Sterility in mutant \((t^{Lx}/t^{Ly})\) male mice

II. A morphological study of spermatozoa

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SUMMARY

A comparative light and electron microscopic study was done on cauda epididymal spermatozoa obtained from correspondingly-aged sterile \(t^{Lx}/t^{Ly}\), and fertile \(T/t^{w32}\), \(T/t^{L}\) and BALB/c, mice. The results show that all of the males, regardless of age, contained defective gametes and that all contained the same types of aberrant gametes. The oldest males of each genotype contained more abnormal gametes than the younger males of the same genotype. No unique spermatozoan defect and no increased frequency of a specific spermatozoan defect was noted which could be correlated with the sterility of the \(t^{Lx}/t^{Ly}\) animals.

INTRODUCTION

Comparative studies of spermatozoa from sterile \(t/t\) males and fertile control males have produced conflicting data concerning the correlation between sterility and the frequency of abnormal spermatozoa. Bryson (1944) compared spermatozoa from the vasa deferentia of sterile, intercomplement \(t^{Lx}/t^{Ly}\), and fertile \(T/t^{w32}\), \(T/t^{L}\) and BALB/c, mice. He found that males of all genotypes contained morphologically abnormal spermatozoa but that sterile males usually contained a higher percentage of abnormal gametes. In addition, he noted that the distribution of abnormalities in spermatozoa from \(t^{Lx}/t^{Ly}\) males differed from that in wild-type sterile males. The mutant animals always contained significantly more spermatozoa with defective head morphology. He suggested, therefore, that heterozygosity for two lethal alleles might effect abnormal spermatogenesis. This hypothesis was supported by Dooher & Bennett (1977) who reported that intercomplement males differed from control fertile males in that most of the spermatozoa (over 70\%) from the intercomplement animals had abnormally-shaped heads.

Rajasekarasetty (1951) reported that sterile \(t^{L}/t^{V}\) males contained a significantly higher averaged percentage of abnormal spermatozoa than did \(t^{L}/t^{V}\) quasi-sterile males. He also noted, however, that the ranges of the absolute numbers of abnormal spermatozoa from the two groups of males overlapped.

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A similar observation was reported by Braden & Gluecksohn-Waelsch (1958). In their study of spermatozoa obtained from sterile (tLx/tLy) males, quasi-sterile tVx/tVy and tVx/tVz males and fertile +/tL and +/+ males, they found no consistent correlation between the numbers of abnormal spermatozoa and either sterility or quasi-sterility. Although sterile and quasi-sterile males always contained a higher percentage of abnormal spermatozoa than did fertile males, quasi-sterile males often contained more abnormal gametes (including those with abnormal head morphology) than did sterile males. In addition, Olds (1973), in a comparative study of spermatozoa obtained from T/tu and tLx/tLy animals, noted that most of the spermatozoa from all of the males were normal. Moreover, she found no differences in the types of spermatozoan defects found in sterile and fertile animals.

There exist, therefore, conflicting reports from investigators who have attempted to relate reduced fertility with either specific spermatozoan defects or numbers of abnormal gametes in homozygous and heterozygous recessive tu genotypes. For this reason, we have undertaken a comprehensive light and electron microscopic study of epididymal spermatozoa obtained from sterile intercomplement males and fertile control males in order to determine if a specific defect, or the increased relative frequency of a specific defect, can be correlated with the observed sterility.

MATERIALS AND METHODS

Cauda epididymal spermatozoa were obtained from 6-, 10-, 14- and 17-month-old T/tu, T/tu32, t*/tuZ2 and BALB/c (+/+ ) mice. Six mice of each genotype were examined at each age. These same animals were also used for the spermiogenesis studies reported elsewhere (Hillman & Nadijcka, 1980).

The protocols for preparing the spermatozoa for the light and electron microscopic studies followed those previously reported (Hillman & Nadijcka, 1978). Briefly, aliquots of spermatozoa were obtained from the epididymides extirpated from three males of each genotype at each specified age. Spermatozoan samples were prepared for light microscopy using the technique of Wyrobek, Heddle & Bruce (1975). The technical limitations imposed by electron microscopy necessitated our using light microscopy to quantitate the types and frequencies of spermatozoan abnormalities. One thousand spermatozoa from each male were scored for their morphologies, and the number and distribution of abnormal spermatozoa within and between the four groups of males were compared using a contingency $X^2$ test. The remainder of each of these spermatozoan samples was prepared for electron microscopic studies.

The epididymides from the remaining three mice of each genotype and age were prepared for ultrastructural studies. Ultrathin sections were stained with uranyl acetate (Watson, 1958) and lead citrate (Venable & Coggeshall, 1965) and examined with a Philips 300 electron microscope. We utilized the ultra-
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Table 1. Total numbers of intact, abnormal spermatozoa* in males of different genotypes at different ages

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age (months)</th>
<th>(t^x/t^{w32})</th>
<th>BALB/c</th>
<th>(T/t^{w32})</th>
<th>(T/t^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>991</td>
<td>1135</td>
<td>688</td>
<td>746</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1449</td>
<td>1010</td>
<td>1049</td>
<td>779</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1346</td>
<td>1077</td>
<td>1767</td>
<td>896</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>1618</td>
<td>1583</td>
<td>1077</td>
<td>630</td>
</tr>
</tbody>
</table>

* 3000 spermatozoa were scored for each genotype at each age.

structural studies by Afzelius (1959), Fawcett & Ito (1965), Fawcett & Phillips (1969), Stefanini, Oura & Zamboni (1969), Zamboni & Stefanini (1971) and Fawcett (1975) to distinguish between normal and abnormal spermatozoa as well as for the morphological terminology used in the present investigation.

RESULTS

Light microscope studies

All of the males contained intact abnormal spermatozoa. In only three samples, however, was the frequency of abnormal spermatozoa greater than 50% (Table 1). These exceptional cases were \(T/t^{w32}\) males at 14 months of age and both \(t^6/t^{w32}\) and BALB/c males at 17 months of age. In addition, the data show that males of each genotype which were 10 months of age or older generally contained more abnormal spermatozoa than did males of the same genotype at 6 months of age. The exceptions to this general observation were BALB/c at 10 and 14 months of age and \(T/t^6\) at 17 months of age. Each of these groups of males contained significantly fewer abnormal spermatozoa than younger males of the same genotypes. The data in Table 1 show that, overall, \(t^6/t^{w32}\) and BALB/c males averaged more abnormal spermatozoa than did \(T/t^6\) and \(T/t^{w32}\) males. These combined observations support both (1) our subjective observation that 6-month-old animals contain fewer abnormal spermatids than do older animals of the same genotype and (2) our subjective ranking of the males based on the frequency of observing aberrant spermatids in randomly selected thin sections of their seminiferous tubules (Hillman & Nadijcka, 1980).

It can be noted from Table 2 that all of the classes of spermatozoan abnormalities were present in males of each genotype at all ages. The intercomplement sterile and BALB/c males had significantly more spermatozoa with misshaped heads (column 1, Table 2) at each age than either \(T/t^{w32}\) or \(T/t^6\) animals at corresponding ages. At 6 and 10 months of age, \(t^6/t^{w32}\) and BALB/c had equivalent numbers of spermatozoa with misshaped heads \((P > 0.01)\) while...
Table 2. Distribution of intact, abnormal spermatozoa in mice of different genotypes at different ages

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age (months)</th>
<th>Head defects</th>
<th>Tail defects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Misshaped</td>
<td>Other*</td>
</tr>
<tr>
<td>t&lt;sup&gt;a&lt;/sup&gt;/&lt;sup&gt;v&lt;sub&gt;32&lt;/sub&gt;&lt;/sup&gt;</td>
<td>6</td>
<td>713</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>735</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>459</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>551</td>
<td>178</td>
</tr>
<tr>
<td>BALB/c</td>
<td>6</td>
<td>650</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>686</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>713</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>1193</td>
<td>159</td>
</tr>
<tr>
<td>T/&lt;sup&gt;v&lt;sub&gt;32&lt;/sub&gt;&lt;/sup&gt;</td>
<td>6</td>
<td>235</td>
<td>55</td>
</tr>
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<td></td>
<td>10</td>
<td>430</td>
<td>126</td>
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<td>14</td>
<td>301</td>
<td>301</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>400</td>
<td>248</td>
</tr>
<tr>
<td>T/&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6</td>
<td>143</td>
<td>52</td>
</tr>
<tr>
<td></td>
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<td>117</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>227</td>
<td>290</td>
</tr>
</tbody>
</table>

* The category ‘other’ includes those spermatozoa with heads which were bifid, bifurcated, micro-, or contained in cytoplasmic droplets.
† The category ‘multiple defects’ includes those spermatozoa with both head and tail defects.

at 14 and 17 months, BALB/c males had significantly more spermatozoa with misshaped heads than did t<sup>a</sup>/<sup>v<sub>32</sub></sup> animals (P < 0·01).

Comparisons of the numbers of spermatozoa with total head defects (column 3, Table 2) show, with one exception, that t<sup>a</sup>/<sup>v<sub>32</sub></sup> and BALB/c males contained significantly more spermatozoa with abnormal heads than did T/<sup>v<sub>32</sub></sup> and T/<sup>a</sup> males of corresponding ages. The exceptional cases, t<sup>a</sup>/<sup>v<sub>32</sub></sup> and T/<sup>v<sub>32</sub></sup> at 14 months of age, contained equivalent numbers of spermatozoa with head defects. Additional analyses of these data also show that t<sup>a</sup>/<sup>v<sub>32</sub></sup> and BALB/c males had equivalent numbers of spermatozoa with head defects at 6 and 10 months of age and that BALB/c males contained significantly more spermatozoa with head defects at 14 and 17 months of age than did t<sup>a</sup>/<sup>v<sub>32</sub></sup> males of comparable ages. T/<sup>a</sup> males contained significantly fewer spermatozoa with abnormal heads than the other males at any of the ages represented.

Since spermatozoa which were scored as being multiply defective had both head and tail defects, this category should also be considered when determining
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the distribution and frequencies of head defects. A comparison among the numbers of spermatozoa with both total head and multiple (head and tail) defects show that (1) at 6 months of age both BALB/c and \(t^6/t^{uv32}\) males had equivalent numbers of spermatozoa with head defects and both had significantly more spermatozoa with head defects than did \(T/t^{uv32}\) and \(T/t^6\) males, (2) at 10 months and 14 months of age, \(t^6/t^{uv32}\) males had significantly more spermatozoa with head defects than did the other three groups of males and (3) at 17 months of age, BALB/c males contained significantly more spermatozoa with head defects than did the other three groups of males. At 17 months, \(t^6/t^{uv32}\) and \(T/t^{uv32}\) males had equivalent numbers of spermatozoa with abnormal heads. At all ages, \(T/t^6\) males had the least numbers of spermatozoa with head defects and these numbers were significantly lower than the numbers found in the other animals of comparable ages.

A comparison of the numbers of spermatozoa with tail defects (Table 2) shows that there was no direct correlation between the total numbers of tail defects and the age of the animal. The preponderant defect here was a coiling or folding of the tail. This coiling or folding may, however, be a temporary abnormality and not a true aberration. This suggestion comes from our previous ultrastructural studies which showed that the coiling or folding occurred within a common cytoplasm and that this cytoplasm vacuolated in some spermatozoa (Hillman & Nadijcka, 1978). We have suggested that the vacuolization is the normal process for disrupting the droplet. Continued vacuolization would result in the removal of the cytoplasm allowing the tail to either unfold or uncoil. By this process, the spermatozoa would become morphologically normal gametes. If the coiling and folding defect is trivial, the elimination of this category of abnormalities would reduce the numbers of abnormal spermatozoa to less than 50\% in both \(T/t^{uv32}\) and \(t^6/t^{uv32}\) males at 14 months of age (Table 3). As a consequence, the only animals which would contain a majority of abnormal spermatozoa would be 17-month-old BALB/c males. Also, this elimination would remove the exceptions from the observation that males that are 10 months of age or older contain more abnormal spermatozoa than did 6-month-old males of the same genotype. Since testicular spermatids do not have coiled and folded tails, treatment of the data in this way reflects more accurately the incidence of abnormal spermatids within the different males at different ages.

A category not included in the above tabulations of spermatozoon defects is that of ‘headless’ spermatozoa. At 6 months of age, all of the males contained incomplete spermatozoa, that is, separate tails and heads. At 6 months of age, the occurrence of this defect was infrequent with one exception. In \(t^6/t^{uv32}\) males 27\% of the spermatozoa were headless. The percentage of broken spermatozoa increased in all of the older animals; and by 17 months of age \(t^6/t^{uv32}\) males contained 53\% broken spermatozoa; \(T/t^6\) males, 51\%; BALB/c males, 41\%; and \(T/t^{uv32}\) males, 34\%. Although processing the spermatozoa for light microscopy might cause fragmentation, the fact that older animals of all genotypes
showed an increased number of broken gametes suggests that processing alone can not account for the observed differences.

**Ultrastructural studies**

All of the males contained spermatozoa with the same types of ultrastructural defects. The frequency of ultrastructurally aberrant spermatozoa in each group of males had a positive correlation with the incidence of abnormal spermatozoa recorded from the light microscopic studies. All of these animals had the identical categories of defects and the same range of the defect within each category as we described in an earlier study (Hillman & Nadijcka, 1978). Because the defects were identical, no photomicrographs are included with these descriptions.

**Head abnormalities.** There are four categories of head defects, the same as those which can be distinguished at the light microscope level and the same as previously described. These categories are: (1) misshaped heads, (2) microheads, (3) bifurcated and bifid heads, and (4) heads contained in cytoplasmic droplets. The types of misshaped heads are too numerous and non-distinctive to allow their being arbitrarily grouped into subclasses. In general, however, the misshapen heads are shorter and wider than the heads of normal spermatozoa. The reduction in length from the implantation fossa to the most anterior tip of the rostrum causes the head to appear wedge-, or club-shaped. Although the reason for this defect is not known, we suggest that the misalignment of the relative positioning of the developing acrosome and implantation fossa during spermogenesis could produce most of the myriad types of misshaped heads observed (Hillman & Nadijcka, 1980). The fact that most of the spermatozoa which have misshaped heads also have abnormal acrosomes lends support to the hypothesis that the abnormal development or positioning of the acrosome during spermogenesis would lead to aberrantly-shaped heads (Hollander, Bryan & Gowen, 1960; Hunt & Johnson, 1971). No type of misshaped head was unique to any group of males examined.

The other categories of spermatzoan head defects were present in all of the males and did not differ in type or appearance from those already described.

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**Table 3. Total numbers of abnormal spermatozoa excluding coiled and folded tails**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>$t^6/t^m32$</th>
<th>BALB/c</th>
<th>$T/t^m32$</th>
<th>$T/t^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>803</td>
<td>827</td>
<td>419</td>
<td>274</td>
</tr>
<tr>
<td>10</td>
<td>1147</td>
<td>879</td>
<td>797</td>
<td>381</td>
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<tr>
<td>14</td>
<td>1157</td>
<td>872</td>
<td>971</td>
<td>512</td>
</tr>
<tr>
<td>17</td>
<td>911</td>
<td>1472</td>
<td>883</td>
<td>573</td>
</tr>
</tbody>
</table>
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(Hillman & Nadijcka, 1978). Although the absolute numbers of these defects differed among the different groups of males, their distribution was the same in all males. In the order of the observed incidence, males contained more spermatozoa with either microheads (normally shaped but smaller than usual) or with heads in cytoplasmic droplets than spermatozoa with bifid or bifurcated heads.

**Neck defects.** Morphological defects of the neck region were never observed. The presence of spermatozoa without heads, however, suggests that either the neck, the implantation fossa, or both, were defective. The separation of the heads from the tails always occurred between the caputulum of the connecting piece and the fossa.

**Tail defects.** In addition to the tail defects which were observed at the light microscope level (i.e. coiled and folded tails and double tails) spermatozoa from males of all genotypes and all ages had axonemal and outer dense fiber defects. These defects were, however, ubiquitous. Because of technical limitations imposed by electron microscopy and because these defects are not visible at the light microscope level, it was impossible to quantify the numbers of spermatozoa containing defective tail axonemal doublets and outer dense fibers. The specific defects found in transverse sections of spermatozoa from all males were (1) a decrease in the number of doublets and/or outer dense fibers, (2) excessive doublets, (3) misplaced doublets and misplaced outer dense fibers.

**Abnormal mitochondrial sheath configurations.** All of the males contained gametes in which the mitochondria were aberrantly arranged around the mid-piece axoneme and its associated outer dense fibers. This defect, although common to all males, was infrequently observed.

**Multiple defects.** While the head and certain tail abnormalities (coiled, folded, double) are easily discerned at the light microscope level, certain tail defects (e.g. missing axonemal components) can only be resolved at the electron microscope level. As a consequence, spermatozoa which appeared to have only head defects when grossly examined often contained tails with aberrant components. Because of the limitations imposed by both electron and light microscopy, the exact incidence of multiply defective gametes could not be determined.

**DISCUSSION**

All of the animals contained abnormal epididymal spermatozoa and all contained the same types of defective gametes. These defects have been found in other genetic and inbred strains of mice (Bryson, 1944; Rajasekarasetty, 1954; Braden & Gluecksohn-Waelsch, 1958; Hollander, Bryan & Gowen, 1960; Hunt & Johnson, 1971; Krzanowska, 1972; Hillman & Nadijcka, 1978). The fact that these defects were found in non-\(r^m\)-bearing mice negates the hypothesis that recessive \(t^L\) alleles, either in a homozygous or heterozygous condition, evoke a specific spermatozoan abnormality.

The present study also shows that the infertility of intercomplement \(t^{Lx}/t^{Ly}\)
males cannot be related to their having an excessive number of spermatozoa with misshaped heads. Although \( t^6/t^{u32} \) males have a higher frequency of spermatozoa with misshaped heads than do males of most other genotypes and inbred strains (data in this paper and in Hillman & Nadijcka, 1978), they do not contain more spermatozoa with this defect than do fertile BALB/c males at comparable ages. A comparison of the total number of defective spermatozoa from representative males shows that at certain ages fertile BALB/c and \( T/t^{u32} \) males have either the same or significantly more aberrant gametes than do sterile \( t^6/t^{u32} \) males. Our overall observations support the statements by Braden & Gluecksohn-Waelsch (1958) and by Bryson (1944) concerning male sterility in \( t/t \) mice. The former investigators concluded that ‘there (is) no correlation between fertility and the proportion of morphologically abnormal sperm produced’, and Bryson cautioned that ‘the presence of abnormal sperm in animals of any genotype should not be regarded as the cause of sterility’.

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