

The effects of some Robertsonian chromosome combinations on the seminiferous epithelium of the mouse

C. A. REDI*, S. GARAGNA

Dipartimento di Biologia Animale e Centro di Studio per l'Istochimica del C.N.R., Università di Pavia, Piazza Botta 10, 27100 Pavia, Italy

B. HILSCHER

Abteilung für Experimentelle Pathologie, Universitätstrasse 1, D-4000 Düsseldorf, West Germany

AND H. WINKING

Abteilung für Biologie der Medizinische Hochschule, Ratzeburger Allee 160, D-2400 Lübeck, West Germany

SUMMARY

In Mammals, structural rearrangements of the karyotype cause considerable trouble to the spermatogenic process. Making use of an experimental animal model of Robertsonian chromosomal variation in the house mouse (Gropp, Winking & Redi, 1982a) the effects of these chromosome structural rearrangements on the spermatogenic process were studied in fertile and chromosomally derived subfertile and sterile mice. Each karyotype condition was related to the cytological composition of the twelve stages of the seminiferous epithelium, studied in PAS-haematoxylin-stained testicular sections, with the following results:

1) in subfertile males there is a depletion of spermatogonia in the regenerating compartment but their differentiation is not affected. In the sterile males there is degeneration of primary and secondary spermatocytes and massive spermatid degeneration.

2) Spermatocyte development is retarded in nearly 50% of the spermatocyte population in subfertile males. Moreover the ratio between primary spermatocytes and spermatids is reduced to about 1:2 in subfertile males, while the few spermatids produced in sterile males had degenerated during stages I to VIII.

3) The number of Sertoli cells/100 μm throughout the cycle of spermatogenesis is the same in the three conditions studied. These data indicate that the spermatogenic process is affected by structural changes not only at the meiotic level (primary spermatocyte failure to follow the normal pattern of differentiation and occurrence of defective spermatids) but also at the premeiotic stage, when undifferentiated spermatogonia are regenerating.

INTRODUCTION

The morphological cell types that follow each other during differentiation from undifferentiated spermatogonia to spermatozoa are arranged in the mammalian

* Author for reprint requests.

Key words: Seminiferous epithelium, Robertsonian chromosome, spermatogenesis, spermatogonia, spermatozoa, Sertoli cells, mouse.

seminiferous tubules in a strictly determined order of cell associations. These cell associations are called 'stages' and are present in a limited and very specific number in each species (Roosen-Runge, 1977). The regular pattern observed in both the temporal appearance (cycle) and the spatial succession (wave) of the stages is determined by rigorous factors of coordination and synchronization that control spermatogenesis (Leidl, 1968; Parvinen, 1982). Alterations in testicular histology, and particularly in the cytological composition of the seminiferous epithelium (mainly based on male germ cell losses), can be due to physiological or pathological causes. Some good examples of changes in cytological composition of the seminiferous epithelium due to the action of intrinsic and extrinsic factors are naturally occurring phenomena, such as the existence in some species of an annual cycle of reproduction (Lloyd & Englund, 1973), age-dependent changes in the gonads (Holstein & Hubmann, 1981) or the action of exogenous agents such as heavy metals (Hilscher, Hilscher & Passia, 1978). Among intrinsic factors, karyotype anomalies can very effectively disturb the spermatogenic process (de Boer & Speed, 1982; Gropp *et al.* 1982a; Redi, Hilscher & Winking, 1983a; Redi *et al.* 1983b; Speed & de Boer, 1983) but have a broad range of effects. For example, in the case of reciprocal translocations and tertiary trisomies in the mouse, some show an early and total onset of spermatogenesis, others an impairment of spermiogenesis and it is not possible to predict which karyotype constitution will be fertile and which will be sterile (Searle, 1982). Similarly, whole-arm translocations in the mouse (Robertsonian, Rb, process of chromosomal centric translocation; Robertson & Rees, 1916; John & Freeman, 1975) can also have a variable effect with the fertility or otherwise of carriers dependent on the combinations of those chromosomes which are involved (Gropp *et al.* 1982a). These authors have postulated two mechanisms, acting to a different extent in the two sexes, as being chiefly responsible for the impairment of the gametogenic process and the reduction in fertility of Robertsonian heterozygote carriers:

a) meiotic non-disjunctional events, which are the most important contributors in females.

b) a non-specific depressor effect (similar to the 'male hybrid sterility' described by Haldane, 1922) which, in males, acts in addition to the meiotic non-disjunctional events to impair spermatogenesis.

The disturbances in the regular course of cytological meiotic events induced by structural chromosomal heterozygosities (de Boer & Speed, 1982; Forejt, 1979, 1982; Gropp, 1973; Gropp & Winking, 1981) are undoubtedly responsible for non-disjunctional events. These, in turn, lead to the formation of chromosomally unbalanced gametes, which, although capable of fertilization, lead to postzygotic losses. On the other hand, non-disjunctional events can only partially explain the general breakdown of spermatogenesis, since very little information is available about the premeiotic stages of male germ cell development in structurally heterozygous carriers. We have studied the effects of Rb heterozygosity on the histological constitution of the testis in an experimental model of chromosomal

Robertsonian chromosomes and spermatogenesis in the house mouse 3

variation in the mouse. The European long-tailed house mouse has very complex Rb variations (Gropp, Winking, Zech & Müller, 1972). Several wild mouse populations have already been described (Capanna *et al.* 1976; Gropp *et al.* 1982b) each characterized by a different set of Rb metacentric chromosomes (Gropp & Winking, 1981). With this mouse model, we can breed mice carriers of predictably determined rearranged karyotypes.

MATERIALS AND METHODS

Breeding for heterozygosity

Four different Rb-homozygous stocks (CD; CB; Rb7Bnr; Rb1Iem) were used as progenitors for the induction of heterozygosity. The CD and CB stocks were identified among feral mice from Cittaducale (CD, Central Italy) and Campobasso (CB, Central Italy). Both stocks contain a series of nine pairs of Rb-chromosomes (CD = Rb1Rma - Rb9Rma; CB = Rb10Rma - Rb18Rma). The single Rb(16·17)7Bnr chromosome has been isolated from the 'tobacco mouse' (*Mus musculus poschiavinus*) by repeated backcrosses to all acrocentric laboratory mice, whereas the Rb(8·17)1Iem chromosome was originated in a laboratory mouse genome (Gropp & Winking, 1981). F-1 individuals of the cross CD × CB show in the prophase of meiosis I a megaring of 16 Rb chromosomes plus an Rb bivalent, the bivalent n. 19 and the XY bivalent. The formation of the complex ring is based on alternate arm homologies of the following Rb chromosomes 1·7/7·6/6·13/13·3/3·8/8·14/14·12/12·10/10·11/11·4/4·15/15·5/5·17/17·2/2·18/18·1/ (Fig. 1B). The offspring of the cross Rb7Bnr/Rb7Bnr × Rb1Iem/Rb1Iem exhibit in meiosis a small chain of four partners, two Rb and two acrocentric chromosomes in the following order 16/16·17/17·8/8 (Fig. 1A). In Figs 1A and 1B, complete diakinetid plates of both crosses after C-band staining are shown (Sumner, 1972). NMRI/HAN male mice with the standard karyotype of 40 all acrocentric chromosomes were utilized as control group. In Table 1 the origin and the cytogenetic characterization of the three groups are listed.

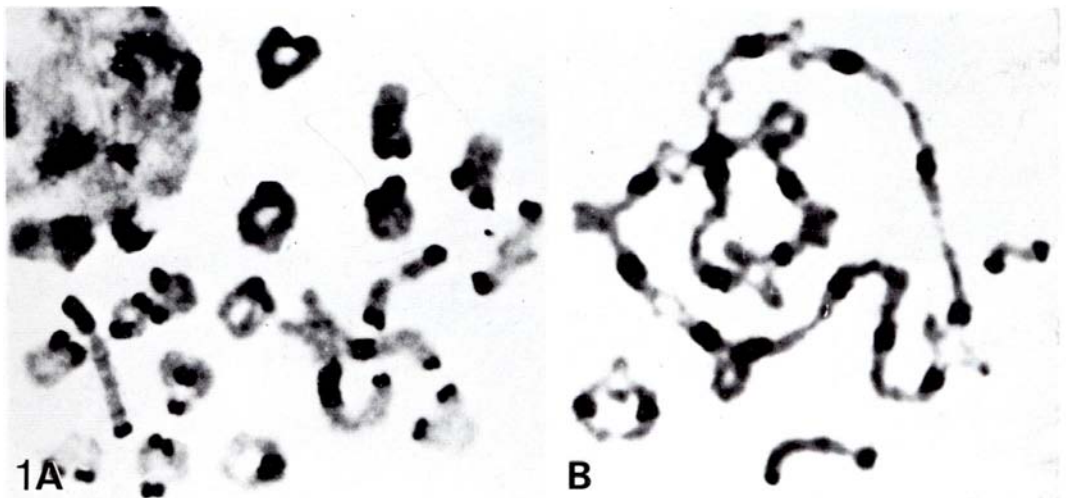


Fig. 1. Meiotic diakinesis/metaphase I of heterozygous male showing: (A) a quadrivalent with a chain shape involving two Robertsonian chromosomes with partial arm homology and two acrocentrics; 16 bivalents are also present; (B) a multivalent with a ring shape involving 16 Robertsonian chromosomes with partial and alternating arm homology; two bivalents plus the sex bivalent are present. C-banding staining.

Testicular histology, staining and cell evaluation

Three individuals, 3 to 6 months of age, from each animal group were killed by cervical dislocation and the testes immediately removed. After a small cut was made in the tunica albuginea, they were placed in Bouin's fixative for 24 h. After paraffin embedding, routinely prepared serial sections ($8\ \mu\text{m}$) were obtained from each testis and placed in a progression of five sections per slide. The seminiferous epithelium was scored after Periodic acid-Schiff and haematoxylin staining. Only perfect cross sections of seminiferous tubules were counted. The twelve stages of the seminiferous epithelium cycle were identified according to the criteria of Oakberg (1956) and Leblond & Clermont (1952): i.e., on the basis of acrosome formation and its progressive maturation. When cells were found which did not belong to one classification stage (defined on the basis of the acrosome formation of the young spermatids), the stage was defined as 'atypical'. The cells were considered to be 'degenerating' when there were obvious signs of morphological abnormality or staining properties clearly different from the average characteristics of the cells in the same cytodifferentiation phase. In order to avoid repeated counting of a given cell in successive sections, we counted the first and the fourth sections of each slide using the other sections only for resolution of doubtful identifications.

In order to determine individual animal variability, all the data from the histological investigation were first plotted for the left and right testis of each animal. Since no large intra- and inter-animal variations were observed, the data were replotted together for each animal group. At least 30 cross-sectioned tubules for each of the twelve stages of the seminiferous epithelium cycle were considered per animal group, 10 for each individual, combining both testes.

The perimeters of the cross-sectioned tubules and spermatocyte perimeters were measured by a digitizer connected with an Apple II microcomputer using a microscope with a drawing mirror and a $\times 56$ objective. The instrument was set to give the measurements in μm . All the cell counts

Table 1. *Description of the three experimental mouse groups*

Animal groups	Origin	Chromosome complement	References
Control NMRI/HAN	Outbred laboratory mouse strain	All acrocentrics $2n = 40$	Committee on Standardized Genetic Nomenclature, 1974
Double Rb heterozygote Rb7Bnr/Rb1Iem	Laboratory F_1 hybrid between homozygous strain Rb(8·17)1Iem and Rb(16·17)7Bnr	2 Rb metacentrics with one arm homology $2n = 38$	Gropp <i>et al.</i> 1982b
Complex Rb heterozygote CD \times CB	Laboratory-bred F_1 hybrid of wild <i>M.m. domesticus</i> from Cittaducale (CD) and Campobasso (CB) Apennine populations	16 Rb metacentrics with alternating arm homologies plus 1 Rb bivalent $2n = 22$	Capanna <i>et al.</i> 1976

Robertsonian chromosomes and spermatogenesis in the house mouse 5

were performed under a $\times 100$ objective and $\times 10$ ocular and the Abercrombie correction (1946) was applied.

Spermatogonia were classified according to their nuclear morphology, as follows: A_0 (regenerating) flattened nucleus without heterochromatin; the nucleus is homogeneously stained and the cells are isolated. A_1 , A_2 , A_3 and A_4 (proliferating) are characterized by increased numbers of heterochromatin clumps and chain formation. Intermediate and B spermatogonia (proliferating) show highly heterochromatinized nuclei, oval (Im gonial) or spherical (B gonial).

Cell counts were always expressed per cross section. Cell counts per 100 Sertoli cells gave equivalent results. A third solution (i.e. cell counts per $100 \mu\text{m}$) was discarded, because it gives numbers too small for statistical analysis for all cell types except for Sertoli cells.

RESULTS

Figs 2A,B and C show the testicular histology of control (NMRI/HAN), subfertile (CD \times CB) and sterile (Rb7Bnr/Rb1Iem) mice. As expected, well-recognizable and defined stages, with the proper cell-to-cell associations are present in the control mice. In subfertile (CD \times CB) mice, some normally arranged stages, some stages with almost normal histology and some stages with severe cell depletion are present. These types of stages are mixed, with alternate normal and defective spermatogenic areas in a mosaic pattern of distribution over the entire cross section of the testis.

In the sterile mice, almost all tubules show severe cell depletion with tubular shrinkage (as observed for other chromosome translocations by de Rooij, 1980) and the tubules with normal histology are very rare. In Fig. 3 the percent frequencies of normally arranged stages (i.e. stages without atypical cellular associations or degenerating areas with cell depletion) for all 12 stages in the three animal groups are shown stage by stage. In the control mice, all the 12 stages were 100 % normal. In the NMRI/HAN mice, there were only four unidentifiable stages with necrotic cells and almost complete cell depletion among more than 3000 identified stages. Subfertile mice show variable amounts of different atypical stages: correctly assembled postspermiation stages (IX, X, XI and XII) are less frequent (less than 50 % frequency) than in the first eight stages of the cycle, for which the frequencies of normally assembled stages range between 55 % and 73 %. In sterile mice there were no stages with correct cell associations.

Atypical stages are mainly due to cell alterations that can occur at any step in cytodifferentiation, but in most of the cases, spermatids are the cell types primarily involved in the degenerative process. Nevertheless a certain proportion of atypical stages involve abnormally small primary spermatocytes.

Fig. 4 shows the growth pattern of primary spermatocytes of fertile mice throughout the 12 stages. Up to stage VI, spermatocyte growth is slow in comparison with its growth pattern from stage VI to stage XII. When the spermatocyte growth pattern of subfertile mice is compared with that of fertile ones (Fig. 5), it can be clearly observed that in subfertile mice not all the primary spermatocytes have the same size range as in the controls. A large percentage (up

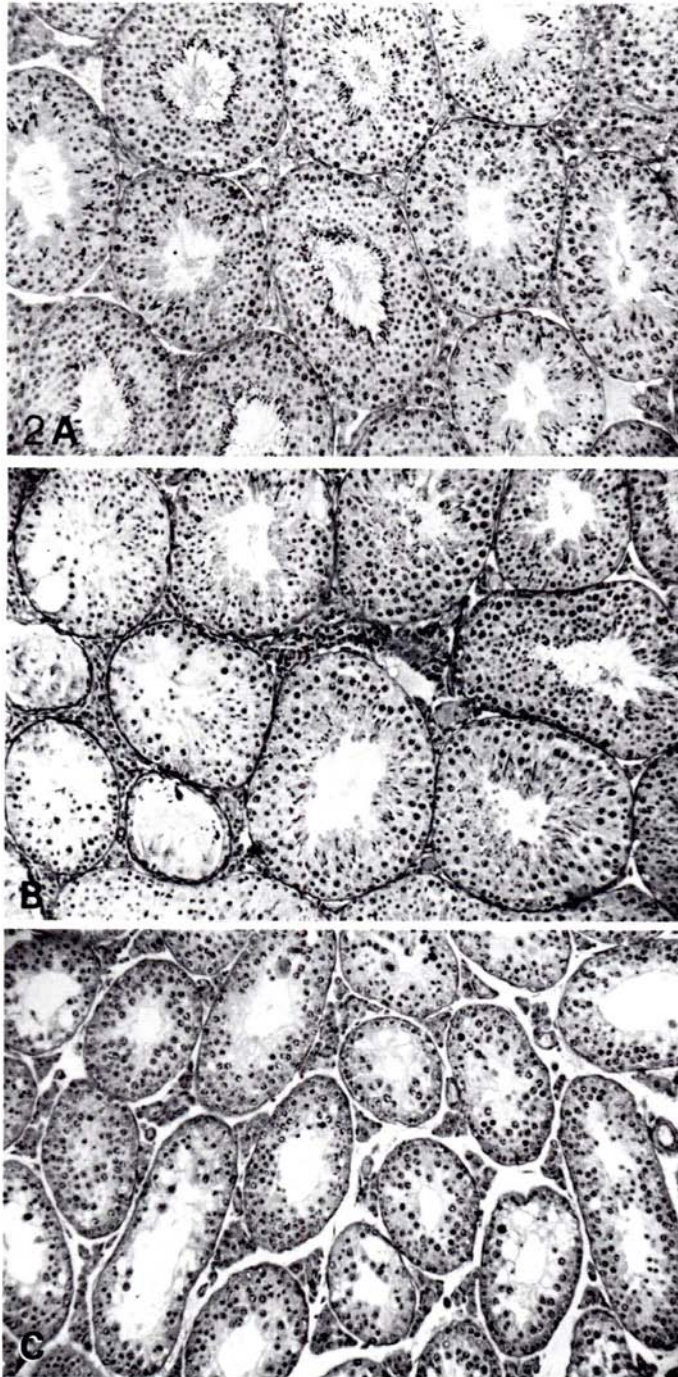


Fig. 2. Histological sections of testes of (A) fertile (NMRI/HAN) mice with normal tubules and abundant spermatozoa; (B) subfertile (CD×CB) mice with some depleted tubules and others with almost normal histology; (C) sterile (Rb7Bnr/Rb1Iem) mice, showing a complete breakdown of spermatogenesis.

Robertsonian chromosomes and spermatogenesis in the house mouse 7

to 45 % of the control value in stage XII) are smaller. The spermatocytes with smaller perimeters do not show more frequent than normal signs of degeneration and, apparently, are only retarded in their volumetric development.

This delayed development of a fraction of primary spermatocytes is not clearly detectable at stages earlier than IX. This fact, among others, can account for the higher proportion of atypical stages detected in subfertile mice from stage VIII onwards (Fig. 6A,B). Degeneration of spermatids and delay in spermatocytic development are very often observed in the same tubule cross section. In subfertile mice at stage XII degenerating spermatocytes are more numerous than in controls, but they do not correspond to the number of retarded primary spermatocytes (10 % versus 45 %). However retarded spermatocytes were never found beyond stage XII: i.e., stages I or II with primary spermatocytes belonging to the last stages of the cycle, were never found. When present, delayed spermatocytes do not occupy the whole cell layer of a tubular cross section, but are clustered in segments of the tubule.

The numerical ratios between primary spermatocytes and spermatids in control, subfertile and sterile mice have already been reported (Gropp *et al.* 1982a) and will be discussed later (see Fig. 8). The numbers of the different types of spermatogonia evaluated for all twelve stages are shown in Fig. 7A, for controls, and Fig. 7B for subfertile mice. In the sterile mice, despite the massive cell degeneration occurring at any moment of the spermatogenesis, there are a few

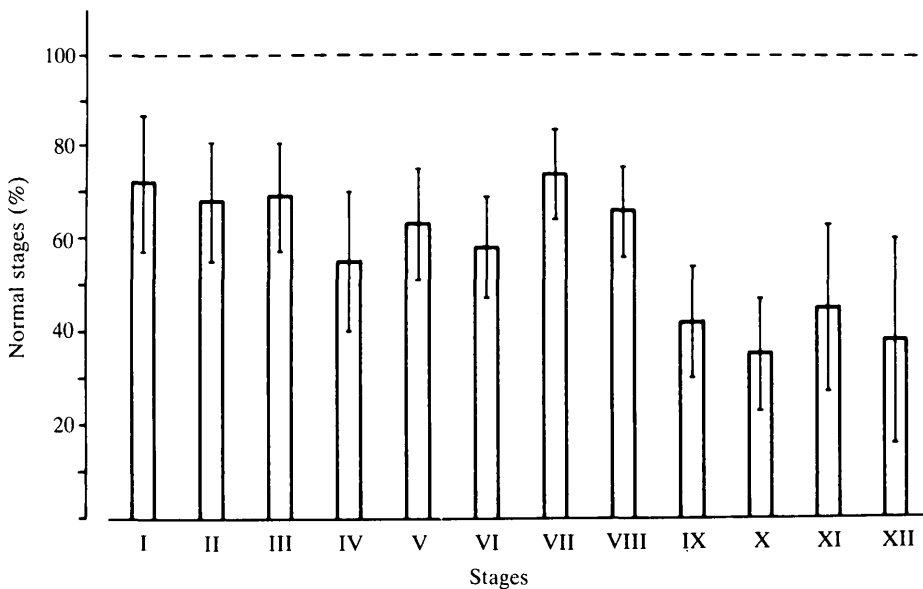


Fig. 3. Relative frequencies of normal stages throughout the seminiferous epithelium cycle, for control (- - -) and subfertile mice (—). The values for sterile mice are nearly 0%. For subfertile mice the lowest values are seen in the stages after spermiation (IX, X, XI and XII).

stages (as already stated) in which the differentiation process continues up to the formation of spermatids that degenerate very soon after arising. It was therefore impossible to count a sufficient number of the different cell types for a mathematical analysis. For this reason the proliferative kinetics of spermatogonia for the sterile mice is not reported (the few data obtained are shown in the histogram in Fig. 8). Only tubular cross sections with definitely identifiable spermatogonia were taken into account.

In control mice, the number of A_0 spermatogonia (Fig. 7A) was two to three cells per cross section through all 12 stages, reaching a maximum at stage VII (3.2 ± 1.3) and without any significant differences among stages. At least 100 spermatogonia were counted, from a minimum of 43 cross-sectioned tubules for stage VII and a maximum of 65 for stage XII. At stage VIII, proliferative spermatogonia begin to differentiate and the A_1 spermatogonia slowly increase in number until stage II when A_4 spermatogonia divide and produce intermediate spermatogonia (Oakberg, 1956; Monesi, 1962). At the beginning of the differentiation process the number of A_1 spermatogonia is 3.0 ± 0.3 (stage VIII) and these will produce 5.8 ± 0.8 A_4 spermatogonia. This slow numerical increase probably reflects physiological losses of spermatogonia during the differentiation process. On the other hand, A_4 and intermediate spermatogonia nearly double in number, producing 19.2 ± 1.2 B spermatogonia. At stages VII–VIII, B spermatogonia divide to produce preleptotene spermatocytes. The trend in subfertile mice is similar although the number of A_0 spermatogonia is smaller, especially

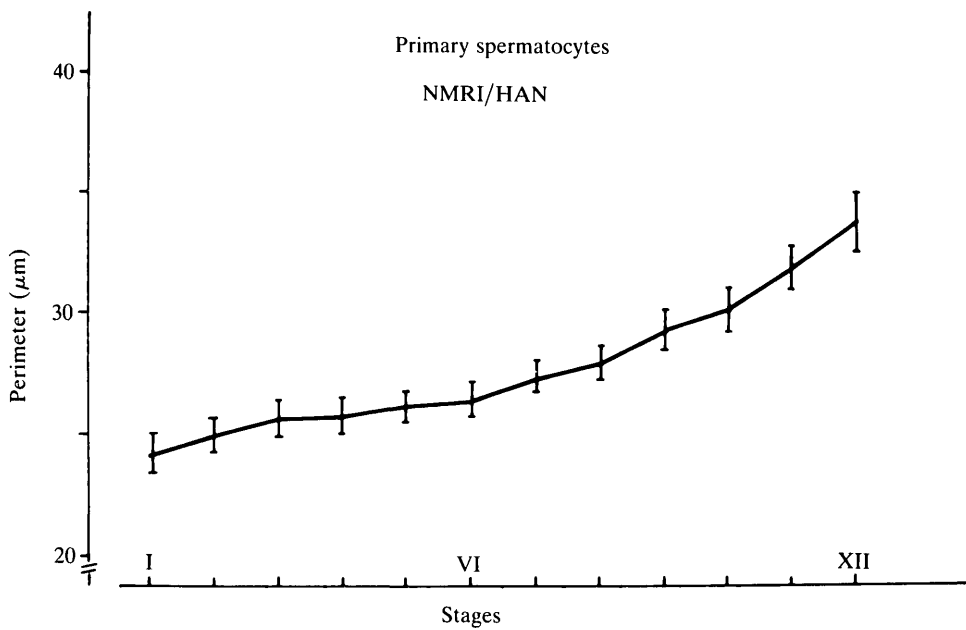


Fig. 4. Perimeters of primary spermatocytes in NMRI/HAN mice throughout the twelve stages of the seminiferous epithelium cycle. A constant increase in perimeter values is clearly detectable and faster from stage VI than from stages I to VI.

Robertsonian chromosomes and spermatogenesis in the house mouse 9

from stage I to stage VII of the cycle. This decrease is not statistically significant except in stages V and VI ($P < 0.05$). The number of differentiating

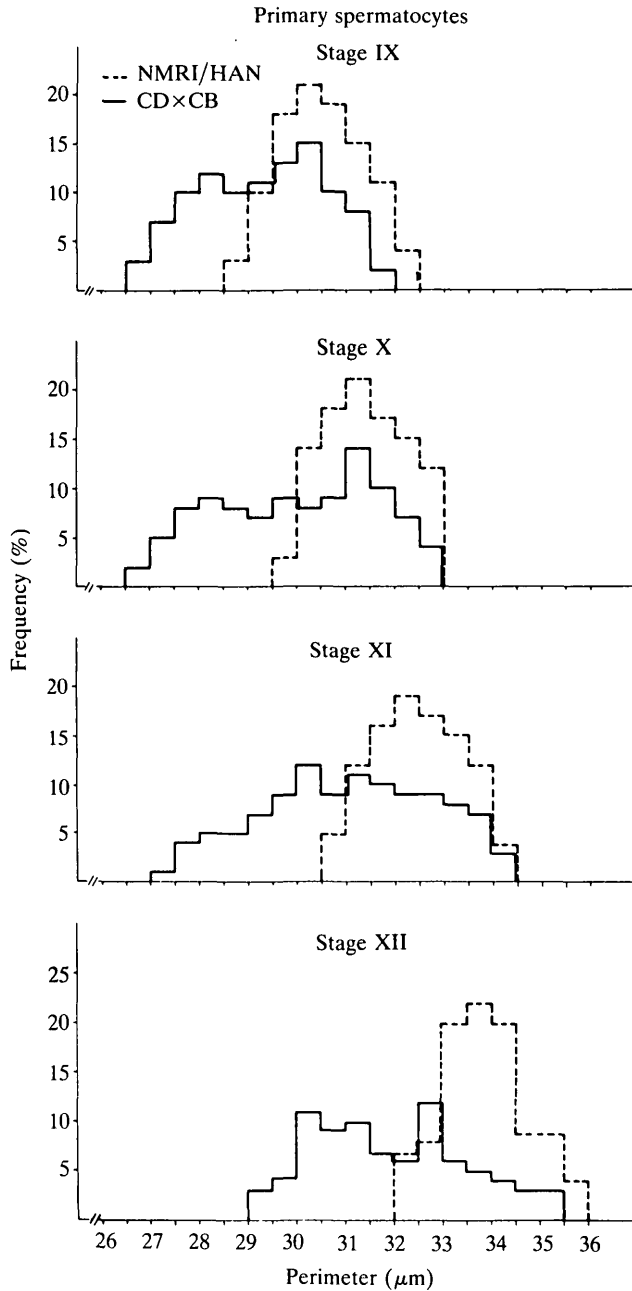


Fig. 5. Histogram of distribution of primary spermatocyte perimeters (μm) in stages IX, X, XI and XII. The fertile mice show a narrow distribution of the data as compared to subfertile mice, in which perimeter values are smaller, clearly detectable at stage XII.

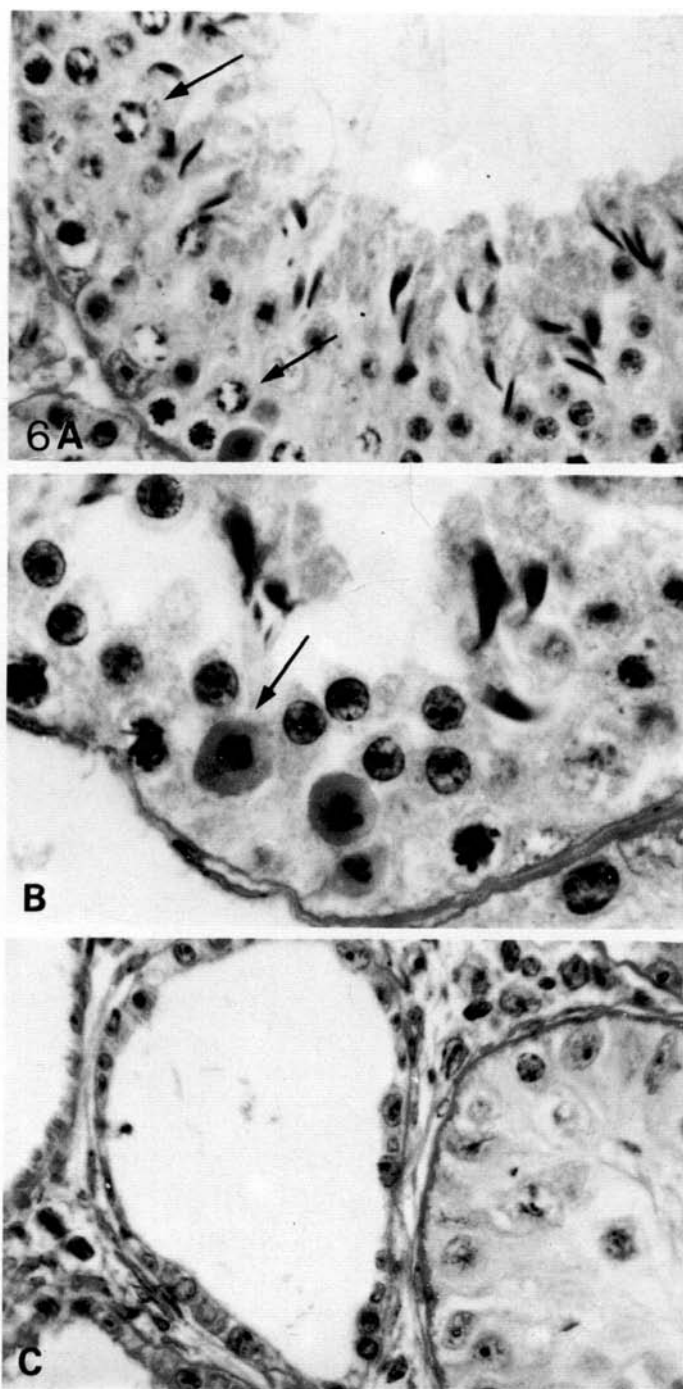


Fig. 6. Histological sections of the testes of subfertile mice: (A) stage XII with primary spermatocytes of different perimeter lengths (→); (B) stage XII with degenerating cells (→); (C) tubules with different degrees of cell depletion.

spermatogonia, especially that of intermediate and B remains lower than in NMRI/HAN mice but not significantly.

Schematic drawings of male germ cell cytodifferentiation for all three conditions are reported in Fig. 8. On the right of the figure the mean number of spermatogonia for the three conditions are given (columns 1, 2, 3) obtained by multiplying the number of spermatogonia (columns on the left side) by the relative frequency of normally assembled tubules found in the three conditions. In this way, the spermatogonia losses occurring in subfertile and sterile conditions are expressed and the final graph gives a more immediate idea of the statistical significance of the spermatogonia losses ($P < 0.05$; regardless of the stage or the type of spermatogonia) in the subfertile and sterile testes. Moreover, it can be seen that the number of spermatids produced at the end of the process is 1:4 (as theoretically expected) for fertile mice, whereas in subfertile mice it is about 1:2. In sterile mice, the few round spermatids that arise at stage XII degenerate very shortly after their appearance.

The number of Sertoli cells remains almost constant throughout the cycle of the seminiferous epithelium although minor oscillations can be detected (Fig. 9). No statistically significant differences can be detected either within or among the mouse groups. The spatial orientation of the Sertoli cell nuclei in the cross-sectioned tubule changes cyclically from stage to stage, being more horizontal at about stages IX to XII and more perpendicular in respect to the basal lamina during stages I to VIII. This cyclic modification is seen even in subfertile mice, but in the sterile mice such observations were impossible because of the severe cellular depletion of the tubules. In Fig. 9, the number of Sertoli cells is expressed per 100 μm perimeter (and not per cross section) in order to show better that there were no numerical differences among the three different animal groups.

DISCUSSION

The presence of structural karyotype heterozygosities of Robertsonian (Rb) origin (i.e., due to whole arm translocations) has two main consequences for the gametogenic process: 1) segregational impairment, based on meiotic anaphase malsegregation, which leads to the formation of aneuploid zygotes, and 2) male-limited breakdown of gametogenesis (Gropp *et al.* 1982a). The breakdown of spermatogenesis can vary from total arrest (primary sterility) to greater or lesser impairment of the process.

How chromosome translocations affect the spermatogenic process is still not known, although in the case of reciprocal translocations some hypotheses have been proposed (altered expression of some genes: Ford, Searle, Evans & West, 1969; Cacheiro, Russel & Swartout, 1974; or interference in X chromosome inactivation: Lifschytz & Lindsley, 1972; Forejt, 1982).

In any case, control mechanisms specific for spermatogenesis must be disturbed in some way, even though the male limitation of the phenomenon becomes doubtful after the finding of Mittwoch, Mahadevaiah & Setterfield (1984) that in

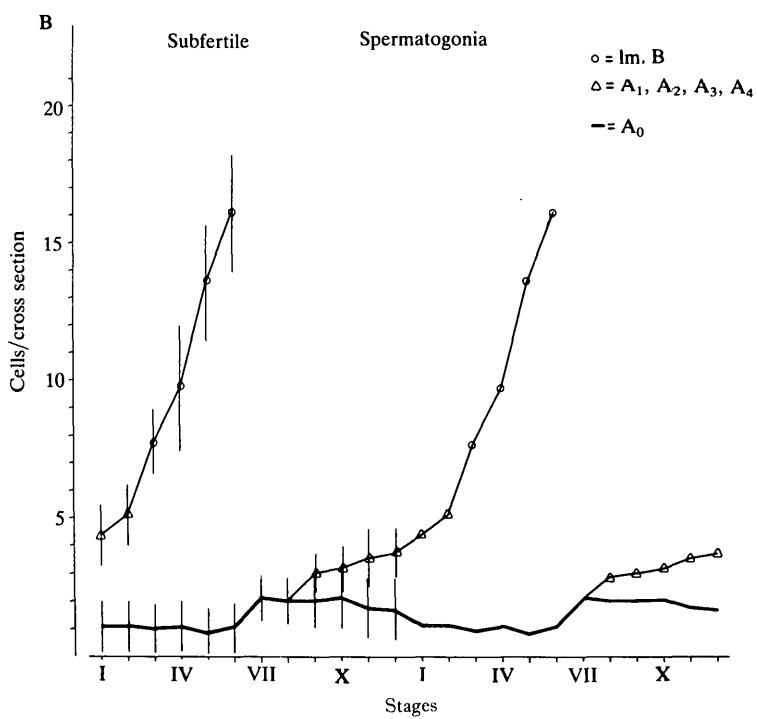
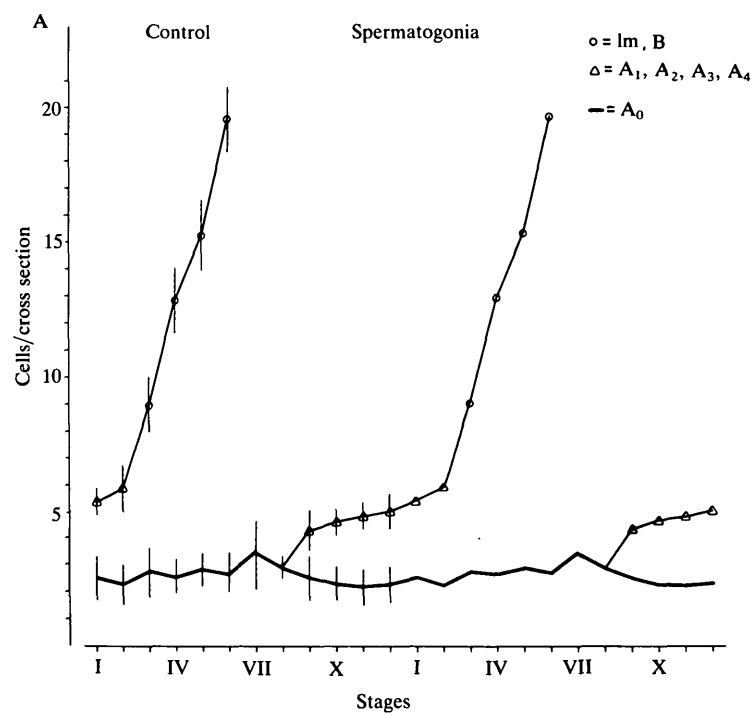


Fig. 7.

some chromosomal anomalies (including reciprocal translocations) known to affect male gametogenesis, ovariogenesis is troubled. Nevertheless we do not yet know whether or not the reduction of ovary size is associated with a reduction in the number of oocytes.

The two cytogenetic conditions we have investigated give rise to sterility or subfertility in males, whereas in the female heterozygotes no abnormalities can be detected in histological sections of the adult ovary (Gropp *et al.* 1982a). Thus the impairment of male fertility in these two types of heterozygotes can be tentatively

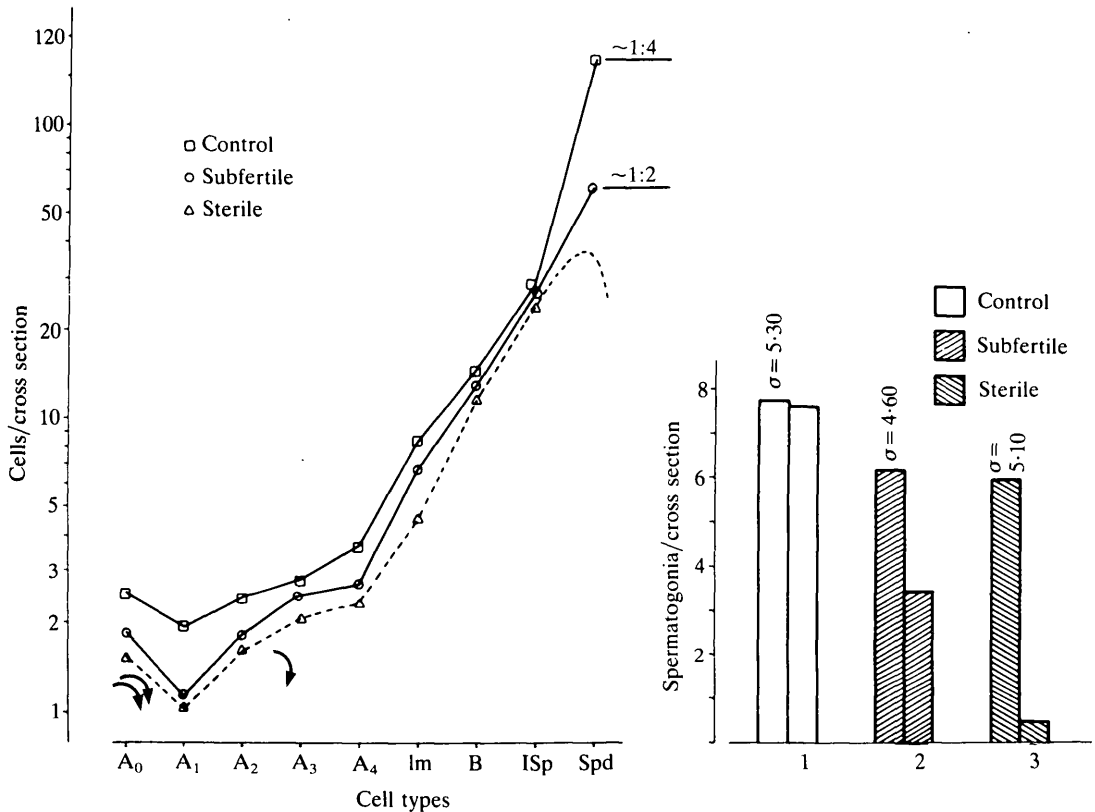


Fig. 8. Cytodifferentiation of the sperm cells in fertile, subfertile and sterile mice. The losses at the spermatogonia level (statistically significant, $P < 0.05$) are better shown in the histogram, in which columns 1, 2 and 3 represent the number of spermatogonia in normally assembled tubules (columns of the left side) multiplied by the relative frequency of normally assembled tubules in fertile, subfertile and sterile mice. Subfertile mice have a low production of spermatids (which consist partly of unbalanced cells). The few round spermatids of the sterile mice die shortly after arising.

Fig. 7. Number of spermatogonia throughout the twelve stages of the seminiferous epithelium cycle, for fertile (A) and subfertile (B) mice. In the first part of the curves the standard deviations of the values are reported. The main difference between the two curves is the lower number of A_0 spermatogonia in first seven stages for subfertile mice.

explained on the basis of X-chromosome inactivation interference exerted to different extent by the chain or ring diakinetid configurations. Winking & Johannisson (1980) found that chain configuration is a more asymmetrical configuration than the ring and it presents more unsynapsed regions (e.g. at the two extreme ends of the chain) that potentially can contribute to X-chromosome interference, whereas the closed configuration of the ring's meiotic pairing has a lower tendency to interfere with X-chromosome inactivation. In both cases a non-permissible gene product of the X chromosome (activated at the chromosomal level) would then be responsible for impaired spermatogenesis. Nevertheless, at present, the association of unpaired regions with the X-Y bivalent is a possible cause rather than an established cause. The difficulties in correct spermatocyte development caused by the heterozygosity could account for spermatocyte degeneration or for errors in the segregational machinery that lead to the production of chromosomally unbalanced gametes.

Some molecular biological data are now available for explaining abnormal primary spermatocyte development in heterozygotes. Hotta *et al.* (1979) analysed the DNA metabolism of pachytene primary spermatocytes in mice that were carriers of reciprocal translocations. They were able to show normal premeiotic DNA metabolism but a high level of endonuclease nicking activity. With autoradiographic methods, Forejt (1980) observed a lengthening of the time of

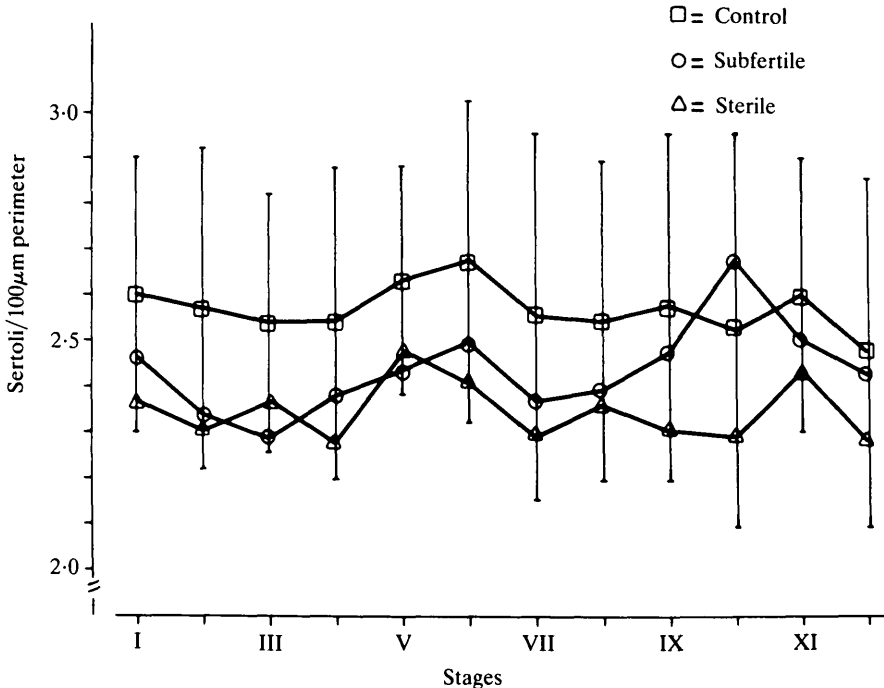


Fig. 9. Number of Sertoli cells (and standard deviation) per 100 μm perimeter of the tubule, throughout the seminiferous epithelium cycle. No differences can be detected either among the three animal groups or along the twelve stages for each animal group.

Robertsonian chromosomes and spermatogenesis in the house mouse 15

pachytene and diakinesis, and in accord with this, Speed & de Boer (1983) reported a delay of meiotic spermatocyte development in cases with impaired spermatogenesis due to chromosomal abnormalities. Redi *et al.* (1983a), in a histoenzymological investigation, showed a deregulation of the pattern of the thiamine pyrophosphatase (TPPase) in pachytene spermatocytes in subfertile and sterile mice.

Even in the two karyotype constitutions investigated here, spermatocytes show retarded development, with a slow acquisition of the correct dimensions. The consequences of such a delay in primary spermatocyte development are the appearance of morphologically atypical stages and cell degeneration. The zonal distribution of spermatocyte abnormalities inside a cross-sectioned tubule supports the hypothesis that the defect arises on a clonal basis. This pattern is particularly evident in subfertile mice for the simple reason that an almost normal histology of the tubule is retained, thus favouring detection of lesser abnormalities. Not all the spermatocytes, thought to be carriers of the same cytogenetic constitution, will be affected to the same extent by the deregulatory factors and this will consequently cause variegated expression of the defects. Moreover, because of the particular cytoarchitecture of the seminiferous tubule (i.e., the presence of plasmodesmata bridges between cells), some germ cells that were already started on a disturbed pattern of differentiation can be rescued by normally developing cells (Redi & Capanna, 1978; Speed & de Boer, 1983; Redi *et al.* 1983a). This allows cells which should be predetermined to die to survive for a longer time, which could explain spermatocyte degeneration at any stage of the cycle and the massive spermatid degeneration.

From the kinetics of premeiotic stages of male germ cell differentiation, subfertile mice (and even more so sterile mice) have mean numbers of spermatogonia per cross section (regardless of the stage or the type of spermatogonia) that are statistically significantly lower than in fertile mice. This shows the influence of the karyotype as early as the premeiotic stage, even though other factors may also be involved (such as tubular shrinkage; de Rooij, 1980) in disturbing spermatogonial proliferation. In any case, the losses that occur even in the regenerative compartment of spermatogonia do not prevent almost identical numbers of these spermatogonia entering differentiation in subfertile and fertile mice. This is probably achieved by triggering an increased number of spermatogonia to enter differentiation. Redi *et al.* (1983a) found that subfertile and sterile mice have increased numbers of alkaline-P-ase- and TPPase-positive spermatogonia. These can be regarded as signs of triggering into the differentiation process. This is strongly supported by the data of Hilscher (1981) for interactions between the regenerating and differentiating spermatogonia that seem to be able to control spermatocyte formation (and then sperm production). Apparently, the Sertoli cell is the only cell type in the seminiferous epithelium that is not affected by chromosomal rearrangement. They are present in equal numbers in the three conditions. The full morphological and numerical normality of Sertoli

cells, together with the few data available about the extra tubular compartment in heterozygous mice (Redi *et al.* 1983a) (as well as about lymphocytes in heterozygous mice; Redi & Capanna, 1978) seem to indicate a substantial viability of the somatic cell line in structural chromosomal rearrangement carriers.

Thus, Rb chromosomes seem to act on spermatogenesis at two distinct levels. The first level is at the spermatogonia, at which the Rb chromosome constitution first acts to produce a reduction in number of the regenerative spermatogonia. The second level is at meiosis, with primary spermatocyte failure to follow a normal pattern of differentiation. This failure first becomes evident at the pachytene stage, producing spermatocyte degeneration and/or errors in segregation which often lead to the appearance of defective spermatids that will soon die. Nevertheless, spermatids are very often able to overcome the cytodifferentiation process and produce completely viable spermatozoa (Redi *et al.* 1983b) giving rise to postzygotic losses (Gropp *et al.* 1982a). These two levels, acting separately or, more frequently, in conjunction, are responsible for the appearance of the typical

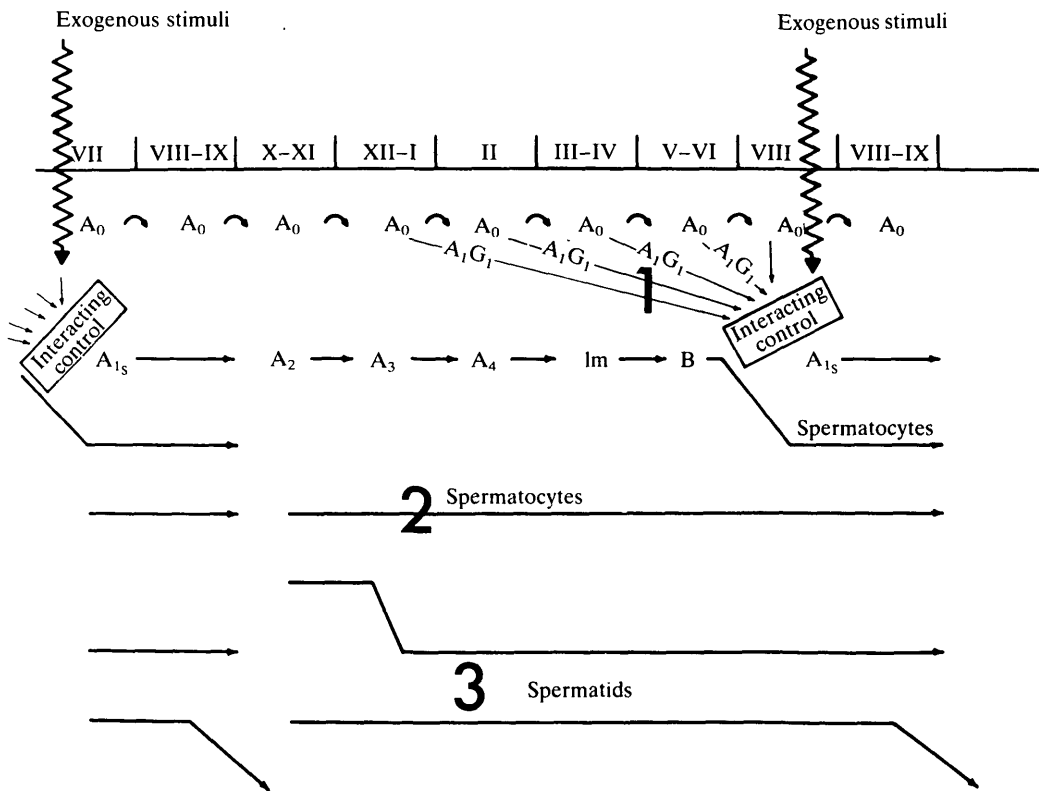


Fig. 10. Scheme for spermatogenesis in chromosomally heterozygous mice with chain or ring diakinetid configuration. The spermatogenic process is disturbed at three main points: (1) transition of spermatogonia from regeneration to differentiation; (2) pachytene spermatocyte development and metaphase I division; (3) spermatid differentiation.

Robertsonian chromosomes and spermatogenesis in the house mouse 17

features of the impaired testis, with variegated expression of damage and arrest of spermatogenesis at any phase of the process.

Fig. 10 shows a proposed scheme for spermatogenesis in conditions of chromosome variability, as derived from the data reported in the present paper (and based on the model of regeneration of the mouse seminiferous epithelium of Oakberg, 1956). There are three critical moments at which cellular deviation from the normal pattern of development or cell degeneration occur and the effects (i.e., the presence of variable moments in the onset of disturbance of spermatogenesis) account for the different expression of damaged spermatogenesis under the influence of chromosomal variability. Since the errors at the meiotic level can probably be accounted for by the particularly critical phenomena linked to segregation (i.e., chromosomal distribution), autoradiographic studies are in progress to elucidate the cell cycle characteristics of the different types of spermatogonia.

The authors wish to thank Professor M. G. Manfredi Romanini, Dipartimento di Biologia Animale, Università di Pavia and Professor W. Hilscher, Abteilung für Experimentelle Pathologie, University of Düsseldorf, for useful discussion throughout this work.

REFERENCES

- ABERCROMBIE, M. (1946). Estimation of nuclear population microtome sections. *Anat. Rec.* **94**, 238–248.
- CACHEIRO, N. L. A., RUSSEL, L. B. & SWARTOUT, M. S. (1974). Translocations, the predominant cause of total sterility in sons of mice treated with mutagens. *Genetics* **76**, 73–91.
- CAPANNA, E., GROPP, A., WINKING, H., NOACK, G. & CIVITELLI, M. V. (1976). Robertsonian metacentrics in the mouse. *Chromosoma* **58**, 341–353.
- COMMITTEE ON STANDARDIZED GENETIC NOMENCLATURE FOR MICE. (1974). Standard karyotype of the mouse. *M. musculus*. *Mouse News Letter* **50**.
- DE BOER, P. & SPEED, R. M. (1982). Meiosis of T70H translocation trisomic male mice. II. Meiotic rate, spermatocyte interactions and fertility. *Chromosoma* **87**, 315–325.
- DE ROOIJ, D. G. (1980). Spermatogenesis in mice heterozygous for a male-sterile translocation (T31H, T32H, T40H or T42H). *Cytogenet. Cell Genet.* **27**, 210–211.
- FORD, C. E., SEARLE, A. G., EVANS, E. P. & WEST, B. J. (1969). Differential transmission of translocations induced in spermatogonia of mice by irradiation. *Cytogenetics* **8**, 447–470.
- FOREJT, J. (1979). Meiotic studies of translocations causing male sterility in the mouse. II. Double heterozygotes for Robertsonian translocations. *Cytogenet. Cell Genet.* **27**, 204.
- FOREJT, J. (1982). X-Y involvement in male sterility caused by autosome translocations. A hypothesis. In *Genetic Control of Gamete Production and Function*. Sero Clinical Colloquia on Reproduction, Vol. 3 (eds P. G. Crosignani, B. L. Rubin & M. Fraccaro), pp. 135–151. London, New York: Academic Press/Grune and Stratton.
- GROPP, A. (1973). Fetal mortality due to aneuploidy and irregular meiotic segregation in the mouse. In *Accidents chromosomiques de la reproduction* (eds A. Boué & C. Thibault), pp. 255–269. Paris: I.N.S.E.R.M.
- GROPP, A. & WINKING, H. (1981). Robertsonian translocation: Cytology, meiosis, segregation patterns and biological consequences of heterozygosity. In *Biology of the House Mouse*, Vol. 47 (ed. R. J. Berry), pp. 141–181. London: Academic Press.
- GROPP, A., WINKING, H. & REDI, C. A. (1982a). Consequences of Robertsonian heterozygosity: segregational impairment of fertility versus male-limited sterility. In *Genetic Control of Gamete Production and Function*, Sero Clinical Colloquia on Reproduction, Vol. 3 (eds P. G. Crosignani, B. L. Rubin & M. Fraccaro), pp. 115–134. London, New York: Academic Press/Grune and Stratton.

- GROPP, A., WINKING, H., REDI, C. A., CAPANNA, E., BRITTON-DAVIDIAN, J. & NOACK, G. (1982b). Robertsonian karyotype variation in wild house mice from Rhaeto-Lombardia. *Cytogenet. Cell Genet.* **34**, 67–77.
- GROPP, A., WINKING, H., ZECH, L. & MULLER, H. J. (1972). Robertsonian chromosomal variation and identification of metacentric chromosomes in feral mice. *Chromosoma* **30**, 265–288.
- HALDANE, J. B. S. (1922). Sex ratio and unisexual sterility in hybrid animals. *J. Genet.* **12**, 101–109.
- HILSCHER, B. (1981). Spermatogenesis, an interacting proliferating process between stem cell spermatogonia and differentiating spermatogonia. In *Stem Cells in Spermatogenesis, Adv. Androl.*, Vol. 7 (eds A. F. Holstein & C. Schirren), pp. 46–57.
- HILSCHER, B., HILSCHER, W. & PASSIA, D. (1978). Histochemische Untersuchungen des Samenepithels des Ratte unter pathologischen Bedingungen. *Veterinär-Humonimed.* Tagung, Giessen, Feb. 1978.
- HOLSTEIN, A. F. & HUBMANN, R. (1981). Spermatogonia in old age. In *Stem Cells in Spermatogenesis. Adv. Androl.*, Vol. 7 (eds A. F. Holstein & C. Schirren), pp. 77–88.
- HOTTA, Y., CHANDLEY, A. C., STERN, H., SEARLE, A. G. & BEECHEY, C. V. (1979). A disruption of pachytene DNA metabolism in male mice with chromosomally-derived sterility. *Chromosoma* **73**, 287–300.
- JOHN, B. & FREEMAN, M. (1975). Causes and consequences of Robertsonian exchange. *Chromosoma* **52**, 123–136.
- LEBLOND, C. P. & CLERMONT, Y. (1952). Spermatogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid" technique. *Am. J. Anat.* **90**, 167–215.
- LEIDL, W. (1968). Steuerung und Beeinflussung der Samenbildungsprozesse. *Z. Tierzüchtung u. Züchtungsbiologie* **84**, 273–289.
- LIFSCHYTZ, E. & LINDSLEY, D. L. (1972). The role of X-chromosome inactivation during spermatogenesis. *Proc. natn. Acad. Sci., Wash.* **69**, 182–186.
- LOYD, H. G. & ENGLUND, J. (1973). The reproductive cycle of the red fox in Europe. *J. Reprod. Fertil.*, Suppl. **19**, 119–130.
- MITTWOCH, U., MAHADEVIAH, S. & SETTERFIELD, L. A. (1984). Ovarian involvement in chromosome anomalies leading to male sterility in the mouse. *Genet. Res.* **43**, 206–207.
- MONESI, V. (1962). Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine. *J. Cell Biol.* **14**, 1–18.
- OAKBERG, E. F. (1956). A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ renewal. *Am. J. Anat.* **99**, 391–414.
- PARVINEN, M. (1982). Regulation of the seminiferous epithelium. *Endocrine Reviews* **3**, 404–417.
- REDI, C. A. & CAPANNA, E. (1978). DNA content variation in mouse spermatozoa arising from irregular meiotic segregation. *Boll. Zool.* **45**, 315–332.
- REDI, C. A., HILSCHER, B. & WINKING, H. (1983a). Stage-dependent enzymatic activities in spermatogenesis of mice with the standard karyotype and of chromosomal variants with impaired fertility. *Andrologia* **15**, 322–330.
- REDI, C. A., GARAGNA, S., PELLICCIARI, C., MANFREDI ROMANINI, M. G., CAPANNA, E., WINKING, H. & GROPP, A. (1983b). Spermatozoa of chromosomally heterozygous mice and their fate in male and female genital tracts. *Gamete Res.* **9**, 240–254.
- ROBERTSON, W. M. & REES, B. (1916). Chromosome studies. I. Taxonomy relationship shown in the chromosomes of Lettigidae and Acrididae. *Chromosomes and variations. J. Morph.* **27**, 179–331.
- ROOSEN-RUNGE, E. C. (1977). *Developmental and Cell Biology*, Vol. 5: *The Process of Spermatogenesis in Animals*. Cambridge: Cambridge University Press.
- SEARLE, A. G. (1982). The genetics of sterility in the mouse. In *Genetic Control of Gamete Production and Function*. Sero Clinical Colloquia on Reproduction, Vol. 3 (eds P. G. Crosignani, B. L. Rubin & M. Fraccaro), pp. 93–114. London, New York: Academic Press/Grune and Stratton.

Robertsonian chromosomes and spermatogenesis in the house mouse 19

- SPEED, R. M. & DE BOER, P. (1983). Delayed meiotic development and correlated death of spermatocytes in male mice with chromosome abnormalities. *Cytogenet. Cell genet.* **35**, 257-262.
- SUMNER, A. T. (1972). A simple technique for demonstrating centromeric heterochromatin. *Expl Cell Res.* **75**, 304-306.
- WINKING, H. & JOHANNISSON, R. (1980). Pattern of pachytene pairing in mouse hybrids with chain and ring multivalent. *Clin. Genet.* **17**, 94.

(Accepted 8 October 1984)