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

I. A morphological study of spermiogenesis

By NINA HILLMAN and MARY NADIJCKA

From the Department of Biology, Temple University, Philadelphia

SUMMARY

The results from a comparative ultrastructural study of spermiogenesis in 6-, 10-, 14- and 17-month-old sterile \(t^W/t^{w32}\), and fertile \(T/t^W, T/t^{w32}\) and BALB/c, mice are reported. The studies show that all of the males contained the same types of defective spermatids and that defects were not limited to specific spermatid stages. Younger males had fewer abnormal spermatids than older males of the same genotype and at each age the BALB/c and \(t^W/t^{w32}\) males appeared to contain more abnormal spermatids than the other males. No unique spermatid defect or increased frequency of a specific defect was found which can be correlated with infertility of the \(t^W/t^{w32}\) males.

INTRODUCTION

The \(T/t\) complex in the house mouse is located on chromosome 17 and includes a series of recessive \(t^n\) mutations, referred to as \(t\)-haplotypes (Artzt & Bennett, 1975). Detailed mapping and genetic studies of the recessive mutations have recently been reported (Lyon, Evans, Jarvis & Sayers, 1979). The categories of the recessive mutations – lethal \((t^L)\), semi-lethal \((t^{SL})\), and viable \((t^V)\) – are based on their effect on embryo viability in a homozygous condition. The lethal recessive mutations have been further subdivided into six classes according to their abilities to partially complement each other genetically (Bennett, 1975). A number of the \(t^n\) mutations have also been shown to cause a reduction in male fertility when present as a homozygous \((t^n t^n)\) or heterozygous \((t^n t^{w2})\) genotype. Various combinations of the recessive mutations always result in male sterility (Bryson, 1944; Braden & Gluecksohn-Waelsch, 1958; Dunn, Bennett & Beasley, 1962; Dunn, 1964). These combinations are (1) homozygosity for a semi-lethal mutation \((t^{SL}/t^{SL})\); (2) heterozygosity for semi-lethal mutations \((t^{SLx}/t^{SLy})\); (3) heterozygosity for a lethal and a semi-lethal mutation \((t^L/t^{SL})\) and (4) heterozygosity for lethal mutations from different complementation groups \((t^{Lx}/t^{Ly}; t^V/t^V; t^{Lx}/t^{Ly}; t^V/t^{SL})\) can result in male sterility, quasi-sterility or fertility (Rajasekarasetty, 1954; Braden & Gluecksohn-Waelsch, 1958; Dunn & Bennett, 1969).

Spermiogenesis in \(t^W/t^{w32}\) intercomplement males has been described by

\[1\] Authors' address: Department of Biology, Temple University, Philadelphia, Pennsylvania 19122, U.S.A.
Dooher & Bennett (1977). They noted that spermatid development is completely normal prior to Stage 11 of development. At Stage 11 and in later developmental stages, however, spermatids show nuclear and head abnormalities. Dooher & Bennett suggested that these defects are caused by the t mutations, stating that ‘genetic factors other than T/t haplotypes are insignificant in determining the morphological features of the spermatids’. However, these investigators did not report the results of comparative ultrastructural studies of spermiogenesis in control fertile males. Since most of the spermatid defects described by Dooher & Bennett have been found among the spermatids of other non-t"-bearing genetic and inbred strains of mice (Johnson & Hunt, 1971; Bennett, Gall, Southard & Sidman, 1971; Hillman & Nadijcka, 1978), we undertook the present comparative study of spermiogenesis in correspondingly-aged T/t₆, T/tᵦ₃２, t₆/tᵦ₃２ and BALB/c mice to determine first, if intercomplement males have unique spermatid defects and second, if these defects become apparent only in the late stages of spermatid development.

MATERIALS AND METHODS

The comparative electron microscopic studies were done on 6-, 10-, 14- and 17-month-old T/t₆, T/tᵦ₃２, t₆/tᵦ₃２ and BALB/c (+/+ ) mice. The T/t₆, T/tᵦ₃２ and BALB/c males were obtained from inbred stocks maintained by brother-sister matings. The intercomplement sterile males were obtained from matings between T/t₆ males and T/tᵦ₃２ females. This cross produces T/T embryos which die in utero (Chesley, 1935) and viable T/t₇ and t₆/tᵦ₃２ offspring. The T/t₇ offspring are tailless while the t₆/tᵦ₃２ animals have normal tails. Those animals which were classified by their phenotype to be intercomplement males were tested for ten mating units (Bennett & Dunn, 1967) with fertile females to assure that they were genotypically t₆/tᵦ₃２ males and sterile. The control males were tested for their levels of fertility and all were classified as being normal fertile prior to being used for this study. Six males of each genotype were examined at each age.

The males were sacrificed by cervical dislocation and their testes removed and processed for electron microscopic studies. The protocol used for preparing the tissues for study followed that described by Hillman & Nadijcka (1978). Ultrathin sections were stained with either lead citrate (Venable & Coggeshall, 1965) or with both lead citrate and 2% uranyl acetate (Watson, 1958). The sections were examined with a Philips 300 electron microscope.

We do not include, in this report, a detailed description of normal mouse spermatid development. Our criteria for normal spermatid morphology were obtained from the following studies: Fawcett & Phillips, 1969; Fawcett, Eddy & Phillips, 1970; Sandoz, 1970; Bennett et al., 1971; Fawcett, Anderson & Phillips, 1971; Bryan & Wolosewick, 1973. The spermatids were classified according to the staging by Oakberg (1956) as modified by Dooher & Bennett (1973).
RESULTS

Spermatid abnormalities

General observations

The same types of abnormal spermatids were found in males representing all of the genotypes examined. Spermatids at all stages of development showed aberrant morphologies, with no specific defect limited to the intercomplement males. Both control and intercomplement males contained aberrant spermatids which were clustered in the seminiferous tubules. This clustering has been reported by Bryson (1944), Rajasekarasetty (1954) and Hillman & Nadicjcia (1978). Because of this clustering and because the sections of seminiferous tubules were randomly selected for microscopic examination, it was not possible to quantitate the numbers of abnormal spermatids in the intercomplement males. However, the relative ease of observing aberrant spermatids in the 6-month-old intercomplement males suggested that the sterile males contained a high frequency of abnormal spermatids. This frequency was apparently comparable to that in BALB/c animals, but greater than that in the correspondingly-aged T/t^6 and T/t^w32 males.

The types of spermatid abnormalities present in the youngest animals (six months) were found also in 10-, 14- and 17-month-old t^6/t^w32, T/t^w32, T/t^6 and BALB/c mice. However, based on the relative frequencies of finding abnormal spermatids, there appeared to be increased numbers of defective spermatids in males from each of the four genotypes when they were examined and compared at older ages. The BALB/c and intercomplement males appeared to have higher frequencies of aberrant spermatids than either the T/t^w32 or the T/t^6 mice at all of the ages examined.

Specific observations

All males contained defective young spermatids. For example, all of the animals contained Stage-3 spermatids which had duplicated proacrosomal vesicles and granules. We suggested earlier that this duplication may lead to the rostral and lateral bifidity and bifurcation of spermatid nuclei (Hillman & Nadicjcia, 1978). Bifid and bifurcated spermatids were present in all males. The extent of the bifidity and bifurcation varied from only a slight bifidity to a cleft or bifurcation which extended posteriorly beyond the level of the perinuclear ring to the caudal-most portion of the nucleus. Males of all genotypes and ages contained spermatids which showed the complete range of this defect.

An additional early spermatid defect was the presence of binucleated and multinucleated cells. Of these cells, only those in which two or more nuclei share a single acrosome give rise to aberrantly-shaped spermatids. These abnormal cells have been found in numerous inbred and random-bred strains of mice. The effect of this condition on the morphological development of the
Spermatid defects in mutant \((t^{Lx}/t^{Ly})\) male mice

Fig. 5. A longitudinal section through two normal and one abnormal Stage-11 spermatids. Note, in the abnormal spermatid, that the apex of the acrosome (arrow) is adjacent to the perinuclear ring (PR). \(\times 7000\).

Fig. 6. This photomicrograph shows sections of two late Stage-9 blunted spermatids. Note that the acrosomal apices (arrows) are abnormally positioned in both spermatids. \(\times 5600\).

Fig. 1. A section through a normal Stage-6 spermatid. Note the spatial relationship of the acrosomal cap (A) with the implantation fossa (arrow). \(\times 7000\).

Fig. 2. A longitudinal section through a normal Stage-9 spermatid. Note that the rostral apex (arrow) of the acrosome is midway between the extreme edges of the acrosomal cap. In serial sections of this spermatid, the implantation fossa of the flagellum was found to be positioned directly opposite the apex of the acrosome. \(\times 9400\).

Fig. 3. This micrograph shows an abnormal Stage-5 spermatid. Note the misplacement of the acrosomal cap and the future rostrum of the acrosome (arrow) relative to the implantation fossa (IF) (cf. Fig. 1). \(\times 10600\).

Fig. 4. A longitudinal section through an abnormal Stage-9 spermatid. Note that the apex of the rostrum (arrow) has formed opposite to the implantation fossa but does not bisect the acrosomal cap. This abnormal positioning of the apex causes the acrosomal portion of the nucleus to be misshaped and the entire nucleus to become shortened and wedge-shaped (cf. Fig. 2). \(\times 9400\).
Spermatid defects in mutant \((t^{Lx}/t^{Ly})\) male mice

Fig. 11. A longitudinal section through a multiply defective early Stage-14 spermatid. These defects include nuclear bifidity, retraction of the condensed chromatin from the nuclear membrane and, possibly, an extensive perinuclear ring with excessive microtubules. \(\times 13000\).

Resultant spermatids has been discussed in detail (Hunt & Johnson, 1971; Johnson & Hunt, 1971; Bryan & Wolosewick, 1973; Johnson, 1974).

A third spermatid defect, found in all males, was an aberrant spatial relationship of the developing component parts of the spermatid. The most frequently encountered defect in this category was the incorrect relative positioning of the Golgi apparatus and its structural derivatives, the proacrosomal vesicle and its granule, with the positioning of the implantation fossa of the developing flagellum. Normally the vesicle and the fossa form at opposite poles of the spermatid nucleus, establishing, respectively, the future rostral and caudal regions of the elongated nucleus. Subsequent to proacrosomal vesicle formation, the vesicle spreads in all directions to cover the anterior one-third of the nucleus,

Figures 7–10

Fig. 7. A cross section through an aberrantly-shaped Stage-5 spermatid nucleus at the level of the acrosomal cap. \(\times 10400\).

Fig. 8. A micrograph of an abnormal Stage-5 spermatid. The portion of the nucleus subjacent to the acrosomal cap has a normal configuration. The remainder of the nucleus is aberrantly shaped. Note the disrupted and reflected nuclear membrane (arrow). \(\times 7200\).

Fig. 9. A section through an aberrant Stage-11 spermatid. Note the presence of Sertoli cell cytoplasm projecting into both the acrosomal and postacrosomal regions of the nucleus (arrows). The projection in the rostral region of the nucleus is circumscribed by the nuclear, acrosomal and plasma membranes. \(\times 14000\).

Fig. 10. A section of an extremely bizarre nucleus of a Stage-12 spermatid. Sertoli cell cytoplasm (arrows) is projected into the nucleus at points both anterior and posterior to the perinuclear ring (PR). \(\times 14000\).
forming the acrosomal cap. During normal development, the centre of this cap is positioned directly opposite the implantation fossa (Fig. 1). This central area of the cap becomes the apex of the acrosome (Fig. 2). In the aberrant spermatids the acrosomal cap is shifted so that it covers the lateral portion of the nucleus (Fig. 3). This shift also misaligns the positioning of the presumptive apex of the developing rostrum relative to the implantation fossa, resulting in spermatid heads in which the acrosomal portion of the nucleus is blunted and widened (Fig. 4). Spermatids with blunted, wedge- or club-shaped nuclei were, in fact, found in all males (Figs. 5 and 6), and it is possible that the initial defective positioning of the acrosomal cap produced this defect.

Early-staged spermatids with irregular nuclear shapes were present in all males. In a number of cells, the shape of the entire nucleus was extremely distorted (Fig. 7) while in other cells only a portion of the nucleus had an aberrant configuration. For example, Figure 8 shows a Stage-5 spermatid in which the nuclear area subjacent to the acrosomal cap is normally shaped whereas the remainder of the nucleus is distorted. The reason for this type of abnormality is not known; however, it could result from Sertoli cell cytoplasm projecting into early-staged spermatids, consequently distorting the configuration of the nuclei. If these projections persist, portions of the nuclei would continue to develop abnormally, resulting in older spermatids which have indented and irregularly-shaped heads. Figures 9 and 10 are typical examples of spermatids in which both the rostral and caudal portions of the nuclei are abnormally shaped. In both spermatids, indentations of the Sertoli cell cytoplasm are seen projecting into the nucleus at points both anterior and posterior to the perinuclear ring. Posterior to the rings, the cytoplasm projects into the otherwise normal manchette.

Additional types of head defects were found among the spermatids in animals of every genotype at all ages. These defects, all of which have been previously described (Hillman & Nadijcka, 1978), include abnormal chromatin condensation, disrupted nuclear membranes, dehiscence of the nuclear membrane and the condensed chromatin, non-sequential development of spermatid component parts and possibly, manchette abnormalities. Besides containing spermatids which have abnormal protrusions of the microtubules of the manchette into the nucleus (which may be a passive response to external pressure exerted by Sertoli cell projections), all males had spermatids whose manchettes appeared to contain excessive numbers of microtubules. Since the nuclei of the latter spermatids were always aberrant, the seemingly excess numbers of microtubules may be illusory.

Frequently a single spermatid demonstrated more than one type of nuclear and head abnormality (Fig. 11). Moreover, defective development was not necessarily limited to the head region. All of the males contained spermatids with duplicated implantation fossae and flagella. Also, all contained mature spermatids in which the doublets and/or dense fibres of the tail were either
Spermatid defects in mutant \((t^{Lx}/t^{Ly})\) male mice

missing, duplicated or disorganized. These defects were the same as those described in wild-type males and males carrying a single recessive \(t^L\) mutation (Hillman & Nadijcka, 1978). In all males, tail defects were encountered less frequently than head defects.

**DISCUSSION**

Our study shows that the sterility of intercomplement males is not caused by a unique spermatid defect. The intercomplement males contain a broad spectrum of spermatid abnormalities (both head and tail defects), all of which can be found in fertile control animals. The same types of aberrant spermatids are present in all of the males regardless of their age; however, in older males, these defects are seen with increasing frequency. The latter observation lends support to the hypothesis that aberrant spermiogenesis is age-related and cautions investigators to use correspondingly-aged animals for comparative and definitive spermiogenesis studies (Bryson, 1944; Hancock, 1972; Krzanowska, 1972).

Our survey also shows that defects in spermatid morphology can be found at all stages of spermiogenesis. We suggest that the types of defects present in the younger spermatids could lead to many of the head and nuclear defects which are apparent in the older spermatids. This suggestion supports the hypothesis that most abnormalities in spermatid head shapes are caused by aberrantly formed acrosomes (Hollander, Bryan & Gowen, 1960; Hunt & Johnson, 1971) or by the impingement of Sertoli-cell cytoplasm, either alone or together with the manchette, into the developing spermatid nucleus (Bennett *et al.*, 1971; Johnson & Hunt, 1971).

Locating defects of the younger spermatid stages often requires the study of serial sections or the fortuitous selection of sections which are cut in the correct plane to include several developing component parts of the spermatid (e.g. the acrosome and the implantation fossa). Because of the inherent limitations imposed by electron microscopy, defective morphologies of the younger-staged spermatids are not as easily discerned as are the more obvious developmental and morphological defects of older spermatids. This difference in the ease of identifying defects could explain the fact that Dooher & Bennett (1977) observed no abnormalities in the spermatid morphology of \(t^{Lx}/t^{Ly}\) males until Stage 11 of spermiogenesis. Alternatively, intercomplement males of the genotype \(t^6/t^{w32}\) may differ from \(t^6/t^{w32}\) males in the time of expression of abnormal spermatid development. However, since \(t^6\) and \(t^0\) belong to the same complementation group (Bennett, 1975) it is probable that males of these two genotypes contain the same multiple types of spermatid aberrations.

This research was supported by the United States Public Health Service Grants nos. HD 00827 and HD 09753. The authors would like to thank Marie Morris and Geraldine Wileman for their technical assistance.
REFERENCES


N. HILLMAN AND M. NADIJCKA
Spermatid defects in mutant \( t^{Lx}/t^{Ly} \) male mice


(Received 27 July 1979, revised 4 March 1980)