Sperm morphology and cytogenetic studies in ageing C57BL/6 mice

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Summary. A dose–response relationship was observed in the percentage of morphologically abnormal spermatozoa as a function of age for 2-, 6-, 12-, 18- and 25-month-old C57BL/6 male mice. Compared with values for younger mice, 25-month-old mice had a significantly ($P < 0.005$) higher percentage of abnormal spermatozoa ($23.9 \pm 2.3\%$) and the percentages in mice aged 12 ($12.9 \pm 0.7\%$) and 18 ($14.3 \pm 1.3\%$) months were significantly ($P < 0.05$) different from those of 2-month-old animals ($10.5 \pm 0.7\%$). Significant differences were not observed in the cytogenetic studies for 6-, 12-, 18- and 25-month-old mice, although 2-month-old mice had a lower frequency ($P < 0.05$) of chromosomal aberrations compared to 12- and 18-month-old animals.

Introduction

A relationship has been shown between paternal ageing and certain human diseases, such as achondroplasia, Apert syndrome, Marfan syndrome and fibrodysplasia ossificans progressiva (Tunte, Becker & Von Knorre, 1967; Murdock, Walker & McKusick, 1972; Smith, 1972; Erickson & Cohen, 1974). While the aetiological basis of these disorders is still unknown, it has been suggested that accumulated exposure to mutagenic chemical agents and increased susceptibility to mutation of spermatogonial stem cells from aged men may be causes. The ‘cyclic’ epidemic pattern for Down’s syndrome may also be explained by male transmission, in that differential rates of environmentally induced chromosomal non-disjunction occur in male germ cells and not in females (Bennett & Abroms, 1979). In a clinical study of over 1500 men ranging from 20 to 50 years of age, it was reported that the ease with which conception is achieved decreases after 25 years (MacLeod & Gold, 1953). MacLeod & Gold (1953) did not observe a relationship between age and sperm morphology, but Natoli, Riondino & Brancati (1972) reported that the number of normal spermatozoa (motility and morphology) per ejaculate decreased with age in men 45–91 years of age.

Among certain strains of mice an increased frequency of morphologically aberrant spermatozoa are associated with early maturity (Krzanowska, 1972) and the number of spermatozoa of normal appearance increased from 2.8 to 82.2% in 6- and 10-week-old C57BL/Kw mice, respectively (Krzanowska, 1981). However, whether sperm head morphology is altered in senescent male mice has not been adequately examined. Krzanowska (1981) reported that the percentage of normal spermatozoa in one 15-month-old KE mouse was 61.5% compared to 81.3% found in five 3-month-old KE mice. In this same study 10-month-old CBA/Kw mice had 90.4% normal spermatozoa compared to 93.2% in 10-week-old mice. Since some abnormal spermatozoa are viable and may be capable of fertilization, thereby contributing to the paternal age effect, it was of interest to determine whether the sperm population was affected in older mice.

Materials and Methods

Male C57BL/6 mice were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts, and aged at The University of Texas Medical Branch, Galveston, Texas. The mice were housed under artificial lighting (lights on 06:00–20:00 h) and provided with food and water *ad libitum*.

Sperm morphology. The cauda epididymidis was removed from each side and minced in a phosphate-buffered saline solution (PBS) and passed through an 80-mesh stainless-steel filter. Smears were made of the suspension and allowed to dry in air. Slides were stained in 1% eosin-Y solution for 45 min. A total of 666 spermatozoa was observed at $\times 480$ for each animal; the spermatozoa were scored 'blind' as normal or abnormal, and abnormal spermatozoa were classified according to the criteria established by Wyrobek & Bruce (1978) but the specific type of abnormality was not categorized within each group. Only intact spermatozoa were included in the analyses. All slides were scored by the same individual.

Cytogenetics. Each animal was injected with 2 mg colcemid/kg 2 h before death. Bone marrow was flushed from both femurs of each mouse with PBS in a 1-ml syringe (30-gauge needle). The cells were then collected by centrifugation at 5000 g for 5 min and resuspended in a hypotonic solution (0.075 M-KCl) for 18 min at 37°C. They were then recentrifuged and fixed in Carnoy's fluid (methanol:acetic acid, 3:1 v/v). Air-blown slides were prepared and stained in a 2% Giemsa solution. When possible, 100 metaphase cells were analysed per animal.

Histology. After excision of the caudae, the testes of each mouse were removed, weighed and fixed in 10% neutral formalin or Bouin's fluid. After dehydration, embedding in paraffin wax, sectioning at 5 μ m and staining with haematoxylin and eosin, the slides were examined for morphological differences.

Results

The data in Table 1 demonstrate that there was a progressive increase in the percentage of abnormal spermatozoa with age. The percentage of aberrant metaphases was not different in 6- to 25-month-old mice, but the frequency of chromosome breaks in 2-month-old mice was significantly lower than that in all other age groups (Table 2). Testicular weight increased up to 12 months of age and then declined (Table 1). The testes from most 25-month-old mice were not as firm or turgid as those of mice in the other age groups. Histologically it was apparent that the

Table 1. Effects of ageing on the percentage of morphologically abnormal spermatozoa in C57BL/6 mice

Age (months)	No. of mice	Mean ($\pm$ s.e.m.) wt of testes (mg)	No. of spermatozoa		Mean ($\pm$ s.e.m.) % aberrations
			Analysed	Abnormal	
2	8	151.3 $\pm$ 9.0	5328	561	10.52 $\pm$ 0.72
6	8	183.5 $\pm$ 3.4*	5328	650	12.20 $\pm$ 1.00
12	8	193.2 $\pm$ 5.1*	5328	686	12.88 $\pm$ 0.72*
18	8	159.0 $\pm$ 7.9†	6400	986	14.30 $\pm$ 1.30*
25	5	140.8 $\pm$ 12.5†	3330	796	23.90 $\pm$ 2.30‡§

* Significantly different from value for 2-month-old mice: $P < 0.05$.

† Significantly different from values for 6- and 12-month-old mice:
 $P < 0.01$.

‡ Significantly different from values for 2-, 6- and 12-month-old mice:
 $P < 0.001$.

§ Significantly different from value for 18-month-old mice: $P < 0.005$.

Fig. 1. Tunica albuginea (T) and seminiferous tubules from the testis of a 6-month-old C57BL/6 mouse. $\times 160$.

Fig. 2. Tunica albuginea (T) and seminiferous tubules from the testis of a 25-month-old C57BL/6 mouse. The tunica albuginea is thicker than in testes of younger mice (Fig. 1) and the epithelium of some of the seminiferous tubules has degenerated (*). $\times 160$.

(Facing p. 486)

Table 2. Effects of ageing on the percentage of chromosomal aberrations in the bone marrow of C57BL/6 mice

Age (months)	No. of mice	No. of metaphases	No. of metaphases with					Mean ($\pm$ s.e.m.) % aberrant metaphases
			Gaps	Breaks, fragments	Exchanges	Deletions	Multiple aberrations	
2	8	769	0	1	0	0	0	0.13 $\pm$ 0.13
6	8	689	0	8	3	0	0	1.64 $\pm$ 0.68
12	8	523	0	7	4	0	0	2.02 $\pm$ 0.72*
18	8	684	2	8	3	0	2	1.81 $\pm$ 0.55*
25	5	850	0	17	2	0	0	2.24 $\pm$ 0.77

* Significantly different from value for 2-month-old mice: $P < 0.05$.

tunica albuginea was thicker in 25- than in 2- and 6-month-old mice. Some of the seminiferous tubules in testes of 25-month-old mice were abnormal in that there was an absence of spermatogenesis (Pl. 1, Figs 1 and 2).

Discussion

The present results of increased sperm head aberrations as a function of ageing are consistent with a theory of decreased repair ability in cells from aged animals (Alexander, 1967). While spermatogenesis is a continuing event involving a constant turnover of germ cells, the germ cells are still derived from an aged animal. Hence, the increase in aberrations could reflect DNA repair deficiencies in the germ cells from the aged animals. Unscheduled DNA synthesis which reflects DNA repair has been identified in pre-meiotic germ cells (Sotomayor, Sega & Cumming, 1979), although age-related studies have not yet been reported. If sister chromatid exchange represents a form of DNA repair (Latt, 1974), then there could be decreased DNA repair ability in ageing cell populations because the frequencies of induced sister chromatid exchange by mitomycin C are higher in human cell cultures of younger donors than in those from older donors (Schneider & Gilman, 1979). Similar results have been obtained from in-vitro studies on human diploid fibroblasts at different stages of cellular passage (Schneider & Monticone, 1978) and from in-vivo studies on ageing mice (Kram, Schneider, Tice & Ganas, 1978). Other reports have indicated that cellular DNA lesions such as strand breaks (Price, Modak & Makinodan, 1971), DNA-protein crosslinks (Archarya, 1972) and spontaneous aberrations (Curtis & Miller, 1971) may be more frequent in ageing cell populations. Aged animals demonstrating an increased frequency of morphologically abnormal spermatozoa may therefore be simply unable to repair gene damage responsible for sperm head morphology.

Mice treated with chemical mutagens and radiation have an increased incidence of morphologically abnormal spermatozoa (Wyrobek & Bruce, 1975). This incidence is dose-related and appears to be genetically inherited (Staub & Matter, 1976; Hugenholtz & Bruce, 1977; Wyrobek, 1979). The basis for these abnormal spermatozoa, while not completely understood, may be related to cellular changes caused by chemical carcinogens and/or ageing (Pitot, 1978).

The mitotic studies revealed differences in frequency of chromosome aberrations in the 2-month-old mice when compared to the other groups. This technique was included in this study to compare ageing effects using two independent measurements. From these results for the C57BL/6 mouse strain, sperm morphology assessment appears to be a more sensitive technique to monitor ageing effects than is analysis of the frequencies of chromosomal aberrations.

The same mutagenic exposure which might increase the frequency of mutation for genetic diseases could also act on the genes responsible for determining sperm head shape, thus

increasing the numbers of spermatozoa with abnormalities, but this is a very difficult theory to test. The present results, however, certainly demonstrate a significant increase in the frequency of abnormal spermatozoa as a function of paternal ageing and this increase may be due to environmental factors or an inherent susceptibility to mutation in the ageing germ cells. It is not known, however, exactly what percentage of these abnormal spermatozoa are actually capable of entering the oviduct and fertilizing the ovum. Krzanowska (1974) reported that C57BL/Kw mice contain fewer abnormal spermatozoa in the oviduct than in the uterus.

Neaves & Johnson (1981) studied 4- and 21-month-old mice and reported differences in the thickness of the tunica albuginea and a significant decline in Leydig cell number with advancing age. The incidence of atrophic seminiferous tubules increases with age in men (Honoré, 1978; Hatakeyama, Takizawa & Kawahara, 1979) and CBA/Ca mice (Gosden, Richardson, Brown & Davidson, 1982). The lack of spermatogenesis in some seminiferous tubules of 25-month-old C57BL/6 mice could be explained by a decline in testosterone levels as reported for CBF₁ (Bronson & Desjardins, 1977) and CBA/Ca (Gosden *et al.*, 1982) strains.

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