THE SPERMATOZOA OF STERILE BULLS

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(With Plates 3 and 4)

INTRODUCTION

This paper is concerned with a morphological abnormality in the spermatozoa of certain sterile bulls and the identification of this abnormality when specimens are prepared for microscopical examination.

In certain cases sterility in the bull can be associated with the presence of an abnormal acrosome, the abnormality taking the form of an eccentrically placed thickening of this structure (Teunissen, 1946; Blom, 1948; Hancock, 1949; Rollinson & Makinson, 1949; Bretschneider, 1950). A number of photographs of it are given later in this paper. In the United Kingdom it has been recognized in seventeen Friesian bulls, sixteen of which were closely related. The clear-cut nature of the defect and its transmission by apparently normal parents suggests that it is due to a recessive gene.

Recent work on the preparation of spermatozoa for microscopical examination shows that considerable care must be taken to preserve the morphological characteristics of the living spermatozoa before fixation. This question has been discussed in detail in a recent paper (Hancock, 1952), and here it is only necessary to say that unless special precautions are taken to avoid damage from such causes as sudden cooling, fixed preparations are morphologically very different from living ones. When examining spermatozoa for evidence of the genetical defect referred to above, it is essential to take precautions to avoid accidental damage if the correct incidence of this defect is to be estimated.

The acrosome is situated at the anterior half of the head. It is a compact refractile structure which in the living spermatozoon is closely applied to the surface of the sperm head. After maltreatment or in certain pathological cases, it may appear as a fragile loose envelope. Blom (1945) calls this structure the galea capitis, but for reasons discussed in detail elsewhere the word 'acrosome' is preferable.

The spermatozoa of bulls with this genetical defect may appear to be physiologically normal (Rothschild, 1950), which makes accurate morphological information about them of special importance. The experiments described in this paper were designed to investigate the effects of post-mortem changes, caused by various treatments, on the morphology of spermatozoa with this defect.

When spermatozoa are examined in a dry film under the microscope they are of course dead. Some of these may have been dead originally, in the sample of semen;
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others may have been killed during the treatment of the material before the film was prepared, while others may have been alive until the moment when the film was fixed or dried. In investigating the microscopical or sub-microscopical morphology of spermatozoa, whether originally normal or abnormal, a clear distinction must be made between these three classes; otherwise, as has happened in the past, it is difficult to decide which morphological structures were originally present, which were induced by the treatment prior to examination, and which were masked by the preparatory treatment. In order to maintain these distinctions special meanings have been given to certain words used in this paper. A spermatozoon is (1) living if it is alive when it is examined with the microscope; a spermatozoon is (2) 'live' if it has been alive up to the moment of fixation (or drying). A spermatozoon may have been (3) originally dead when the sample was collected or it may have been (4) killed during the preparatory treatment, e.g. by 'temperature shock'. In cases where it is unnecessary or impossible to distinguish between (3) and (4) the word (5) dead is used. When a spermatozoon shows the eccentrically placed thickening of the acrosome, with which this paper is concerned, it is referred to as (6) abnormal.

MATERIALS AND METHODS
The semen of three Friesian bulls (numbered 1, 2 and 3), all with clinical histories of virtual sterility, was collected in the usual way and stained with nigrosin-eosin, Giemsa and Heidenhain's iron haematoxylin, as described in an earlier paper in this Journal (Hancock, 1952). Studies of dead spermatozoa were made on samples killed by rapid cooling as previously described. The procedure for electron microscopy was the same as before, except that the semen was fixed by dilution with an equal volume of neutral formal saline (5% formaldehyde) and washed after fixation for 1 hr. The electron micrography was carried out by Mr R. W. Horne, Cavendish Laboratory, University of Cambridge.

The percentage of morphologically abnormal forms was determined from counts of four hundred spermatozoa per sample.

RESULTS
In smears of abnormal spermatozoa, stained by the nigrosin and eosin method, the 'live' spermatozoon (which do not take the stain) are seen to possess a highly refractile area at the distal end of the acrosome; in dead spermatozoa (which do take the stain) there is little evidence of this abnormality. The characteristic appearances of 'live' and dead spermatozoa are seen in Pl. 3a, b. Both these preparations were made from the same ejaculate, (a) being stained at 30° C. and (b) being stained at 10° C. and thus subjected to temperature shock. In (a) the abnormality is clearly seen in all four spermatozoa; in (b) it is seen in one spermatozoon which is unstained and therefore 'live' whereas it is not seen in the other two which have taken the stain and were presumably killed by temperature shock.

Differential counts made from smears prepared after staining at different temperatures showed that the proportion of clearly abnormal spermatozoa declined as the temperature difference between semen and stain was increased. This decline
was associated with a decrease in the percentage of spermatozoa which survived the temperature shock imposed during staining. The results of one experiment, using the semen of bull 1, are summarized in Table 1.

Studies of unfixed material with the phase-contrast microscope confirmed that the appearance of the abnormal acrosome is modified by the death of the spermatozoon. Using this technique the acrosome appears as a sharply defined dark refractile area in the living spermatozoon; but it is quite inconspicuous in dead cells. Its appearance in living and dead spermatozoa viewed by this method is shown in Pl. 3 e, d.

Table 1. The effect of stain temperature on the staining affinity of bull spermatozoa and on the abnormal acrosome (nigrosin-eosin)

<table>
<thead>
<tr>
<th>Stain temperature (°C)</th>
<th>No. of spermatozoa in 200</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>A</td>
</tr>
<tr>
<td>30°0</td>
<td>130</td>
<td>85</td>
</tr>
<tr>
<td>20°5</td>
<td>75</td>
<td>69</td>
</tr>
<tr>
<td>15°0</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>2°5</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Live unstained, L; visibly abnormal, A, temperature of semen, 30° C.

In haematoxylin-stained smears of 'live' spermatozoa, the abnormality is seen as an intensely stained area of the acrosome. It is less conspicuous in dead spermatozoa, but is not infrequently recognizable as a fragile prominence at the distal border. 'Live' and dead spermatozoa in smears stained with Giemsa or with Indian ink show no marked difference in microscopical appearance. Pl. 3 e, f, show smears of dead spermatozoa stained by these techniques. Electron micrographs of the abnormality in 'live' and dead spermatozoa are shown in Pl. 4 a, b.

The proportion of visibly abnormal spermatozoa was determined in 'live' and killed samples of seven ejaculates from two other Friesian bulls (bulls 2 and 3), using a number of different techniques. The results are summarized in Table 2.

Statistical analysis, for which I am indebted to Mr R. C. Campbell of this Unit, shows that the abnormality is equally apparent in 'live' and dead spermatozoa stained by the Indian ink and Giemsa techniques. This is not, however, the case when the nigrosin-eosin or phase-contrast techniques are used, the abnormality being invisible in dead spermatozoa prepared by these methods. The same applies in the case of haematoxylin-stained material though the relationship between the percentage of 'live' spermatozoa, and the percentage of visibly abnormal spermatozoa is not the same as when the nigrosin-eosin and phase-contrast techniques are used.

These relationships also apply when the data for the 'live' and killed samples are studied separately. There is therefore no evidence that the cytological changes which occur in spermatozoa killed by rapid cooling ('temperature shock') differ from those which distinguish living from dead spermatozoa in freshly ejaculated semen.
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Table 2. Comparison of different methods of examining the abnormal acrosome in 'live' (L) and dead (D) spermatozoa

<table>
<thead>
<tr>
<th>Bull</th>
<th>Ejaculate</th>
<th>Sample</th>
<th>Live (%)</th>
<th>Abnormal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nigrosin-eosin</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>L</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>L</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>L</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>L</td>
<td>77</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>49</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>L</td>
<td>81</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>L</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>L</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

The abnormal features of living and dead spermatozoa are compared in the column headed Phase contrast.

DISCUSSION

Diagnosis of sterility in bulls whose spermatozoa exhibit this acrosomal abnormality can at present only be based on microscopical examination. After the death of the spermatozoa, changes occur in the acrosome which render the abnormality indistinct or unrecognizable by certain microscopical techniques. Smears of killed spermatozoa stained with nigrosin and eosin or with Heidenhain's iron haematoxylin show little evidence of the abnormality: it is also indistinct in dead spermatozoa examined with the phase-contrast microscope. In smears stained by the Giemsa and indian ink techniques the abnormality is equally evident in both 'live' and dead spermatozoa.

Freshly ejaculated semen contains a variable proportion of originally dead spermatozoa; living spermatozoa are readily killed by such treatments as rapid cooling. Of the techniques used only the Giemsa and indian ink methods will enable an accurate estimate to be made of the proportion of abnormal spermatozoa in a sample.

SUMMARY

1. Sterility in certain Friesian bulls is associated with a specific morphological abnormality affecting the acrosomes of their spermatozoa.

2. This abnormality is visible in living spermatozoa and is also visible in fixed spermatozoa when precautions are taken to preserve the morphology of the cell.

3. If such precautions are not taken, and the spermatozoa are subsequently stained by the nigrosin-eosin method, or with Heidenhain's iron haematoxylin, or
when such spermatozoa are examined with the phase-contrast microscope, the
abnormality is inconspicuous.

4. These difficulties do not arise when either the Giemsa stain or indian ink is used.

I wish to thank Mr J. Smiles, National Institute for Medical Research, Mill Hill,
London, for the photomicrographs in Pl. 3 a, b, Mr A. V. Guntrip for those in
Pl. 3 c-f, and Miss P. J. Hennessy for her technical assistance.

The bulls were made available through the collaboration of Mr E. J. Simmons,
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I am indebted to Lord Rothschild, Department of Zoology, University of
Cambridge, for reading the manuscript of this paper and for many helpful
suggestions.

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APPENDIX

Although this investigation was primarily concerned with the morphology of
spermatozoa it has brought to light a certain body of information which bears upon
the inheritance of the abnormality and may be of interest to geneticists.

An examination of the pedigrees of the sixteen sterile bulls shows that they are
all related to two bulls which we will call A and B. The relationships are sum-
marized in a chart (Text-fig. 1). Many people find this presentation difficult
because they try to interpret Text-fig. 1 as a pedigree chart of the orthodox type.
To understand how the chart is to be read the reader is advised simply to work
through the following examples in the exact order indicated in the instructions and
omitting none of the steps.

Take first the relationship of sire. Find the line labelled ‘sire’ on the chart.
Note that associated with ‘sire’ are the symbols A (1, 2, 3, 4, 8, 10, 11, 12, 13). This
means that bull A was the sire of the sterile bulls 1, 2, 3, 4, 8, 10, 11, 12 and 13.
Next, take the relationship of maternal grandsire. Find the line labelled ‘maternal
grandsire’ on the chart and note that it bears the symbols A (4, 11, 12) B (1, 2, 3, 7,
13). This means that bull A was the maternal grandsire of sterile bulls 4, 11 and 12
and that bull B was the maternal grandsire of sterile bulls 1, 2, 3, 7 and 13. Next
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find the line at the top left-hand corner of the chart which is unlabelled but bears the symbols A (7). The proper label for this line is 'father of the father of the paternal grandsire', as the chart indicates. The symbols A (7) in association with this line mean that bull A was the father of the father of the paternal grandsire of sterile bull 7. Now search the chart and note all the positions in which the figure 7 occurs. There are three positions where 7 occurs in brackets after A and four positions where 7 occurs in brackets after B.

The three positions where 7 occurs in brackets after A indicate that bull A was:

(i) the father of the father of the paternal grandsire,
(ii) the father of the paternal granddam,
(iii) the father of the maternal granddam,

of sterile bull 7.

The four positions where 7 occurs in brackets after B indicate that bull B was:

(iv) the father of the mother of the paternal grandsire,
(v) the father of the mother of the paternal granddam,
(vi) the father of the mother of the maternal granddam,
(vii) the maternal grandsire,

of sterile bull 7.

It should now be possible for the reader to take any other sterile bull, say sterile bull 5, find the positions where the figure 5 occurs on the chart and thereby discover where bulls A and B occur in the family history of sterile bull 5.
EXPLANATION OF PLATES

PLATE 3
(a) 'Live' spermatozoa showing abnormal acrosome. Nigrosin-eosin. \( \times 950 \).
(b) One 'live' and two dead spermatozoa showing altered features of abnormal acrosome in dead spermatozoa. Nigrosin-eosin. \( \times 950 \).
(c) Living spermatozoa showing abnormal acrosome. Phase-contrast. \( \times 800 \).
(d) Dead spermatozoa showing altered features of abnormal acrosome. Phase-contrast. \( \times 800 \).
(e) Dead spermatozoa showing abnormal acrosome. Giemsa. \( \times 680 \).
(f) Dead spermatozoa showing abnormal acrosome. Indian ink. \( \times 680 \).

PLATE 4
(a) 'Live' spermatozoon showing abnormal acrosome. Electron micrograph. Shadowed gold-palladium. \( \times 14,000 \).
(b) Dead spermatozoon showing abnormal acrosome. Electron micrograph. Shadowed gold-palladium. \( \times 16,500 \).
HANCOCK—THE SPERMATOZOA OF STERILE BULLS
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