CHANGES OCCURRING IN THE HEAD OF BOAR SPERMATOZOA: VESICULATION OR VACUOLATION OF THE ACROSOME?

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The sequence of structural changes in the head of mammalian spermatozoa which have been associated with ageing or death (see review by Hancock, 1966) were determined in bull spermatozoa by Saacke & Marshall (1968). Bedford (1970) considered this acrosomal disintegration to be a 'nonspecific degeneration' or 'false' acrosomal reaction, quite different from the 'true' acrosomal reaction (Barros, Bedford, Franklin & Austin, 1967) which involves vesiculation of the opposing acrosomal and plasma membranes. These reactions do not, however, account for all morphological modifications of the acrosome and the subject has therefore been examined further. This report summarizes the results of a number of studies, and records for boar spermatozoa the degenerative changes already described in bull spermatozoa by Saacke & Marshall (1968), which involved vacuolation of the acrosome. Another sequence of changes, not previously reported and which involves vesiculation of the acrosome, is also described.

Experiment 1 involved further examination of the electron micrographs of spermatozoa from the head, body and tail of the epididymis used by Jones (1971b). Ejaculates collected with an artificial vagina were used in the other experiments, in which the storage of semen was studied; Exp. 2 (Table 1) concerned the storage of spermatozoa in a surgically prepared pocket in the uterus of a sow; Exp. 3 (replicated with ejaculates from two boars) involved the storage of semen for up to 10 hr in fluid collected from the lumen of the ligated uterus or the Fallopian tubes; Exp. 4 (Table 1) concerned semen stored for 6 hr at 20° C or 5° C in a solution of 20% v/v egg yolk, 266 mM-glycine and 20 mM-tris (adjusted to pH 7-2 with HCl) and containing 0, 5 or 10% v/v glycerol; in Exp. 5, ejaculates from six boars were examined at various times during incubation at 5° C and 20° C for up to 62 hr in the diluent containing 10% glycerol which was used in Exp. 4.

Spermatozoa were fixed initially in glutaraldehyde–cacodylate, then osmicated. Sub-samples of some treatments in Exp. 5 were also fixed directly in osmium cacodylate in order to confirm that the structures observed were not due to glutaraldehyde fixation. Subsequent procedures for the preparation and examination of samples for electron microscopy have been described by Jones (1971a, b, 1973). They included coding and randomization of treatments and preparation of low power (× 5000 initial magnification) electron micrographs.

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from different areas of an Araldite section from each treatment. These were subsequently studied and the different modifications of the acrosome or mitochondria were scored for counting (Table 1).

The acrosomes of spermatozoa in freshly collected semen contained homogeneous material within a bilaminar membrane. The plasma membrane was usually intact and closely apposed to the acrosomal membrane (Pl. 1, Fig. 1). A sequence of changes involving vacuolation of the acrosome was observed (Pl. 1, Figs 6 to 8). They were similar to the changes described in bull spermatozoa by Saacke & Marshall (1968) except that usually the first sign of deterioration in the boar spermatozoon was breakage of the plasma membrane over the acrosome (Pl. 1, Fig. 6). Swelling and vacuolation of the acrosome was then observed (Pl. 1, Fig. 7) and finally, complete disruption of the plasma membrane and of the anterior end of the outer acrosomal membrane and loss of acrosomal contents (Pl. 1, Fig. 8). About 5% of spermatozoa from the different regions of the epididymis contained advanced stages of vacuolation of the acrosome (see Table 2 in Jones, 1971a) and about the same proportion was

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**EXPLANATION OF PLATE 1**

Figs 1 to 5. Sagittal sections through the anterior end of boar sperm heads, showing successive stages of vesiculation of the acrosome. × 40,000.

Fig. 1. Intact acrosomal and plasma membrane.

Fig. 2. Slight swelling of acrosome and some invagination of the outer acrosomal membrane into the apical ridge.

Fig. 3. Vesicles (arrows) formed within the acrosome from the outer acrosomal membrane.

Fig. 4. Acrosomal contents contained within the inner acrosomal membrane and the plasma membrane after much of the outer acrosomal membrane has been used during formation of the vesicles (arrows).

Fig. 5. Only the inner acrosomal membrane remains over the nucleus; this is covered by a layer of vesicles.

Figs 6 to 8. Sagittal sections through the anterior end of boar sperm heads showing successive stages of vacuolation of the acrosome that follow from the stage shown in Fig. 1. × 22,500.

Fig. 6. Unchanged acrosome and broken plasma membrane.

Fig. 7. Swollen and vacuolated acrosome and broken plasma membrane.

Fig. 8. Loss of most of the plasma membrane covering the acrosome, of the outer acrosomal membrane (O) and of the acrosomal contents from the anterior region of the acrosome, leaving the nucleus covered by the inner acrosomal membrane.

Figs 9 and 10. Cross-section through the apical ridge of the acrosome of boar spermatozoa showing the invagination of the outer acrosomal membrane (Fig. 9) and the formation of vesicles (Fig. 10). Spermatozoa like those in Fig. 10 that had vesiculated yet showed signs of vacuolation were very rare. × 45,000.

Figs 11 and 12. More distal regions (equatorial segment of acrosome) of the head of the boar spermatozoon shown in Fig. 4. Acrosomal material is present between the plasma membrane and the outer acrosomal membrane (O) which is intact in the equatorial segment of the acrosome and continuous with the inner acrosomal membrane (arrow, Fig. 12). × 40,000.

Fig. 13. Membrane-bound vesicle (arrow shows bilamina) in the acrosome of a boar spermatozoon at a stage of 'vesiculation' intermediate between the stages shown in Fig. 5 and Fig. 4. × 89,000.

Fig. 14. Part of a cross-section of the anterior region of the acrosome of a boar spermatozoon at a stage of vesiculation intermediate between the stages shown in Figs 2 and 4. The plasma membrane (P) and most of the outer acrosomal membrane (O) is intact. A meshwork of 'channels' has developed throughout the acrosomal contents. × 71,000.
observed in freshly collected semen (Table 1). The incidence was increased by ageing and processing semen (Exp. 4, Table 1 and Exp. 5).

The other type of structural change, vesiculation of the acrosome, involved swelling of the acrosome and invagination of the outer acrosomal membrane (Pl. 1, Figs 2 and 9). Membrane-bound vesicles (Pl. 1, Figs 3, 4, 10 and 13) were then formed within the acrosome from the outer acrosomal membrane (Pl. 1, Figs 3 and 10). Since most of the outer membrane of the anterior region of the acrosome was involved in this process, the acrosomal material was surrounded only by the plasma membrane and inner acrosomal membrane (Pl. 1, Fig. 4).

Table 1. The effect of various treatments on the structure of the head of boar spermatozoa and scores of percentage motile spermatozoa

<table>
<thead>
<tr>
<th>Classification of sperm heads*</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>†</th>
<th>7</th>
<th>8</th>
<th>Mean percentage motile</th>
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<tr>
<td>Plasma membrane: intact</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>broken or lost</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>40</td>
</tr>
<tr>
<td>Acrosome: swollen</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>84</td>
</tr>
<tr>
<td>vesculicated</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>64</td>
</tr>
<tr>
<td>vacuolated</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>76</td>
</tr>
<tr>
<td>loss of outer membrane</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>54</td>
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<tr>
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<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>50</td>
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<tr>
<td>Experiment 2 (1 replicate)</td>
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<td></td>
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<td></td>
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<tr>
<td>Freshly collected semen (n = 82)</td>
<td>38</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Stored in utero</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>(n = 55)</td>
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<tr>
<td>Experiment 4 (4 replicates)</td>
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<tr>
<td>Freshly collected semen (n = 73)</td>
<td>79</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>84</td>
</tr>
<tr>
<td>Diluted: 20° C, 0% glycerol</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>(n = 74)</td>
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<td>25</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>76</td>
<td>84</td>
</tr>
<tr>
<td>20° C, 5% glycerol (n = 71)</td>
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<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>74</td>
<td>82</td>
</tr>
<tr>
<td>20° C, 10% glycerol (n = 83)</td>
<td>46</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>9</td>
<td>73</td>
<td>80</td>
</tr>
<tr>
<td>5° C, 0% glycerol (n = 75)</td>
<td>44</td>
<td>33</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>7</td>
<td>54</td>
<td>80</td>
</tr>
<tr>
<td>5° C, 5% glycerol (n = 93)</td>
<td>31</td>
<td>26</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>9</td>
<td>58</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>5° C, 10% glycerol (n = 73)</td>
<td>16</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>23</td>
<td>4</td>
<td>56</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>S.E. of means (±)§</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>3</td>
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<td>2</td>
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</tbody>
</table>

Samples were fixed fresh or incubated in utero for 4 hr (Exp. 2), or in vitro for 6 hr at 20° C or 5° C in media containing egg yolk and various concentrations of glycerol (Exp. 4). Results are given as percentages; values have been rounded up to the nearest whole number; n = average numbers of spermatozoa counted/replicate.

* Classes 1 to 8 correspond to Figs 1 to 8 in Plate 1.
† Similar to type shown in Pl. 1, Fig. 2, except that the plasma membrane was broken.
‡ Means were equal to 0.25.
§ Calculated from residual mean square in analyses of variance; because of numerous zero values in data for Columns 2 to 5, these values would be a little deflated.

Although these changes occurred throughout the anterior region of the acrosome, the outer acrosomal membrane usually remained intact at the equatorial segment, even though some of the acrosomal contents had moved outside the membrane (Pl. 1, Figs 11 and 12). Initially, the process was most obvious in the apical ridge where it seemed that the ridge folded backwards (Pl. 1, Fig. 2) during the invagination of the outer acrosomal membrane, or the invagination occurred in the dorso-ventral plane (Pl. 1, Fig. 9). This produced a double layer of membrane which fused along its length to form the vesicles shown in Pl. 1,
Figs 3 and 10. A meshwork of 'channels', similar to those described by Phillips (1972), was also observed in the acrosome (Pl. 1, Fig. 14) of some spermatozoa that had proceeded beyond the stage of the reaction shown in Pl. 1, Fig. 2. Plate 1, Fig. 5 shows the last stage of the vesiculation process; the inner acrosomal membrane remained intact, but only a layer of small vesicles marked the region of the rest of the acrosome and plasma membrane.

The immature acrosomes of spermatozoa observed in Exp. 1 (see immature types in Pl. 1, Jones, 1971b) were morphologically similar to the swollen acrosomes (i.e. similar to Pl. 1, Fig. 2) of some spermatozoa observed in Exps 2 to 5 (particularly when cross-sections of sperm heads were compared). Spermatozoa from the epididymis which showed subsequent stages of vesiculation were quite rare. In Exp. 1 (seventy spermatozoa examined for each of the five replicates), vesiculation within the acrosome occurred in only two spermatozoa (similar to Pl. 1, Fig. 3) and only one spermatozoon contained vesicles but the outer acrosomal membrane was no longer intact (similar to Pl. 1, Fig. 4).

A small percentage of spermatozoa showing various stages of vesiculation was observed in freshly collected semen (Exps 2 and 4, Table 1; also Exps 3 and 5). The incidence of these types was increased by incubation of semen for 4 hr in the uterus of a sow (Exp. 2, Table 1) but a similar period of incubation in vitro (37° C) in fluids from the uterus or Fallopian tubes did not have much effect (Exp. 3). Experiment 4 (Table 1) showed that cooling to 5° C and the inclusion of glycerol in the 5° C diluents both increased the incidence of spermatozoa showing stages of vesiculation. The reaction could not be related to the activity of spermatozoa (see scores of percentage motility, Table 1) or to any other structural changes; counts of the incidence of spermatozoa with mitochondrial damage that were made in Exp. 4 showed that this parameter was not affected by any of the semen treatments. Experiment 5 showed that the incidence of spermatozoa showing signs of vesiculation increased with storage time at 5° C.

Until more is known about the structure and nature of the acrosomal membrane, any description of the mechanism of vesiculation would be purely speculative. It may occur in all mammalian spermatozoa; to date, it has been observed in bull, ram and rabbit semen as well as boar semen (R. C. Jones, unpublished data). As the acrosome resembles a lysosome (Allison & Hartree, 1968, 1970; Dott & Dingle, 1968), it is possible that vesiculation is similar to endocytosis but its endocytic activity (De Duve & Wattiaux, 1966) could not be proved on other evidence. I attempted unsuccessfully to prove such activity (R. C. Jones, unpublished data) by demonstrating the uptake of horse radish peroxidase (using methods described by Fahimi, 1970) or ferritin (Casley-Smith, 1969). It is unlikely, however, that these substances could penetrate the intact plasma membrane of the spermatozoon, and, as endocytosis is temperature-dependent, its activity would be arrested (Casley-Smith, 1969) rather than increased (Exps 4 and 5) by cooling spermatozoa to 5° C. The initial invagination of the acrosomal membrane is more probably due to a change in the physical or chemical state of the membrane (Shohet, 1972).

It is not possible to conclude from these studies that vacuolation and vesiculation of the acrosome are different physiological processes. Morphologically,
they are quite dissimilar yet they could be the same cytolitic process, but occurring at different rates. Thus, possibly, when the acrosome swells rapidly, vacuolation occurs but when swelling is slow, vesiculation results. Nevertheless, it is considered noteworthy that the process of vesiculation as here described rarely occurs in the epididymis, yet it may be induced by storage in utero (Exp. 2), and the final stage is morphologically similar (Pl. 1, Fig. 5) to that of the acrosomal reaction which Barros et al. (1967) proposed as taking place during fertilization by mammalian spermatozoon. It is also noteworthy that the semen treatments which caused the acrosomal vesiculation observed in Exp. 4 were used in fertility studies by Wilmut (1971) and did not cause a loss of fertility. Wilmut (1971) found that incubation at 20° C in 10% glycerol for 6 hr reduced fertility whilst the same period of incubation in glycerol at 5° C did not affect fertility. Regardless of whether or not the reactions are the same, a description of each is necessary to account for all the different morphological types of spermatozoa occurring in semen. Indeed, it seems most likely that the 'true' acrosomal reaction that Bedford (1970) claimed to observe in rabbit semen actually involved the acrosomal vesiculation described in this report.

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