm are slowly produced and are produced in the testes (Mercier and Salisbury, 1946; Swanson and Herman, 1941; Branton and Salisbury, 1947).
MATERIALS AND METHODS

The data presented on epididymal spermatozoa were obtained from 29 inbred and linecross bulls raised at Oregon State University in connection with the Western Regional Beef Cattle Breeding Project, W-1. The inbred bulls came from four inbred lines. Three of these lines were Herefords and the other line was Angus.

The inbred lines were not highly inbred. The average inbreeding coefficients for the three Hereford lines were as follows: Lion-heart .1609, Prince .1109, and David .2142. The average inbreeding coefficient for the four lines was .1636, with a range of .1109 to .2226. There were six inbred Herefords and two inbred Angus bulls.

The 21 linecross bulls were produced from reciprocal crosses of the three Hereford lines. The Angus line was not used in any of the crosses.

The bulls were put on test at 450 pounds. They were fed one part of concentrate to two parts roughage up to 800 pounds. The bulls were tested for rate of gain and feed efficiency between 450 and 800 pound body weights. To finish the bulls out to 1000 pounds the concentrates were increased. They were then slaughtered at 1000 pounds. The bulls were sexually rested. Suckling gains were recorded prior to the testing period.
The epididymides were stripped from the testes at the time of slaughter and wrapped in paper towels soaked in cold water. These were then marked as to left and right heads and tails. The epididymides were then taken to the lab to obtain sperm from which slides were made. The differential stain used for characterizing the spermatozoa as dead or alive and for detecting physiological abnormalities was that described by Mayer et al. (1951). This is an eosin-fast green stain which is applicable to the human, ram, bull, rabbit, and stallion. A substance present in the semen plasma of the boar interferes with the differential staining of the spermatozoa.

The slides were made by making a slit, with a knife, in the epididymis and squeezing the sperm out to the surface. The sperm mass was then touched lightly to the face of a slide. A drop of stain was then added and the stain and sperm spread out over the slide with a stirring rod. This slide was then covered by another slide. The slides were then pulled apart and quickly dried on a hot plate heated to 110°F. The slides were then marked as to left or right, head or tail.

Two slides were made for each location on the epididymides. There was a total of 16 slides made for each bull. Eight of these were from the heads of the epididymides and eight from the tails. For instance, there were four slides from the left head, left tail, etc.

The slides were then studied under a microscope. Two hundred
spermatozoa were counted on each slide. Only ten to 25 were counted in one spot in an effort to get a good overall picture of each slide. The number of live, dead, normal and abnormals were recorded. The different types of abnormals counted and recorded were neck beads, tail beads, tailless heads, coiled tails, and bent tails.

The statistical analysis consisted of analysis of variance tests to determine the nature and significance of any differences noted. Variance tests were run for breeding types, sides, and locations. An analysis of variance was also run on all possible interactions between these. Statistical significance was determined by running an F test.

Since there were some significant differences in breeding types and locations, means were calculated for these and appear in table form in the section on results and discussion. Means were also calculated for each inbred line. To estimate general combining ability effects, means of lines I, II, or III combined with each of the other two lines may be compared. To estimate specific combining ability, means of I x II, I x III, and II x III are compared.
RESULTS AND DISCUSSION

Generally speaking no difference was found between inbred and linecross bulls for spermatozoan morphology. The six inbred Herefords and 21 linecross bulls were very similar in all categories studied. The inbred Angus showed some differences but these differences can not be given strong consideration since there were only two Angus bulls in the study.

The analysis of variance indicated that there was a statistically significant difference between breeding types for live spermatozoa, tail beads, and bent tails. There wasn't a significant difference for normals, neck beads, tailless heads, or coiled tails.

The differences in breed types may have resulted from the small numbers in some of the types or they may be true differences. In two of the inbred Hereford lines there was only one bull and in the inbred Angus line only two bulls. If any of these bulls were exceptional in any of the variables, it could have caused a statistically significant difference to exist.

There was a statistically significant difference due to location for live spermatozoa, neck beads, tail beads, tailless heads, coiled tails, and bent tails. The only one that was not significant was percentage of normal spermatozoa.

The differences in location would be expected due to the maturation process that takes place from the head of the epididymis to the tail. As indicated in the literature, many workers have found
For classification purposes all protoplasmic droplets just below the head were called neck beads and all others were called tail beads.

this to be true (Branton and Salisbury, 1947; Hancock, 1955).

The only trait for which there was a significant difference between sides was bent tails. The reason a difference was found in bent tails is not known and no attempt will be made to explain this effect in this paper.

Significant differences were also found on type and location interactions for live spermatozoa and tailless heads. A difference was also found for live spermatozoa on type, side, and location interaction.

As shown in Table 1, very little difference was observed between the six inbred and 21 linecross Herefords. The inbreds had slightly lower percentages of live and normal sperm and higher numbers of tailless and bent tails. They were quite high in neck beads. The inbreds were slightly superior to the linecrosses in having fewer coiled tails and neck beads. In overall merit the differences in semen from inbred and linecross bulls were very small.

The inbred Angus bulls showed larger differences when compared with the linecross Herefords. They had fewer live sperm and more tailless and bent tails than the inbred and linecross Herefords. They had more normals and fewer neck beads and tail beads than both groups of Herefords.

From Table 1 you could not conclude that any one group was superior or inferior in the overall to any other group.

Table 2 is presented to compare the general combining ability of the three inbred lines. No one line seemed to excel any other line in overall merit of morphological characteristics studied.
Table 1. Comparative means of live, normal and abnormal epididymal spermatozoa from Inbred Hereford, Inbred Angus, and Linecross Hereford bulls.

<table>
<thead>
<tr>
<th>Type and Location</th>
<th>No. of Animals</th>
<th>Live</th>
<th>Normal</th>
<th>Neck Bead</th>
<th>Tail Bead</th>
<th>Tailless</th>
<th>Coiled Tail</th>
<th>Bent Tail</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred Hereford</td>
<td>6</td>
<td>152.5</td>
<td>108.0</td>
<td>38.0</td>
<td>21.0</td>
<td>18.5</td>
<td>7.5</td>
<td>7.0</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>148.0</td>
<td>99.0</td>
<td>58.0</td>
<td>3.0</td>
<td>25.0</td>
<td>6.0</td>
<td>9.0</td>
<td>5.0</td>
<td>200</td>
</tr>
<tr>
<td>Tail</td>
<td>157.0</td>
<td>117.0</td>
<td>18.0</td>
<td>39.0</td>
<td>12.0</td>
<td>9.0</td>
<td>5.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Inbred Angus</td>
<td>2</td>
<td>133.5</td>
<td>127.5</td>
<td>8.0</td>
<td>15.5</td>
<td>27.0</td>
<td>6.0</td>
<td>17.0</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>115.0</td>
<td>124.0</td>
<td>8.0</td>
<td>2.0</td>
<td>39.0</td>
<td>5.0</td>
<td>23.0</td>
<td>200</td>
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<tr>
<td>Tail</td>
<td>152.0</td>
<td>131.0</td>
<td>8.0</td>
<td>29.0</td>
<td>15.0</td>
<td>7.0</td>
<td>11.0</td>
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<tr>
<td>Linecross Hereford</td>
<td>21</td>
<td>154.0</td>
<td>111.5</td>
<td>25.8</td>
<td>30.5</td>
<td>16.5</td>
<td>11.0</td>
<td>6.5</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>148.0</td>
<td>109.0</td>
<td>44.0</td>
<td>10.0</td>
<td>21.0</td>
<td>9.0</td>
<td>8.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>160.0</td>
<td>114.0</td>
<td>7.0</td>
<td>51.0</td>
<td>12.0</td>
<td>13.0</td>
<td>5.0</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Means for comparing general combining ability of lines I, II, and III.

<table>
<thead>
<tr>
<th>Type and Location</th>
<th>No. of Animals</th>
<th>Live</th>
<th>Normal</th>
<th>Neck Bead</th>
<th>Tail Bead</th>
<th>Tailless</th>
<th>Coiled Tail</th>
<th>Bent Tail</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line I x Others</td>
<td>7</td>
<td>172.5</td>
<td>99.0</td>
<td>33.5</td>
<td>40.0</td>
<td>14.0</td>
<td>6</td>
<td>4.5</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>170.0</td>
<td>97.0</td>
<td>63.0</td>
<td>12.0</td>
<td>16.0</td>
<td>4</td>
<td>5.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>175.0</td>
<td>101.0</td>
<td>4.0</td>
<td>68.0</td>
<td>12.0</td>
<td>8</td>
<td>4.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Line II x Others</td>
<td>4</td>
<td>149.5</td>
<td>113.5</td>
<td>3.0</td>
<td>33.5</td>
<td>17.0</td>
<td>7</td>
<td>7.0</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>146.0</td>
<td>106.0</td>
<td>4.0</td>
<td>16.0</td>
<td>23.0</td>
<td>3</td>
<td>11.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>153.0</td>
<td>121.0</td>
<td>2.0</td>
<td>51.0</td>
<td>11.0</td>
<td>11</td>
<td>3.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Line III x Others</td>
<td>10</td>
<td>143.0</td>
<td>117.0</td>
<td>19.5</td>
<td>23.0</td>
<td>18.5</td>
<td>9</td>
<td>7.5</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>134.0</td>
<td>115.0</td>
<td>32.0</td>
<td>7.0</td>
<td>25.0</td>
<td>8</td>
<td>8.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>152.0</td>
<td>119.0</td>
<td>7.0</td>
<td>39.0</td>
<td>12.0</td>
<td>10</td>
<td>7.0</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>
There were some differences in individual categories.

Linecrosses of II and III with others were similar in all categories with the exception of neck and tail beads. Line II crosses were slightly superior to line III crosses in percentages of live, tailless, coiled tails, and bent tails. This linecross type was quite superior in neck beads. Line III crosses were slightly superior to line II crosses in normals and quite superior in tail beads.

Line I crosses were superior to both line II crosses and line III crosses in number of live spermatozoa, and slightly superior in tailless, coiled tails, and bent tails. Line I crosses were inferior to both the others in percentages of normals, neck beads, and tail beads.

The specific combining abilities of line I x II and II x I are presented in Table 3. It should be kept in mind that the line II x I contained only one animal.

Line II x I was superior in number of live spermatozoa and slightly superior in neck beads, tailless, and bent tails. Line I x II was superior in normals, tail beads, and coiled tails.

It can not be concluded from Table 3 that either the I x II or II x I linecross is superior in overall merit to the other. The data are handicapped by the small numbers involved.
Table 3. Means for comparing specific combining ability of lines I x II and II x I

<table>
<thead>
<tr>
<th>Type and Location</th>
<th>No. of Animals</th>
<th>Live</th>
<th>Normal</th>
<th>Neck Bead</th>
<th>Tail Bead</th>
<th>Tailless</th>
<th>Coiled Tail</th>
<th>Bent Tail</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line I x II</td>
<td>4</td>
<td>174.0</td>
<td>98.5</td>
<td>34.5</td>
<td>44.0</td>
<td>11.0</td>
<td>8.0</td>
<td>4</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>173.0</td>
<td>98.0</td>
<td>65.0</td>
<td>14.0</td>
<td>14.0</td>
<td>5.0</td>
<td>5</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Tail</td>
<td>175.0</td>
<td>99.0</td>
<td>4.0</td>
<td>74.0</td>
<td>8.0</td>
<td>11.0</td>
<td>3</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Line II x I</td>
<td>1</td>
<td>193.5</td>
<td>81.5</td>
<td>32.5</td>
<td>63.5</td>
<td>6.0</td>
<td>16.5</td>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>194.0</td>
<td>80.0</td>
<td>63.0</td>
<td>48.0</td>
<td>7.0</td>
<td>2.0</td>
<td>1</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Tail</td>
<td>193.0</td>
<td>83.0</td>
<td>2.0</td>
<td>79.0</td>
<td>5.0</td>
<td>31.0</td>
<td>1</td>
<td></td>
<td>200</td>
</tr>
</tbody>
</table>

I = Lionheart  
II = Prince
The specific combining abilities of lines I x III and III x I are compared in Table 4. Neither cross appeared to be superior to the other in overall merit.

Line I x III was superior in percentage of live spermatozoa and slightly superior in tailless, coiled tails, and bent tails. Line III x I was superior in percentage of normals, neck beads, and tail beads.

No conclusion can be made from the data in this table as to which linecross is superior in specific combining ability. Each linecross showed superiority in some of the morphological characteristics and was inferior in others.

The specific combining abilities of line II x III and III x II are compared in Table 5. Line II x III excelled in normals, neck beads, tail beads, and coiled tails. Line III x II excelled in number of live spermatozoa, tailless, and bent tails. No appreciable overall difference was found between the two linecrosses.
Table 4. Means for comparing specific combining ability of lines I x III and III x I.

<table>
<thead>
<tr>
<th>Type and Location</th>
<th>No. of Animals</th>
<th>Live</th>
<th>Normal</th>
<th>Neck Bead</th>
<th>Tail Bead</th>
<th>Tailless</th>
<th>Coiled Tail</th>
<th>Bent Tail</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line I x III</td>
<td>3</td>
<td>165.5</td>
<td>106</td>
<td>32.0</td>
<td>43.5</td>
<td>17</td>
<td>3.5</td>
<td>5.5</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>165.0</td>
<td>103</td>
<td>60.0</td>
<td>9.0</td>
<td>17</td>
<td>3.0</td>
<td>6.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>166.0</td>
<td>109</td>
<td>4.0</td>
<td>78.0</td>
<td>17</td>
<td>4.0</td>
<td>5.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Line III x I</td>
<td>6</td>
<td>134.5</td>
<td>123</td>
<td>17.5</td>
<td>21.0</td>
<td>23</td>
<td>9.0</td>
<td>8.0</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>125.0</td>
<td>120</td>
<td>29.0</td>
<td>5.0</td>
<td>32</td>
<td>8.0</td>
<td>8.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>144.0</td>
<td>126</td>
<td>6.0</td>
<td>37.0</td>
<td>14</td>
<td>10.0</td>
<td>8.0</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

I = Lionheart  
III = David

Table 5. Means for comparing specific combining ability of lines II x III and III x II.

<table>
<thead>
<tr>
<th>Type and Location</th>
<th>No. of Animals</th>
<th>Live</th>
<th>Normal</th>
<th>Neck Bead</th>
<th>Tail Bead</th>
<th>Tailless</th>
<th>Coiled Tail</th>
<th>Bent Tail</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line II x III</td>
<td>3</td>
<td>139.0</td>
<td>124</td>
<td>18</td>
<td>23.0</td>
<td>21.5</td>
<td>3.5</td>
<td>9.5</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>130.0</td>
<td>114</td>
<td>34</td>
<td>5.0</td>
<td>29.0</td>
<td>3.0</td>
<td>15.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>148.0</td>
<td>134</td>
<td>2</td>
<td>41.0</td>
<td>14.0</td>
<td>4.0</td>
<td>4.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Line III x II</td>
<td>4</td>
<td>151.5</td>
<td>108</td>
<td>24</td>
<td>26.5</td>
<td>10.5</td>
<td>25.0</td>
<td>6.5</td>
<td>200</td>
</tr>
<tr>
<td>Head</td>
<td>148.0</td>
<td>108</td>
<td>38</td>
<td>9.0</td>
<td>14.0</td>
<td>24.0</td>
<td>8.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>155.0</td>
<td>108</td>
<td>10</td>
<td>44.0</td>
<td>7.0</td>
<td>26.0</td>
<td>5.0</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

II = Prince  
III = David
This paper clearly points out the fact that no one criterion can be used to determine sperm quality. In many cases if just one morphological characteristic is considered, one bull or group of bulls may appear to be highly superior to another bull or group of bulls. Then when all the morphological characteristics were taken into consideration, the overall difference of sperm quality was little or none. The larger the number of sperm characteristics taken into consideration, the better the chances are for an accurate analysis.

There were a lot of differences between individual bulls in all categories studied. Some of these differences could be due to technique, judgement of the observer, spermatogenic disturbances, or to the fact that some of the bulls may have masturbated, while others didn't, prior to slaughter. Large differences between bulls have been found by other workers also (Mercier and Salisbury, 1946).

In all cases, when comparing any two groups of bulls, where there was a higher number of live spermatozoa, there was also a lower number of normals. This was because where the normals were lower it was usually due to a higher number of neck and tail beads, rather than the other types of abnormalities. These beads or protoplasmic droplets are believed to be a sign of immature spermatozoa and they were almost always unstained or alive. These beaded spermatozoa caused the number of abnormalities to be high and
the number of live spermatozoa to be high.

There was always a higher number of protoplasmic droplets on the neck of the sperm from the head of the epididymis and a higher number of midpiece droplets in sperm from the tail of the epididymis. This is due to maturation of the spermatozoa as it passes through the epididymis. The more immature spermatozoa found in the head of the epididymis contain a higher number of neck beads. As the spermatozoa pass through to the tail of the epididymis they become more mature and the droplet migrates to the tail of the spermatozoa. If ejaculated spermatozoa had been collected, very few beads should have been present. The spermatozoa apparently shed the droplet upon reaching maturity. If this droplet is not shed the spermatozoa is considered abnormal (Branton and Salisbury, 1947).

There was also consistently a higher number of live spermatozoa in the tail of the epididymis than in the head. This could have also been due to the fact that there were more spermatozoa showing protoplasmic droplets here, and as previously stated these were almost always unstained or alive.

The number of normals was also consistently higher in the tail of the epididymis than in the head. This may have been due to the fact that the spermatozoa were being improperly formed
and therefore showing signs of deterioration in the head of the epididymis. Some of them may have deteriorated completely by the time they reached the tail. The steps of deterioration found by other workers are bent tails followed by a complete loss of the tail (Hancock, 1955). Tailless spermatozoa were usually stained or dead. There were more bent tails and tailless heads found in the head of the epididymis than in the tail. This again would point to not producing normal spermatozoa, causing signs of deterioration to show up in the head of epididymis and consequently less normals. Also the number of abnormals with protoplasmic droplets was slightly higher in the tail of the epididymis than in the head.

Coiled tails were always higher in the tail of the epididymis than in the head. This was expected because there was also a higher number of spermatozoa showing tail beads in the tail of the epididymis. The tendency for coiled tails and tail beads to go together seems to exist (Lasley and Bogart, 1944). The tails of the spermatozoa are usually coiled or bent at the spot where the protoplasmic droplet is located on the spermatozoa. These spermatozoa showing coiled tails were almost always unstained or alive. This has been found to be true by other workers as indicated in the literature review (Moule and Waites, 1963).

Harris, Faulkner, and Stonaker (1960) in a study on 311 yearling Herefords found that inbreds were inferior to linecrosses in
estimated breeding soundness. Percent sperm alive and morphology was found to be extremely variable from year to year. Inbreeding was found to adversely affect morphological sperm characteristics more than any other criteria studied. This adverse effect that in-breeding was found to have on sperm morphology would indicate a possible effect on spermatogenesis.

It also appeared that the differences found between the linecross and inbred bulls were partly due to one or two inbred lines that were extremely poor in spermatozoan morphology. This would tend to indicate that if bulls of poor fertility were used in the inbreeding program to begin with, that the fertility problem would be magnified.

These differences found between inbred and linecross bulls for estimated breeding soundness may have been largely due to differences in sexual maturity. There is some suggestion of a retarding effect of inbreeding upon age of sexual maturity. This would cause yearling inbred bulls to appear inferior to yearling linecross bulls in fertility traits.

The sexual maturity of the bulls at a constant weight may be more comparable than that of bulls at the same age. Using weight as a constant, rather than age, little difference was found between inbred and linecross bulls for percent of live spermatozoa or for morphological abnormalities. As long as fertility is one of the criteria selected for during inbreeding, the inbreds may very well compare favorably with linecrosses in breeding soundness.
SUMMARY AND CONCLUSIONS

Generally speaking no difference was found between inbred and linecross bulls for spermatozoan morphology. The six inbred Herefords and 21 linecross bulls were very similar in all categories studied. The inbred Angus showed some differences but these differences cannot be given strong consideration since there were only two Angus bulls in the study.

An analysis of variance indicated that there was a statistically significant difference between breeding types for live spermatozoa, tail beads, and bent tails. No significant difference was found for normals, neck beads, tailless heads or coiled tails. Since a particular breeding type that was superior in one characteristic was also inferior in another, there was no generally superior breeding type.

There was a significant difference due to location for live spermatozoa, neck beads, tail beads, tailless heads, coiled tails, and bent tails. This was believed to be a result of the maturation process of the spermatozoa from the head to the tail of the epididymis.

Significant differences were also found on type and location interactions for live spermatozoa and tailless heads. A difference was also found for live spermatozoa for breeding type, side, and location interaction.
No one line of inbreds seemed to excel any other line, when comparing overall merit or morphology characteristics studied for general combining ability. To estimate specific combining ability, means of I x II, I x III, and II x III were also compared. No significant differences were found here for overall merit.

There were many differences between individual bulls in specific categories studied. There were more differences between bulls than between breeding types.

In all cases, when comparing any two groups of bulls, where there was a higher number of live spermatozoa, there was also a lower number of normals. This was due to the fact that the spermatozoa showing protoplasmic droplets were almost always alive.

A higher number of protoplasmic droplets on the neck of the sperm was found from the head of the epididymis and a higher number of midpiece droplets in sperm from the tail of the epididymis. This was believed to be a result of maturation of the spermatozoan as it passes from the head to the tail of the epididymis. As the spermatozoa matures the droplet migrates to the tail and is eventually lost.

A higher number of live spermatozoa and normals was found in the tail of the epididymis. This was thought to be due to more protoplasmic droplets, causing a higher percentage of live, and to improperly formed spermatozoa being produced. This would cause
an increased number of spermatozoa showing signs of deterioration in the head of the epididymis. More bent tails and tailless heads were found in the head of the epididymis. Both these are considered signs of deterioration of the spermatozoa. Some of the spermatozoa may have deteriorated completely by the time they reached the tail.

More coiled tails were found in the tail of the epididymis. Coiled tails and tail beads are believed to go together and there was also more tail beads in the tail of the epididymis. The tails seem to coil at the point where the protoplasmic droplet is found on the tail of the spermatozoa.

It appears that morphology and percentage of live sperm may be as good in inbred as in the semen of linecross bulls when evaluated at comparable weights if selection has been practiced for improved fertility during the time inbreeding is being done.
BIBLIOGRAPHY


