Sperm head abnormalities in relation to the age and strain of mice

Halina Krzanowska

Department of Genetics and Evolution, Institute of Zoology, Jagiellonian University, Karasia 6, 30-060 Kraków, Poland

Summary. Sperm abnormalities were examined in the samples of the uterine contents collected after copulation. Male mice of the inbred C57BL/Kw (C57), CBA/Kw (CBA) and KE strains were tested at the ages of 6, 7, 8 and 10 weeks. Severely misshapen spermatozoa were produced by 6-week-old males of all strains: 30% of spermatozoa had 'thread-like' heads, devoid of Feulgen-positive material; the total proportion of abnormalities was lowest in CBA males. During maturation the percentages of abnormalities dropped rapidly and the proportion of drastically misshapen spermatozoa diminished in favour of less severe deformations. The frequency distribution of abnormal sperm types was strain specific for adult males but not for very young males. Large spermatozoa were seen in samples from young CBA and KE males (0.5–2%) and sporadically in 10-week-old CBA males. A negative relationship between body weight and the severity of abnormalities was seen for young, but not adult, males reared in litters of different size.

No sperm deterioration was noticed in the first year of life of 5 KE males tested every 3 months until death, but a male surviving to 15 months had increased (38%) proportions of abnormal spermatozoa.

Introduction

Inbred strains of mice are said to differ in the proportion of abnormal spermatozoa produced by adult males (e.g. Beatty & Sharma, 1960; Mori, 1961; Krzanowska, 1962; Bruce, Furrer & Wyrobek, 1974), although the methods of scoring and the strains used by these workers have varied. Nevertheless, the tendency to produce high or low proportions of misshapen spermatozoa seems to be firmly inherent in the genetic constitution of the particular strain (Buda & Krzanowska, 1978). In adult males differences between strains involve not only the total percentage of abnormalities, but also the relative proportions of particular types of sperm head deformation (Krzanowska, 1976a, b; Buda & Krzanowska, 1978).

Most severely misshapen spermatozoa are observed in the semen of young males, when sperm production has just started (Krzanowska, 1974), and extreme forms are found only sporadically in the adult males. However, strain differences in the distribution of these deformations have not been systematically studied. The total percentage of abnormalities, highly elevated in the 6th week of life, diminishes with age, reaching the level characteristic for a given strain in the 3rd month of life (Krzanowska, 1972a, b). Wyrobek (1979) mentioned that no further changes were observed in males up to 6 months of age, but older animals have not been studied.

The main object of the present study was to analyse the types of sperm head deformation and their relative proportions in the adolescent males of three inbred mouse strains, the adults of...
which are known to show different but characteristic degrees of sperm abnormalities (Krzanowska, 1976a). Some males of one strain were reared in small and large litters and compared to determine whether the retardation of growth in the larger litters had any long-term effects on sperm abnormalities. The effects of ageing were studied in a few males whose spermatozoa were investigated throughout their life.

Materials and Methods

Experiments

Experiment 1. Three inbred mouse strains, C57BL/Kw, CBA/Kw (subsequently referred to as C57 and CBA, respectively) and KE, were used. To obtain sperm samples outbred females were mated with experimental males and killed on the morning of the day when the copulation plug was found. A drop of uterine contents was then smeared on the slide and air dried. Each male was tested when 6, 7, 8 and 10-weeks-old. Some males were 1 day older or younger as not all copulated when planned.

Experiment 2. KE males were reared in litters of different sizes (2–10) and were weighed and tested as in Exp. 1 when 51–53 days old and again at 70 days of age.

Experiment 3. Five breeding KE males were sampled as in Exp. 1 once every 3 months until death. Six virgin CBA males were tested when 10 months old.

Analysis of spermatozoa

Air-dried smears were fixed for 1 h in acetic alcohol (3 parts absolute ethylene alcohol, 1 part glacial acetic acid) and stained with 5% (w/v) aqueous eosin. Some smears were stained with Feulgen reagent using the method adapted for testicular preparations (Ford, 1962). Smears were examined under an oil-immersion objective at ×100. The percentages of normal and abnormal heads in 200 spermatozoa from each male were determined. For statistical treatment, percentages were transformed to angles (Snedecor, 1955).

The classification of abnormal forms was based on a previous scheme (Krzanowska, 1976a, b) but with two modifications (Text-fig. 1). Firstly, it was difficult to distinguish Class 1 abnormality (almost normal head but with a slightly changed curvature in the distal and/or proximal part of the head), because in the very young males many sperm heads tended to be thicker, shorter and less smoothly shaped than in adult males. All these heads were classified as ‘near-normal’. Secondly, an additional category of severely misshapen ‘thread-like heads’, typical for very young males, was distinguished as Class 5 (as in an earlier classification; Krzanowska, 1974). Such ‘heads’ contain no or only traces of Feulgen-positive material.

Results

Experiment 1: types of sperm abnormalities in adolescent males

The uterine fluids of females mated with 6-week-old males contained very small numbers of spermatozoa, but 200 per smear could be analysed, except on 3 occasions (Table 1) when copulations were almost completely sterile. In the samples from C57 and KE males only a few spermatozoa with normal heads were found, while in the samples from CBA males there were 21% normal and 23% nearly normal spermatozoa. In all strains the percentages of normal heads rose with age, being highly variable in 7–8-week-old males. The level characteristic for each strain was established by the 10th week of age.
Table 1. Proportions of normal and abnormal spermatozoa in mouse semen recovered from the uterus after mating

<table>
<thead>
<tr>
<th>Strain (no. of males)</th>
<th>Age (weeks)</th>
<th>Normal spermatozoa</th>
<th>%</th>
<th>Angles Mean ± s.d.</th>
<th>Percentages of abnormal spermatozoa in the following classes:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nearly normal</td>
</tr>
<tr>
<td>CBA (6)</td>
<td>6</td>
<td>21-3</td>
<td>27-10 ± 6-00</td>
<td>22-9</td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>70-0</td>
<td>57-52 ± 13-80</td>
<td>9-2</td>
<td>0-7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>81-1</td>
<td>64-79 ± 7-43</td>
<td>5-4</td>
<td>0-8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>93-2</td>
<td>74-72 ± 1-60</td>
<td>0-7</td>
<td>0-6</td>
</tr>
<tr>
<td>C57 (6)</td>
<td>6*</td>
<td>2-8</td>
<td>6-65 ± 7-96</td>
<td>7-6</td>
<td>0-0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>15-3</td>
<td>18-90 ± 15-39</td>
<td>18-2</td>
<td>0-1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>45-8</td>
<td>42-40 ± 12-08</td>
<td>18-6</td>
<td>0-2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>82-2</td>
<td>65-10 ± 2-52</td>
<td>2-8</td>
<td>0-1</td>
</tr>
<tr>
<td>KE (8)</td>
<td>6†</td>
<td>2-7</td>
<td>5-30 ± 8-66</td>
<td>2-2</td>
<td>0-2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>20-0</td>
<td>22-33 ± 17-61</td>
<td>15-4</td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>56-4</td>
<td>48-51 ± 14-39</td>
<td>10-2</td>
<td>0-7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>79-5</td>
<td>63-13 ± 2-02</td>
<td>2-4</td>
<td>1-9</td>
</tr>
</tbody>
</table>

* One male not included (sterile copulation).
† Two males not included (sterile copulations).

The proportions of different sperm head deformations were clearly dependent both on the age and on the strain of the males (Table 1). A frequency distribution of the abnormal types is presented in Text-fig. 2. For 6-week-old males of all strains the most frequent abnormality was that of 'thread-like', Class 5 spermatozoa (reaching 30% in absolute percentages); the frequency diminished rapidly with age and very few were found in the adults.

During maturation of KE and C57 males there was a change in proportion between the severely abnormal spermatozoa of Class 4 and the less deformed ones of Class 3. In CBA males spermatozoa of Class 4 were less frequent even at 6 weeks of age: a sample from one male containing as many as 92% spermatozoa with abnormalities (half of them of Class 5), had only 8% Class 4 spermatozoa.

The most conspicuous interstrain differences appeared for Class 2 spermatozoa (Text-fig. 1). In very young males spermatozoa were counted as belonging to Class 2 when the canal was present, although the shape of the head was sometimes similar to that of Class 3 spermatozoa. In KE males Class 2 spermatozoa were infrequent at 6 weeks of age and then rose in frequency (both in absolute and in relative values) until the 8th week. Although the absolute percentage was lower at 10 weeks, this type of malformation remained predominant in the adult KE males. A similar trend was observed in CBA males but only for the relative proportions of Class 2 spermatozoa; the absolute percentages decreased with age. In C57 males this type of abnormality was only rarely seen.

Text-fig. 1. Shapes of normal and abnormal mouse sperm heads according to the arbitrary Classes 2–5 and subclasses (see text).
Text-fig. 2. Distribution of the relative percentages of mouse spermatozoa in Classes 2–5 in the ejaculates of males tested successively at 6, 7, 8 and 10 weeks of age.

The abnormality represented by nearly normal sperm heads was not included in the calculations of relative percentages because such spermatozoa are a rather heterogeneous group. In 6-week-old CBA males and in 7-week-old KE and C57 males they appeared in proportions similar to those of normal spermatozoa and were characterized by thicker and shorter heads, with 'rough' contours. During maturation near-normal heads became more smoothly shaped and their frequency diminished rapidly in relation to the normal forms. At 10 weeks of age nearly normal spermatozoa did not exceed 3% and the abnormality was expressed only as a slight change in the curvature in the apical or distal part of the head.

In the samples from 6–8-week-old males of the KE and CBA strains 0·5–2% of spermatozoa with large heads, about twice the normal size, probably diploid, were observed. Occasional spermatozoa of this type were seen in samples from 10-week-old CBA (but not KE) males; such spermatozoa were never seen in the C57 samples.
Experiment 2: relation between body weight and sperm abnormalities

The body weights of experimental males, tested when 51–53 days old, ranged from 20 to 26 g and the percentages of sperm abnormalities from 20 to 55%. The regression of the transformed percentages on the body weight (Text-fig. 3) was negative and highly significant ($b = -2.68; P < 0.01$). The types of sperm malformations were related to body weight. Spermatozoa from smaller males showed the frequency distribution of abnormality classes typical for the 7th week of age, while in the samples from larger males the pattern was more like that of the adults.

By 70 days of age there was a positive relationship between the body weights measured at the two ages ($b = 0.68; P < 0.01$), but the percentages of sperm abnormalities (13.5–20%) were neither related to the actual body weight (22–27 g; Text-fig. 3) nor to the values estimated at 51–53 days.

Text-fig. 3. Relation between body weight and the proportion of abnormal spermatozoa of 11 KE males tested when 51–53 days old (O, regression line) and at 70 days of age (×).

Experiment 3: sperm quality of aged males

Of the 5 breeding males of the KE strain tested at 3-month intervals, 4 died at 7, 9, 9 and 12 months and the other at 15 months. None of the males showed any obvious symptoms of illness and they continued to sire litters almost until death (females were replaced by younger animals every 4 months). No effect of ageing on sperm quality was observed during the first year of life (Table 2) and the percentages of sperm abnormalities estimated for individual males were rather stable, the extreme differences between samples not exceeding 5%. Even the spermatozoa collected post mortem from the vas deferens did not show any signs of deterioration. Proportions of abnormality classes were always typical of those for adult KE males.

Table 2. Percentages of abnormal spermatozoa in the ejaculates of ageing male mice tested until death

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age (months)</th>
<th>No. of males</th>
<th>% of abnormal spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>KE</td>
<td>3</td>
<td>5</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>19.1</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1</td>
<td>38.5</td>
</tr>
<tr>
<td>CBA</td>
<td>10</td>
<td>6</td>
<td>9.6</td>
</tr>
</tbody>
</table>
The male tested at 15 months of age had sired a litter 1 month earlier but showed elevated proportions of sperm abnormalities—38%, in contrast to 21–24% during the first year of life. The sample collected post mortem from the vas deferens 2 weeks after the 15-month test contained 45% of abnormalities. There were no changes in the proportions of nearly normal and Class 2 spermatozoa, but Class 4 abnormalities rose in frequency (amounting to 11%) at the expense of Class 3.

The second group investigated in this experiment consisted of 6 CBA males used to test the genetic purity of their strain. At 4 months of age the males were given skin transplants from their brothers (all were accepted) and were kept singly until 10 months of age when they were mated with outbred females for the examination of sperm quality. Although the mean percentage of abnormalities was higher (9.6%, Table 2) than in 10-week-old males (6.8%, Table 1), this difference was caused by one male whose ejaculate contained very low numbers of spermatozoa with 19.5% of abnormal heads. Proportions of abnormality classes were typical for the adult CBA males although 0.5–2% large spermatozoa appeared in all samples.

Discussion

The elevated proportions of abnormal spermatozoa in very young males have been already demonstrated for mice (Krzanowska, 1972a, b) and for hamsters (Hancock, 1972). The results of Exp. 1 suggest that at the onset of spermatogenesis males of all tested strains produced highly abnormal spermatozoa, both in relation to the total percentages and the severity of deformations. About 30% of spermatozoa, belonging to Class 5, contained practically no nucleus, as shown by the lack of Feulgen-positive material.

At the age of 6 weeks, CBA males had the lowest percentages of abnormal spermatozoa which might be ascribed to the earlier maturation in this strain. However, there are 2 reasons for doubting this simple explanation. Firstly, even in 2 CBA males tested at 37 days of age (data not included in Table 1) sperm quality was better than in 42-day-old males of the other strains and sperm samples could not be collected at earlier ages. Secondly, the high frequency of Class 5 spermatozoa in 6-week-old males of the CBA strain suggests that the process of sperm production has just started. It would therefore seem that in the strains characterized by low levels of sperm abnormalities (as in CBA) the process of spermatogenesis may be less affected from the very beginning than in other strains. The comparison of inbred with F_1 hybrid males (Krzanowska, 1972b) suggests the same conclusion.

During maturation the total percentage of abnormalities dropped rapidly and the proportion of drastically misshapen spermatozoa also diminished. It seems that in very young males abnormalities of Classes 2 and 3 cannot be fully expressed while spermatogenesis is very heavily disturbed, leading to the preponderant production of severely misshapen forms (Classes 4 and 5). This would explain the transient rise in frequency (even in absolute percentages) of Class 2 and 3 spermatozoa, as observed in 7–8-week-old males of the KE and C57 strains, respectively.

In the adults the frequency distribution of types of sperm abnormality was strain specific, and each male could be easily assigned to the proper strain after the analysis of a single sperm sample, although this was not possible with very young C57 and KE males for which sperm abnormality types were rather similar (Table 1; Text-fig. 2).

As in the previous investigations (Krzanowska, 1974), 6–8-week-old males (but not 10-week-old) of the KE strain possessed some large spermatozoa which were lacking in the C57 males. Both age and strain are known to affect the incidence of such spermatozoa in rabbits (Beatty & Fechheimer, 1972). In the present study large spermatozoa also occurred in young CBA males and were sporadically found even in the adults. Diploid spermatozoa are rarely produced by adult male mice, as demonstrated by the absence of such forms amongst the 560 spermatozoa from 14 strains (not including CBA) examined by size measurements (Beatty, Lim
& Coulter, 1975) and amongst the 1000 spermatozoa examined by DNA measurements (Carothers & Beatty, 1975).

Among the environmental factors that are likely to affect sperm quality, dietary deficiencies have been mentioned but have not been studied in mice (Wyrobek & Bruce, 1978). In the present Exp. 2 the influence of restricted food supply, caused by the large litter size, was investigated. Young KE males reared in large litters were retarded in growth and produced more abnormal spermatozoa than did the males reared in small litters. Although some differences in body weight persisted until adulthood, as also shown for this strain by Musialek (1967), the differences in sperm quality had disappeared by the 10th week of age (Text-fig. 3). It follows that the undernourishment affected the sperm quality of young males by delaying their maturation rather than by inducing permanent effects on the proportions of abnormalities. This information may be useful to investigators employing sperm-abnormality assays for the detection of mutagenic agents (Wyrobek & Bruce, 1978). However, it cannot be ruled out that the restricted food supply before weaning may later modify the damaging effects of mutagens. An interaction between two environmental factors, dietary deficiency and thermal shock, was shown to be involved in affecting sperm quality of rams (Hancock, 1972).

The effects of ageing (Exp. 3) were not clear-cut. In 5 KE males tested successively until death no signs of deterioration in sperm quality were found during the 1st year of life, but an elevated percentage of abnormalities appeared in one male at 15 months of age. If any deterioration of sperm quality is really dependent on the ageing of males, this seems to start so late that it may be manifested only in some individuals which happen to survive long enough. Among 6 CBA males tested when 10-months-old, one showed a high percentage of abnormalities, outside the range of variation found for 10-week-old males, but it is not known whether this was connected with ageing. On the other hand, a higher frequency of large spermatozoa in aged males may indicate age-related disturbances of meiosis.

Overall, these results suggest that the percentages of misshapen spermatozoa and the relative proportions of abnormal types, once established in an individual male mouse by the 10th week of age, tend to persist throughout the first year of life.

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References


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