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Germ cell migration and early development of the gonads in the trisomy 16 mouse – an animal model for Down's syndrome

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Summary. The aneuploid condition of patients with Down's syndrome (trisomy 21) frequently leads to a sub- or infertility of these individuals. Gonads from adults and fetuses with trisomy 21 demonstrated histologically a remarkable reduction in germ cells. Disorders in the germ cell migration, the early development of the gonads as well as meiotic defects are thought to contribute to this pathomorphology. To gain information about premeiotic defects, investigations on the trisomy 16 mouse, an animal model for Down's syndrome, were carried out. By means of morphometric studies a delay in migration and a reduction in primordial germ cells was evaluated in trisomic mice of embryonic day 11 (E11). At day E13 a generalized growth retardation of the developing gonads was obvious in trisomic animals. Additionally performed electron microscopic examinations revealed signs of germ cell demise in trisomy 16 mice. Thus, the mechanisms of a diminished proliferation capacity, impaired migration and premature death of germ cells represent premeiotic disorders that presumably contribute to the pathomorphology observed in the gonads of individuals with Down's syndrome.

Key words: Down's syndrome – Trisomy 16 mouse – Cell migration – Primordial germ cells

Introduction

In carriers of trisomy 21, the chromosomal aberration leads to sub- or infertility (Finch et al. 1966). Gonads taken from prepubertal female patients showed a reduced follicle number and a retarded follicle growth (Hojager et al. 1978). In the testes of adult individuals, the tubuli seminiferi contain a reduced number of germ cells or are completely devoid of germ cells (Kjessler and De la Chapelle 1971; Johannisson et al. 1983). A huge body of literature suggests that in aneuploid conditions meiotic defects lead to an extensive germ cell loss (Speed 1984; Mittwoch et al. 1984). Morphometric studies on the testes of fetuses with prenatally diagnosed trisomy 21 revealed a reduction in the germ cell count of up to half, compared to controls. These findings indicate premeiotic disorders which contribute to the development of the aforementioned pathomorphology. Therefore, a defect in the migration of primordial germ cells (PGCs) and the early development of the gonads is probable (Coerdt et al. 1985). To gain information about this topic investigations were carried out on the trisomy 16 mouse, which is an animal model for Down's syndrome (Gropp et al. 1983; Epstein et al. 1985 a). In the mouse, as well as in humans, the same migration pathway from the yolk sac to the genital folds has been demonstrated for PGCs. Consequently, this animal model is suitable for utilization in comparative investigations.

The aim of this study was to investigate the final stage of the cell migration as well as the behaviour of premeiotic germ cells during early gonadal development in trisomy 16 mice.

Materials and methods

Breeding design

Doubly heterozygous males Rb (11.16) $2H^{+}$/Rb (16.17) 8 $Lubt^{wLub3}$ were mated with laboratory strain NMRI females possessing 40 acrocentric chromosomes for the induction of trisomy 16 (Gropp et al. 1975; Winking 1987). The first observation of a vaginal plug was regarded as indicating embryonic day 1

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(E1). Females were killed by rapid cervical dislocation and embryos were taken from the uterus. Trisomic and normal littermates were identified by chromosome preparations of the amniotic membranes. Accurate confirmation of trisomy was provided by the demonstration of two Robertsonian metacentric chromosomes at a count of 41 chromosome arms (Miyabara et al. 1982). By staining the chromosome preparations with the C-banding technique the sex of the embryos was determined in order to achieve exact comparison of the randomised trisomic and euploid, male and female embryos. Only animals of the same litter were compared to each other.

Enzyme histochemistry

For enzyme histochemistry, embryos of day 11 to 12 were fixed in toto in 80% ethanol for 1 h and transferred to 70% ethanol at 4 °C. The tissue was dehydrated in graded ethanol, embedded in paraffin and cut in serial transverse sections of 16 µm thickness. Paraffin sections were stained by Gomori's method (1952) for demonstrating primordial germ cells (PGCs), which have a high content of alkaline phosphatase (Chiquoine 1954). The sections were mounted with Aquatex (Merck, Darmstadt).

Transmission electron microscopy

For electron microscopy, the specimens were fixed by immersion in 0.1 M cacodylate buffer containing 2.5% glutaraldehyde and 2% paraformaldehyde at pH 7.4 for 24 h. The embryos were postfixed in 1% OsO_4 and stained 'en bloc' with 2% uranylacetate (in 0.1 M maleate buffer pH 5.0) for 90 min. The specimens were dehydrated in graded ethanol and embedded in Araldite (Fluka, 10951). Semithin sections were stained with methylene blue and azure II (Richardson et al. 1960). Ultrathin sections were obtained with an ultramicrotome (Ultracut, Reichert-Jung), stained with lead citrate and examined under an EM 400 electron microscope (Philips). Light-microscopical observations were documented with an Axiophot photomicroscope (Zeiss).

Morphometry

For morphometric studies the in situ activity of alkaline phosphatase (Gomori's technique) was used. By means of this staining, PGCs of day E11 could be easily identified. The measurements were performed with the interactive digitizer of IDMS (Interaktives Digitizer Meßsystem). The total number of PGCs of each embryo and their spatial distribution were determined. Furthermore, the perimeter, the projection area, minimal as well as maximal diameter, and the factor of circularity (form factor) were calculated by the system. A comparison of randomised euploid, trisomic, male and female mice of the same litter was carried out (six euploid and six trisomic embryos). The mean value and the standard deviation (σ_{n-1}) were calculated. In order to verify the null hypothesis, all measured parameters were tested using the t-test.

Results

Morphometry

In respect of the parameters i. e. perimeter, projection area, form factor, minimal and maximal diameter of PGCs, statistically significant differences have not been found between euploid and trisomic embryos (Table 1). The morphometric evaluation of the distribution of PGCs on day E11 showed a striking difference in the total number of cells and their distribution between trisomic and euploid mice. The mean total number of PGCs in the euploid animals was 642 ± 64. Trisomic animals had statistically significantly fewer PGCs (396 ± 112; $p \leq 0.002$). There was also an obvious difference in the spatial distribution of these cells. In trisomic embryos the mean count of 34 ± 19 PGCs lying in the dorsal mesentery of the gut was statistically significantly higher compared to controls (3 ± 2; p 0.003). Vice versa we found statistically significantly more PGCs in the urogenital region of euploid mice (639 ± 63) as compared to the same area in trisomic mice (362 ± 105; $p \leq 0.001$) (Fig. 1).

Table 1. Overview of the morphological parameters. These parameters do not show statistically significant differences.

Parameter	euploid (n = 6)	trisomic (n = 6)
min. diameter	0.2 µm ± 0.000	0.2 µm ± 0.005
max. diameter	5.7 µm ± 0.516	5.3 µm ± 0.516
perimeter	16.0 µm ± 0.632	15.5 µm ± 0.548
projection area	76.7 µm² ± 5.854	74.0 µm² ± 3.286
form factor	0.87 ± 0.015	0.89 ± 0.021

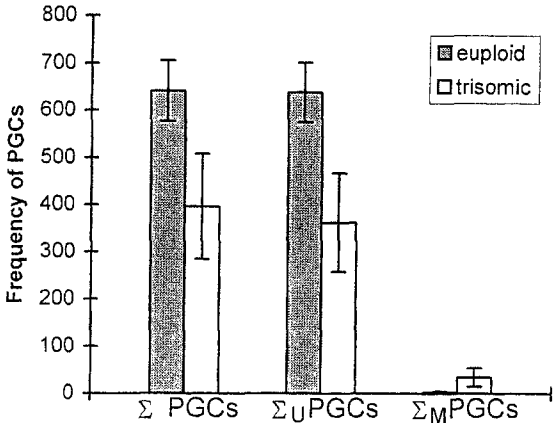

Fig. 1. Frequency and distribution of PGCs on day E11. ΣPGCs = Total number of PGCs; Σ_UPGCs = PGCs situated in the urogenital region; Σ_MPGCs = PGCs situated in the dorsal mesentery of the gut.

Enzyme histochemistry

Corresponding to the results obtained from morphometry, the paraffin sections demonstrated an expanded spatial distribution of PGCs in the case of trisomy 16 embryos on day E11. In these animals, a chain-like line of PGCs between the mesentery root and genital ridge could be seen, resembling a single-file formation (Fig. 2 b). In comparison, the controls showed a clustered PGC formation in the genital ridge, representing a complete migration of PGCs (Fig. 2 a).

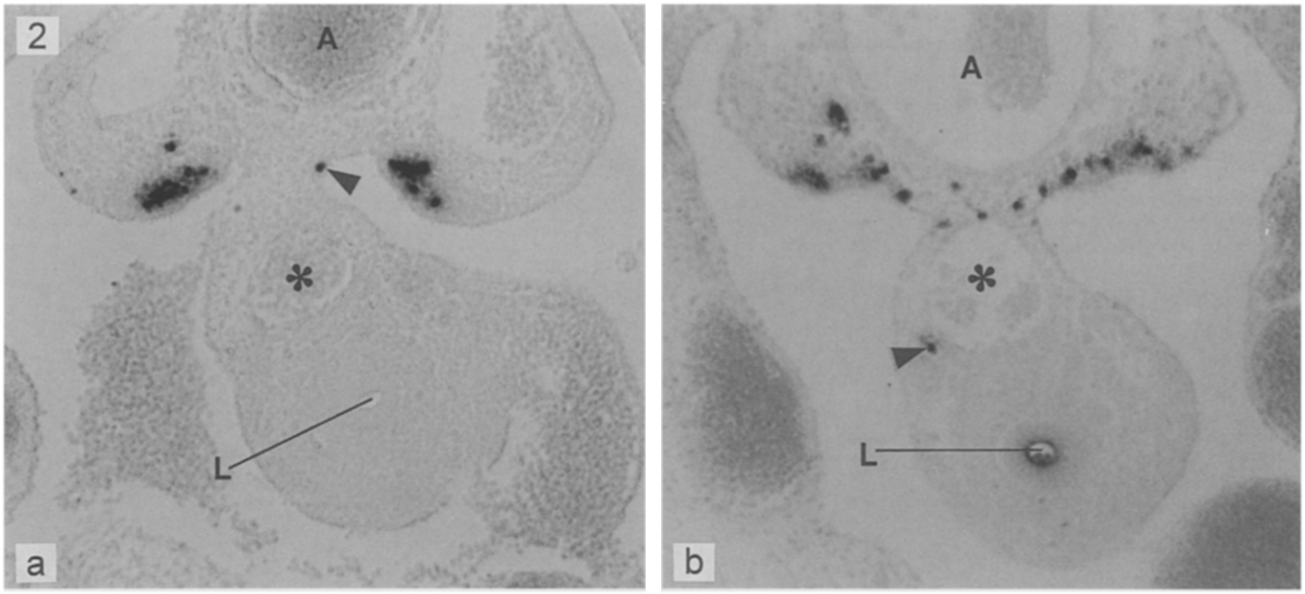

Fig. 2. Enzyme histochemical detection of alkaline phosphatase according to Gomori's method (1952), demonstrating PGCs on day E11. Two embryos are compared regarding the level of the branching of the A. mesenterica superior and the abdominal aorta. **a**) Control: The PGCs form clusters in the genital fold. A single PGC can be observed at the root of the dorsal mesentery (arrow). **b**) Trisomic: The formation of PGCs between the dorsal mesentery and the genital fold is similar to a single-file formation. A PGC is situated within the dorsal mesentery (arrow). Magnification: × 40; A = aorta, L = lumen of the gut, asterisk = arteria mesenterica superior

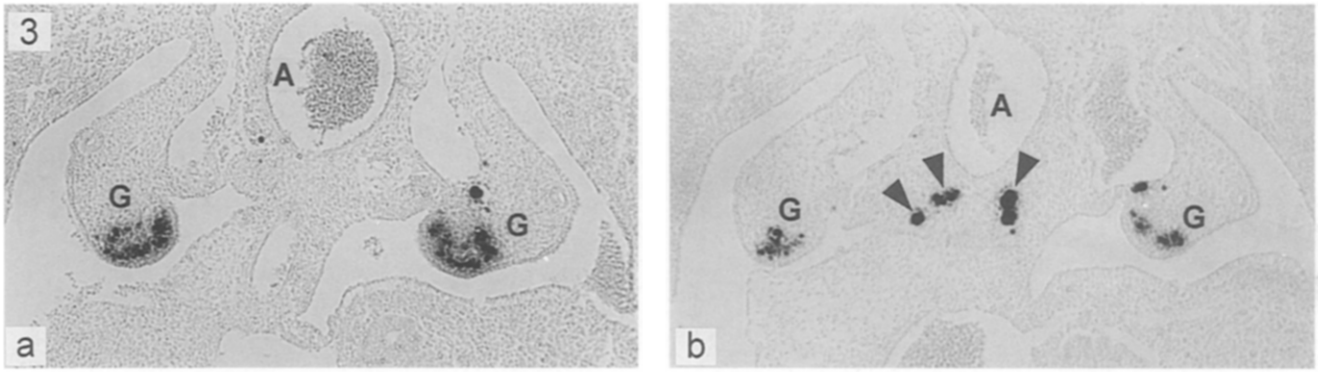

Fig. 3. Enzyme histochemical detection of alkaline phosphatase according to Gomori's method (1952), demonstrating PGCs at day E12. **a**) Control: The PGCs have reached their target tissue and settled in the gonad anlage. **b**) Trisomic: The gonad anlage is sparsely colonized with PGCs. Agglomerations of PGCs (arrowheads) can be observed ventral to the aorta. Magnification: ×20; A = aorta, G = gonad anlage

By day E12, little difference in size between the developing gonads in both groups could be observed. The gonad anlage of trisomic embryos was somewhat smaller and sparsely colonized with PGCs (Fig. 3 b) in comparison to euploid littermates (Fig. 3 a). In trisomic animals, agglomerated PGCs were frequently observed ventral to the aorta. Up to this stage, the development of the gonads was indifferent. It was not possible to distinguish between the sexes from morphological features. In no animal could PGCs be found outside the migratory route.

General morphological features of the gonad anlagen on day E13

On day E13 the main structures of the developing gonad were present in both sexes. In control animals the gonad anlage was attached to the dorsal body wall by means of a small mesenterium urogenitale. A bulge containing nests of germ cells was pointing towards the body centre and could easily be distinguished from the remaining connective tissue of the gonad. Additionally, the Müllerian and Wolffian ducts could be distinguished in an antero-

lateral position (Fig. 4 a). In comparison to euploid animals, the gonad anlage of trisomic animals was smaller, bulky and undifferentiated. The mesenterium urogenitale formed a broad basis to the dorsal body wall. The bulge that contained the germ cells was smaller than in euploid animals. The Wolffian duct could not be detected medial to the Müllerian duct (Fig. 4 b).

Transmission electron microscopy

On day E12 no differences could be found in the gonad anlagen of either group in respect of the ultrastructure and the formation of PGCs. These large, mainly round cells occurred in groups of two to five, which were usually surrounded by only a single layer of connective tissue

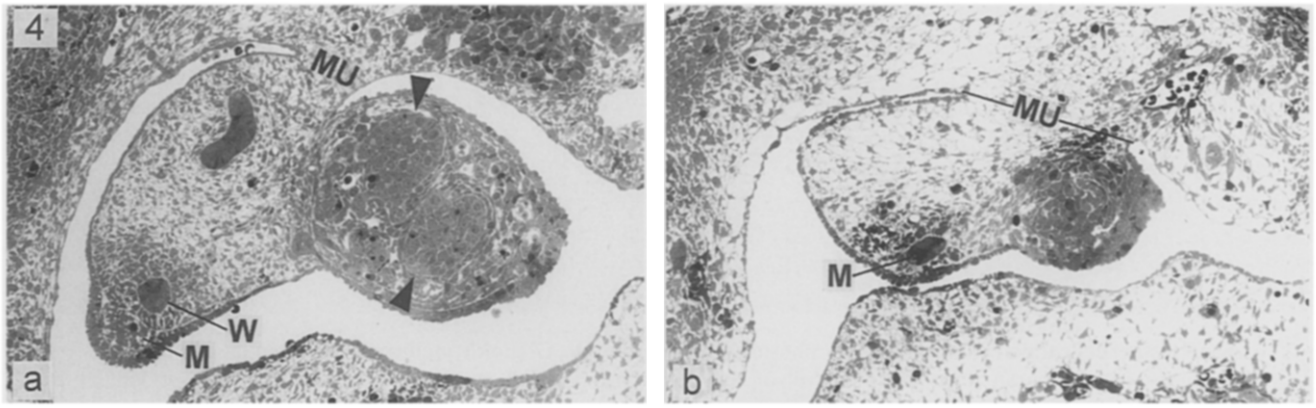

Fig. 4. Semithin sections on day E13 demonstrate the morphology of the developing gonad anlagen in a transverse direction. The gonad anlagen of two male embryos are compared at the level of the Bursa omentalis. **a**) Control: The developing gonad is attached to the dorsal body wall by a small mesenterium urogenitale. The bulge pointing to the body centre contains two cell nests with PGCs (arrowheads). This bulge will develop into a testicle. Antero-laterally, the Müllerian duct can be seen with vacuolized epithelial cells and close to it the Wolffian duct. **b**) Trisomic: In comparison to the control group, the development in trisomic animals appears to be delayed – the gonad anlage is undifferentiated and plump in form. Only the Müllerian duct can be observed on the antero-lateral edge of the gonad anlage. Richardson staining. Magnification: 140; M = Müllerian duct, W = Wolffian duct, MU = mesenterium urogenitale

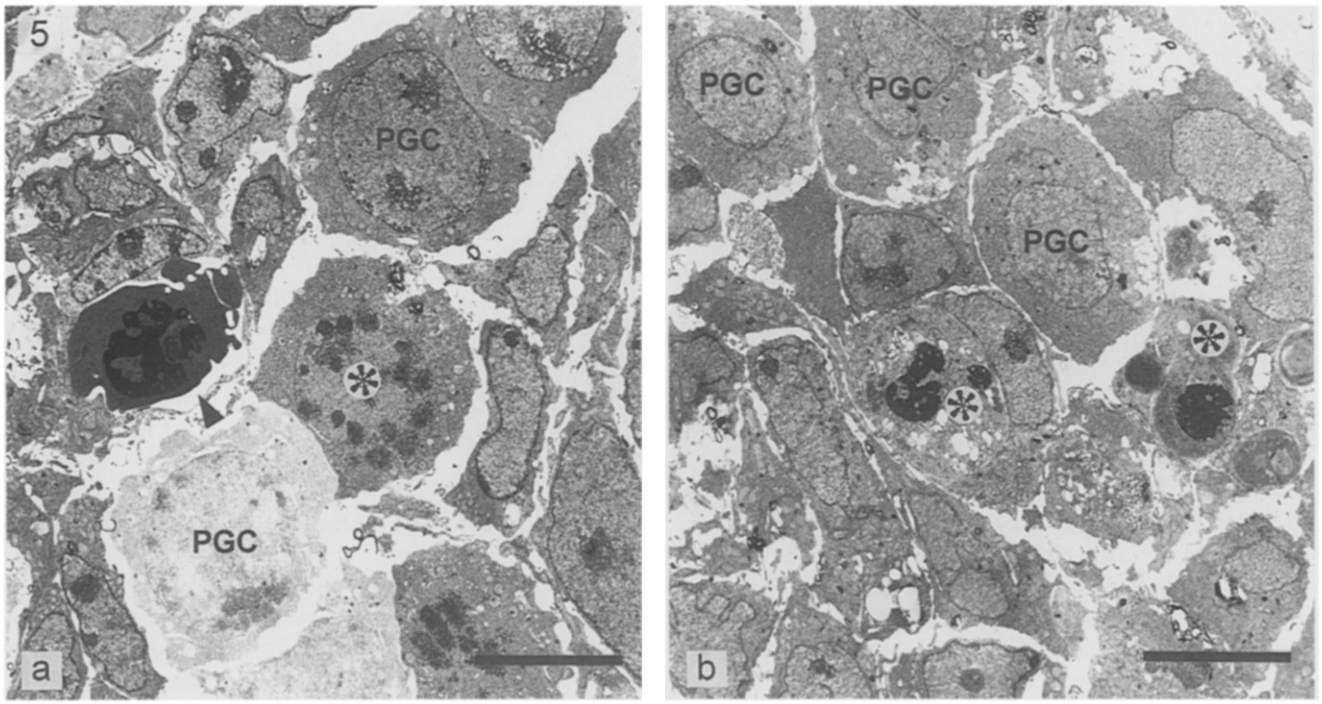

Fig. 5. Electron microscopic photographs showing portions of the gonad anlagen of a female euploid and a male trisomic littermate at day E13. **a**) Control: One PGC in mitosis can be observed between two PGCs of different staining (asterisks). On the left, a vessel containing a blood cell is demonstrated (arrowhead). **b**) Trisomic: In the gonad anlage, cells possessing a strongly condensed nucleus attract attention, whereby their cytoplasm is partially diffuse and infiltrated with vacuoles (asterisks), possibly signs of cell death. In comparison to the control group, the PGCs appear to be smaller in diameter and nucleus. Bar = 5 µm

cells with finger-like processes. Typical for PGCs was the rounded, centrally-situated nucleus, comprising one or two polymorphic nucleoli, varying in size and partly lying at the nuclear membrane. Additional characteristics of PGCs were the mitochondria which appeared to be swollen, and thus, in contrast to the long-stretching small mitochondria of the stroma cells, could easily be used for identification of PGCs.

Remarkable differences between the gonad anlagen of euploid and trisomic animals could be observed on day E13. In euploid animals, the PGCs demonstrated the same morphological characteristics as on day E12. The frequent occurrence of mitotic PGCs indicated an active cell division, even in female embryos (Fig. 5 a). On the other hand, the behaviour of the trisomic littermates was completely different. Here, strongly condensed and partially fragmented nuclei attracted attention. These pyknotic nuclei could be observed in abundance. They were mainly situated in a diffuse vacuolized cytoplasm, in which it was impossible to distinguish single cell organelles. Additionally, it should be mentioned that trisomic PGCs appeared smaller in the diameter and size of nucleus as compared to controls (Fig. 5 b). These changes could be verified in trisomic animals of both sexes.

Discussion

In this study the different histological techniques reveal a delayed migration of the PGCs and a retarded growth and differentiation of the gonads in both sexes of trisomy 16 mice, accompanied by a reduction in germ cells.

A reduction in germ cells in trisomy 16 embryos of day E13 has been reported earlier, but the former studies did not take into account the spatial distribution of PGCs and did not reveal statistically significant differences in germ cell numbers (Epstein et al. 1985 b). It is also important to mention that trisomic embryos show a general growth retardation beyond day E12 (Gropp et al. 1983; Epstein et al. 1985 a; Miyabara 1990). The smaller and less differentiated trisomic gonads, observed in mice of day E13, confirm this finding.

One of our most remarkable findings is the abnormal migration of trisomic PGCs. The distribution of trisomic PGCs along the migratory pathway reveals a delayed cell migration (Fig. 1). The form factor of trisomic PGCs shows no statistically significant difference in comparison to the PGCs of controls, which have already reached the genital ridge. This is interpreted as a premature stopping of migration, possibly caused by a disturbed cell-cell or cell-matrix interaction. An altered expression of components in the extracellular matrix, in particular the glycosaminoglycans, which are of great importance in cell migration and organogenesis (Toole and Underhill 1983; Erickson 1988), was demonstrated in human fetuses suffering from trisomy 21 (Kukharenko et al. 1991; Brand-Saberi et al. 1994; Kukharenko 1994). In vitro findings on fibroblasts taken from the heart and lung of human fetuses with trisomy 21 show an increased intercellular adhesion of these cells. This could also explain a mechanism which prevents a complete migration of PGCs (Wright et al. 1984).

A further factor, which contributes to the reduction of germ cells, may be a reduced mitotic activity of trisomic PGCs. Such a reduced activity has been shown in cultured skin fibroblasts of trisomy 21 individuals (Martin et al. 1970; Segal and McCoy 1973). The methods used in this study could not provide information on this topic.

In a study on trisomy 19 mice a premeiotic germ cell loss has been described (Lorke et al. 1989). In trisomy 16 embryos premeiotic cell death also seems to be responsible for a reduction in germ cells. Transmission electron microscopy shows actively proliferating germ cells in euploid animals of day E13. Though the germ cells of female embryos enter meiosis (preleptotene) at this stage, most of them seem to be still oogonia (Fig. 5 a). These findings are compatible with the literature (Peters et al. 1962; Speed 1982). Trisomic germ cells – especially in male embryos (Fig. 5 b) – show enhanced cell death. Since germ cells in male mice normally show proliferation activity up to day E14 and then undergo divisional arrest (Peters 1970), these results are remarkable and indicate a premeiotic germ cell loss which is rarely found in men and animals (Wartenberg et al. 1971; DeFelici et al. 1992). Since female germ cells just enter preleptotene at day E13, synaptic errors, suspected of leading to an extensive germ cell loss in aneuploid conditions (Speed 1982; 1984), will not occur at such an early stage of meiosis. This may probably be a feature in an accelerated aging process that is well described in patients with a trisomy 21 (Malamud 1972; Lott 1982). With regard to these observations, the high incidence of Alzheimer's disease in trisomy 21 patients above 40 years of age has to be mentioned (Malamud 1972; Sparks and Hunsaker 1992; Mann et al. 1985; Mann 1993). In vitro human skin fibroblasts obtained from individuals with a trisomy 21 show signs of an accelerated aging and a shorter lifetime compared to euploid cells (Martin et al. 1970; Rebhorn and Pfeiffenberg 1982; Flickinger and Culp 1990). The increased incidence of cell demise found in trisomy 16 mice, may therefore be the consequence of an accelerated aging process and a following cell death.

In order to summarize, we suggest – based on the findings of this study – a disturbed cell migration, a diminished proliferation capacity and a premature death of PGCs in the trisomy 16 mouse. In individuals with Down's syndrome these mechanisms presumably contribute to a reduction of PGCs, which finally results – in combination with a meiotic germ cell loss – in a reduction of germ cells in the gonads and an impaired fertility in both male and female.

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