MORPHOLOGY OF SPERMATOZOA FROM DIFFERENT LEVELS OF THE REPRODUCTIVE TRACT OF THE BULL

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In the course of investigations to establish the relationship between measurable characteristics of bull semen and its quantitative fertility in artificial insemination, attention has been focused on the source of morphologically abnormal spermatozoa. Studies in this laboratory (Salisbury et al., 1942, and Mercier, 1944, 1946) have shown that the method used in making, clearing, and staining semen smears may influence markedly the proportion of morphologically abnormal spermatozoa observed. Since the percentage of some types of abnormalities were relatively constant in the semen of any one particular bull, it was believed that the original source of these abnormal types of spermatozoa could be satisfactorily established by studies of the fluids and the spermatozoa at various levels of the male reproductive tract. Only two reports (Sciuchetti, 1938 and Lagerlöf, 1934) concerning the proportion of abnormal spermatozoa at different levels of the excurrent ducts of the bull were found in the literature.

Materials and Methods

The materials for these studies were obtained from the genitalia of 21 bulls slaughtered at a local abattoir. The bulls were either Holsteins or Guernseys with the exception of a few of mixed breeding. They were of various ages and all except 2 were free from disease. Of the 2 bulls, one was a Bang's reactor and the other had a tumor on his penis. In only 3 cases was the fertility of the bulls known.

In most instances the entire reproductive tract of each bull was removed within 15 minutes after slaughter. Fluids from epididymides and ampullae were collected as soon as possible thereafter. The testes were dissected free of the scrotum and tunica albugina. Then, through an incision made into the head of the epididymis, and by gently massaging this region, a drop or two of fluid was obtained upon a clean microscopic slide. The same technique was used to obtain fluid from the body of the epididymis.

The fluid from the tail of the epididymis was obtained by modifications of
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The methods described by Lasley and Bogart (1944) and Lardy et al. (1945). The method most often employed was that of dissecting the tail of the epididymis from the testis and leaving about 6 inches of the vas deferens attached. A physiological saline solution was injected into the vas deferens through a 24-gauge needle attached to a 10 cc syringe. Injection of the saline was continued until the majority of the cauda epididymal fluid was forced into a small test tube. Removal of the test tube before the saline came through the epididymis was necessary to prevent contamination of the sample with saline.

TABLE 1. MEAN PERCENTAGES OF MORPHOLOGICALLY ABNORMAL SPERMATOZOA FROM SIX BULLS

<table>
<thead>
<tr>
<th>Abnormalities of:</th>
<th>Epididymal samples</th>
<th>Ampullar samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head</td>
<td>Tail</td>
</tr>
<tr>
<td>Head</td>
<td>5.23</td>
<td>5.09</td>
</tr>
<tr>
<td>Neck</td>
<td>0.40</td>
<td>0.67</td>
</tr>
<tr>
<td>Mid-piece</td>
<td>1.42</td>
<td>2.09</td>
</tr>
<tr>
<td>Tail</td>
<td>2.24</td>
<td>2.83</td>
</tr>
<tr>
<td>Total</td>
<td>9.28±3.15</td>
<td>10.66±1.22</td>
</tr>
</tbody>
</table>

1 Standard error of a single observation.

The ampulla was isolated from its surrounding membranes and tissues, clamped off from the urethra, and severed from the remainder of the vas deferens. Then the fluid was collected by stripping the ampulla between the index finger and thumb.

The morphological studies of the spermatozoa from the head, body, and tail of the epididymides of 15 bulls were made by the methods described by Salisbury et al. (1942) and Mercier (1944). On the other hand, the technique recommended by Lasley and Bogart (1943) was used to study the morphology of the spermatozoa from the head and body of the epididymides and from the ampullae of 6 other bulls. This latter technique was also used for the purpose of studying the proportion of spermatozoa with attached protoplasmic droplets.

Results

Morphologically Abnormal Spermatozoa

The mean percentages for the totals of the morphologically abnormal spermatozoa, excluding tailless spermatozoa, from the head, body, and tail of the epididymides of the 15 bulls were 13.88, 14.16, and 14.95, respectively. The differences were not statistically significant. However, there was a highly significant statistical difference (significant at 1 percent level of
probability) between bulls. These bulls ranged from 9.8 to 19.5 percent in total morphologically abnormal spermatozoa, excluding tailless spermatozoa.

On the 6 other bulls spermatozoa from the ampullae were observed and the percentages of abnormals compared with the percentages observed in the samples from the head and tail of the epididymides. The comparisons are shown in table 1. Here again no statistically significant differences were obtained. Furthermore, in all cases the predominant types of abnormalities were those affecting the heads of the spermatozoa.

**TABLE 2. AVERAGE PERCENTAGES OF SPERMATOZOA HAVING PROTOPLASMIC DROPLETS ON THEIR NECKS, MID-PIECES, OR TAILS FROM THE HEAD AND TAIL OF THE EPIDIDYMIDES AND AMPULLAE OF SIX BULLS**

<table>
<thead>
<tr>
<th>Location on spermatozoa</th>
<th>Level of tract</th>
<th>Epididymis</th>
<th>Ampulla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level</td>
<td>Head</td>
<td>Tail</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck</td>
<td>60.11 ± 13.98</td>
<td>5.88 ± 1.51</td>
<td>2.61 ± 1.56</td>
</tr>
<tr>
<td>Mid-piece</td>
<td>16.67 ± 8.97</td>
<td>52.40 ± 10.21</td>
<td>12.02 ± 3.81</td>
</tr>
<tr>
<td>Tail</td>
<td>3.54 ± 2.29</td>
<td>4.62 ± 2.40</td>
<td>8.56 ± 3.77</td>
</tr>
<tr>
<td>Totals</td>
<td>80.32 ± 6.291</td>
<td>62.90 ± 2.40</td>
<td>23.19 ± 6.13</td>
</tr>
</tbody>
</table>

*Standard error of a single observation.*

**Protoplasmic Droplets**

Another morphological characteristic of spermatozoa which was observed before and after staining the smears in the studies above was the presence or absence of a protoplasmic droplet on the spermatozoa. As described by Lagerlöf (1934), Selivanova (1937), Donham and Simms (1931), and Fincher et al. (1942), it was observed to be a round mass of cytoplasm or protoplasm which usually surrounded the neck or some part of the mid-piece of the spermatozoa. Although these workers have noted that the proportion of spermatozoa bearing the droplet varies from one level of the reproductive tract of the bull to the other, no data on the relative percentages were published. The question of whether or not all bulls showed comparatively the same percentage of spermatozoa bearing the protoplasmic droplet at any particular level of their excurrent ducts was not satisfactorily answered. Therefore, this morphological characteristic was studied as shown in table 2. These studies were made on samples from the same 6 bulls for whom data in table 1 are shown.

In this group of 6 bulls the analysis of variance revealed highly significant differences between the means of the percentages of protoplasmic droplets
Figure 1. Spermatozoa from different levels of the reproductive tract of the bull, as follows: A—Head of the epididymis; B—Body of the epididymis; C and D—Tail of the epididymis; and E—Ampulla. Spermatozoa in A and C were stained with opal blue-eosin and in B, D, and E with aniline gentian violet and Ziehl's carbol fuchsine.
at all levels of the tract studied. Similarly, in the group of 15 bulls highly significant differences were found for the 3 higher levels of the tract studied: namely, head, body, and tail of the epididymides. Differences between bulls in the percentages of droplets at any particular level of the tract were not statistically significant.

**TABLE 3. TOTAL PERCENTAGES OF THE SPERMATOZOA HAVING PROTOPLASMIC DROPLETS ON THEIR NECKS, MID-PIECES, AND TAILS, FROM THE HEAD AND TAIL OF THE EPIDIDYMIDES AND AMPULLAE OF BULLS A, B, AND C**

<table>
<thead>
<tr>
<th>Bull</th>
<th>Level of tract</th>
<th>Ampulla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head</td>
<td>Tail</td>
</tr>
<tr>
<td>A</td>
<td>79.8</td>
<td>66.7</td>
</tr>
<tr>
<td>B</td>
<td>85.2</td>
<td>65.5</td>
</tr>
<tr>
<td>C</td>
<td>82.3</td>
<td>51.4</td>
</tr>
</tbody>
</table>

**Fertility and Morphological Characteristics**

Very little information could be obtained concerning the fertility of the 21 bulls studied. However, the New York Artificial Breeders' Cooperative Inc., had records on 3 of the group of 15 bulls which will be subsequently referred to as A, B, and C.

The percentages of non-returns for service were calculated for each of these bulls for the 6 months' period prior to slaughter. A, a Bang's reactor, had 56 percent (2 months' non-returns) from 549 first and second services. B, a bull with a tumor on his penis, had 77 percent non-returns from 49 first and second services. C, a bull who was low in fertility, had 46 percent non-returns from 457 first and second services.

Each level of the tract of bulls A, B, and C contained about the same percentage of morphologically abnormal spermatozoa. The average percentage of abnormal spermatozoa, excluding tailless spermatozoa, isolated from the excurrent ducts of A, B, and C was 8.01, 9.23, and 10.44, respectively. The total percentages of spermatozoa from the same bulls having attached protoplasmic droplets are shown in table 3.

**Discussion**

The results reported herein show that the levels of the excurrent ducts of the bull did not differ significantly in the percentages of morphologically abnormal spermatozoa, excluding tailless spermatozoa, when either the staining technique recommended by Salisbury (1942) and Mercier (1944) or
Lasley and Bogart (1943) was used. Since this was the case and since the predominant types of abnormals were those affecting the heads of the spermatozoa, it seems reasonable to conclude that the original source of morphologically abnormal spermatozoa is the testes. This agrees with the conclusion one might draw from the findings of Lagerlöf (1934). He presented quite convincing evidence that there are normally no recognizable changes in the morphology of spermatozoa during their passage through the reproductive tract of the bull.

The percentages of abnormal spermatozoa from the epididymides and ampullae reported by Sciuchetti (1938) are much higher than those reported in this paper. He found that the proportion of abnormal spermatozoa from the epididymides of the bull ranged from 13.07 to 45.33 percent, and from the ampullae the proportion ranged from 19.68 to 45.75 percent. Perhaps the use of different staining techniques and the study of different bulls would explain most of the differences reported by the authors and Sciuchetti (1938).

Three types of spermatozoa, differentiated as to the location of the protoplasmic droplet, were observed at each level of the reproductive tract of the bull (table 2 and figure 1, A to D). The spermatozoa from the head of the epididymides were mostly of the type with the protoplasmic droplets attached to their necks (figure 1, A and table 2). Those from the body of the epididymides had most of the droplets on their mid-pieces (figure 1, B). On the spermatozoa from the tail of the epididymides the droplets were generally attached to the posterior region of the mid-piece (figures 1, C and 1, D), whereas, most of the droplets were absent from the ampullar spermatozoa (figure 1, E). These changes in the location on the spermatozoa and the gradual disappearance of the droplets at the different levels of the reproductive tract further confirm the observations of previous workers (Lagerlöf, 1934; Selivanova, 1937; and Donham and Simms, 1931). As these workers seemed to agree, it is logical to assume that spermatozoa mature as they pass through the epididymis. The droplet finally becomes attached to the posterior region of the mid-piece of the spermatozoa. Before the spermatozoa become a component of the normal ejaculate of the bull, the droplets are detached. Thus, the droplet is usually absent from the ejaculated spermatozoa.

The results reported by the above workers and herein indicate that these changes are stages in the morphological development of spermatozoa and that all normal bulls exhibit these stages.

**Summary and Conclusions**

Studies were made on the morphological characteristics of spermatozoa from the reproductive tracts of 21 bulls of different ages. Fertility of only 3 of these bulls was known. Information on the source of morphologically ab-
normal spermatozoa and the relative proportions of morphologically abnormal spermatozoa, excluding tailless spermatozoa, and spermatozoa with attached protoplasmic droplets was secured.

It was found that the various levels of the reproductive tract of the bull did not differ significantly in the proportions of abnormal spermatozoa and that the predominant types of abnormals were those affecting the heads of the spermatozoa. It was, therefore, concluded that the testis is the original source of morphologically abnormal spermatozoa.

All bulls studied had three types of spermatozoa as to the location of the protoplasmic droplet. There was no significant statistical difference between bulls as to the proportion of spermatozoa with attached droplets at any given level of the tract. However, the droplet was usually located on the neck of spermatozoa from the head of the epididymis, and was usually absent from ampullar spermatozoa. It was concluded that these were morphological stages in the development of the spermatozoa and that all normal bulls show the same stages.

**Literature Cited**


Mercier, E. 1944. The Relationship between the Percentage of Morphologically Abnormal Spermatozoa and Other Criteria of Bull Semen Quality. A thesis presented to the Faculty of the Graduate School at Cornell University in partial fulfillment of the requirements for the degree of Master of Science in Agriculture.

Mercier, E. 1946. The Effect of Season on Spermatogenic Activity and Reproduction in Cattle. A thesis presented to the Faculty of the Graduate School at Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.


