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The incidence of chromosomally unbalanced gametes in T(14; 15)6 Ca heterozygote mice

By M. H. KAUFMAN
Anatomy Department, Cambridge CB2 3DY, U.K.

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SUMMARY

When T6/+ female mice were mated to non-translocation-bearing males, the relative viability of the embryos at 13·5–14·5 days gestation was about 39%. About 36% of the oocytes ovulated by T6/+ females were aneuploid, as a result of non-disjunction at meiosis, the majority having either 19 or 21 chromosomes. However, aneuploidy only accounts for a proportion of the embryonic loss in T6/+ × +/+ matings, as many of the embryos with 41 chromosomes survive postnatally. The present findings indicate that approximately 50% of the oocytes ovulated with the normal haploid number of chromosomes ($n = 20$) were genetically unbalanced as a result of adjacent segregation, and that a high proportion of the resultant embryos die in the early postimplantation period. In the present study non-translocation-bearing mice which were genotypically similar to the T6/+ females acted as controls.

1. INTRODUCTION

The present experiments were undertaken to investigate the genetic constitution and development potential of the eggs ovulated by female mice which were heterozygotes for the T6 translocation. This translocation involves a reciprocal exchange between segments of chromosomes 14 and 15 (Miller *et al.* 1971; Nesbitt & Francke, 1971; Eicher & Green, 1972). During meiosis in T6/+ mice, the small T6 marker chromosome (T6M) and large translocation products associate with their two normal partners either in a quadrivalent or univalent-trivalent configuration, the univalent being the T6M chromosome (Eicher & Green, 1972; Forejt, 1974). T6/+ heterozygote mice ovulate both haploid and aneuploid oocytes as a result of non-disjunction at meiosis I (Eicher & Green, 1972; Kaufman & Sachs, 1975). The majority of the aneuploid eggs ovulated contain 19 or 21 chromosomes with about an equal frequency.

Carter, Lyon & Phillips (1955) had previously noted that the relative viability of the post-implantatin embryos in T6/+ females mated to non-translocation-bearing males was about 36%, while Cattanach (1967) showed that fertilization of the oocytes with 21 chromosomes could lead to the production of viable offspring with 41 chromosomes. The post-natal incidence of mice with 41 chromosomes was 2–7% (Cattanach, 1967; Eicher & Green, 1972). Viable offspring with 39 chromosomes, but excluding the *XO* condition, were not reported in these series. In a more

recent study, Eicher (1973) reported that translocation trisomic offspring were only produced in crosses involving T6/+ females, never T6/+ males, when these were mated to non-translocation-bearing males and females, repectively. In this series, the incidence of translocation trisomic offspring in the T6/+♀ × +/+♂ crosses was about 16 %.

The preimplantation development potential of parthenogenetically activated haploid, hypo- and hyperhaploid oocytes from T6/+ females with 20, 19 and 21 chromosomes, respectively, have recently been examined (Kaufman & Sachs, 1975). These authors found that activated 1-cell eggs, at the first cleavage mitosis, had frequencies of 19, 20 and 21 chromosomes similar to oocytes at ovulation. On the third day of development, few embryos with 19 chromosomes remained, but the frequency of 21-chromosome embryos was similar to the frequency of 21-chromosome oocytes and activated 1-cell eggs. Thus the presence of an additional chromosome did not appear to hinder preimplantation haploid embryonic development. In this series, the incidence of aneuploidy in the oocytes ovulated by the T6/+ females was about 38 %. All the embryos with 21 chromosomes from the T6/+ series contained the T6M chromosome, in contrast to the expected frequency of 50 % if the distribution were random.

The present experiments were carried out to determine the extent of the embryonic loss, and the stage of pregnancy when embryonic death occurs, when T6/+ females are mated to non-translocation-bearing males. The dominant lethal assay was used to determine the stage and extent of the embryonic loss in these matings. Earlier work on translocations in the mouse had suggested that the majority of the genetically unbalanced embryos would die in the pre- or early post-implantation period (Snell, Bodemann & Hollander, 1934; Snell, 1946; Carter *et al.* 1955). Non-translocation-bearing female mice with a similar genetic background acted as controls for these studies. The main function of the controls was to provide information of the level of embryonic loss occurring in the T6/+ females, due to factors unrelated to the T6 translocation.

2. MATERIALS AND METHODS

All the mice used in the present experiments were obtained from the main breeding colony of the Weizmann Institute of Science, Rehovot, Israel. CBA-T6T6 and CBA/Lac male mice were mated to C57BL females. The resultant F_1 hybrid mice are hereafter referred to as F_1T6 and F_1LAC, respectively. The F_1T6 mice were heterozygotes for the T6 translation (T6/+), whereas the F_1LAC mice did not carry the T6 translocation (+/+).

10- to 12-week old F_1T6 and F_1LAC females were given 5 i.u. of pregnant mares' serum gonadotrophin followed 48 hours later by 5 i.u. of human chorionic gonadotrophin (HCG). Females were killed either 8–10 or 14–16 hours after the HCG injection. In the 8–10 hour group the ovaries were removed and pre-ovulatory oocytes obtained by follicular puncture. The chromosome constitution of these oocytes was then determined by air-drying (Tarkowski, 1966). The presence or

absence of a quadrivalent or univalent-trivalent configuration, and the stage of meiotic maturation were determined, namely diakinesis, diakinesis-metaphase I, or metaphase I. Eggs were isolated from the ampullar region of the oviducts of females killed 14–16 hours after HCG. Eggs at metaphase II were examined by air drying, and the number of chromosomes in each egg determined. All preparations were stained with 4% Giemsa following the air-drying procedure, and a maximum of three eggs placed on each microslide. Further groups of 10- to 12-week old F_1 hybrid females of both types were mated to fertile F_1LAC males. Females were checked each morning and those with vaginal plugs were isolated and a maximum of three females caged together. The day on which a plug was observed has been termed the first day of pregnancy. Females were killed at approximately midday on either day 13 or 14 of gestation. The contents of their uterine horns were examined, and the number of resorptions and dead and live embryos noted. The ovaries were then examined to determine the number of corpora lutea present.

Reciprocal skin grafts were performed between groups of CBA-T6T6 and CBA/Lac males as a gross means of assessing their genetic similarity. No rejection was observed in any of the recipients when the sites were examined at 100–120 days after the grafting procedure. This suggests that these two strains were probably either identical, or at least very similar at all of the major and minor loci.

3. RESULTS

(*a*) *The chromosome complement of oocytes during meiotic maturation*

Follicular oocytes from F_1T6 females isolated between 8 and 10 hours after the HCG injection for superovulation were analysed by air-drying. Classification of oocytes was made on morphological criteria alone, so that chromosome preparations were divided into diakinesis, diakinesis-metaphase I, or metaphase I groups.

Table 1. *Chromosome configurations observed in oocytes at meiosis I*

F_1 hybrid	Stage of meiosis	Total oocytes examined	Quadrivalent present	Univalent-trivalent present	Bivalents only
F_1T6	Diakinesis	16	15	1	0
	Diakinesis-metaphase I	19	16	3	0
	Metaphase I	42	31	11	0
F_1LAC	Diakinesis, diakinesis-metaphase I, metaphase I	46	0	0	46

This classification was therefore independent of the time after HCG when oocytes were isolated. All preparations contained either a single quadrivalent or a univalent-trivalent configuration. The incidence of univalent-trivalent configurations appeared to increase as meiotic maturation progressed. Thus in only 6·3% of diakinesis preparations was a univalent-trivalent configuration found, whereas 26·2% of metaphase I preparations were of this type (Table 1). If a number of

oocytes at a more advanced stage of meiotic maturation had been obtained, a higher proportion of univalents might possibly have been observed. A few preparations of this type were examined, but found to be technically very difficult to analyse.

In many of the chain quadrivalents in which the T6M was terminal, its association appeared to be very loose. This was especially marked at metaphase I. Thus in many of these preparations the T6M chromosome seemed only to be linked by a single chromatid to the sub-terminal member of the chain. A possible sequence of events in the formation of univalents from chain quadrivalents in T6/+ females is illustrated (Fig. 1). Only bivalent configurations were observed in 46 diakinesis-metaphase I preparations isolated under similar conditions from the control F_1LAC females.

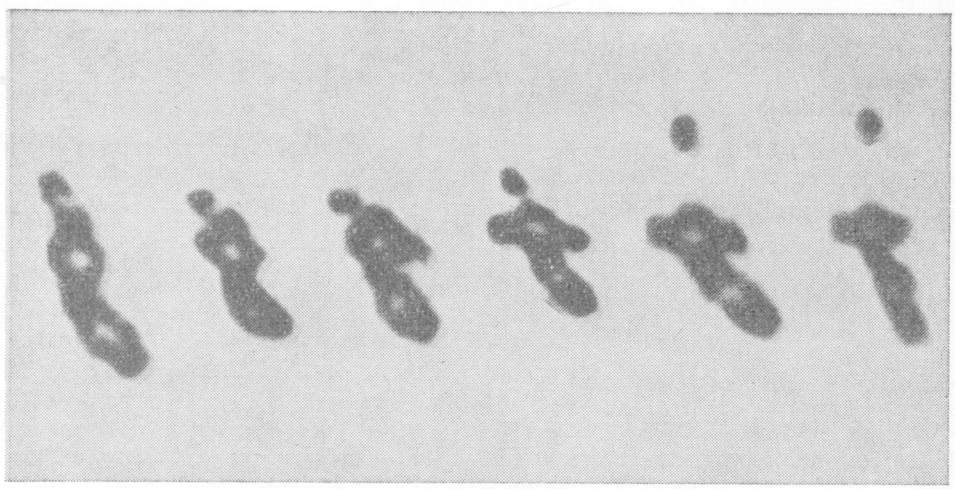

Fig. 1. Possible sequence of events in the formation of univalents from chain quadrivalents during meiotic maturation in T6/+ oocytes.

(*b*) *The chromosome complement of F_1T6 and F_1LAC oocytes at ovulation*

The chromosome complement of recently ovulated oocytes from F_1T6 translocation-bearing and F_1LAC non-translocation-bearing mice is presented in Table 2. 37·8% of the oocytes ovulated by F_1T6 females had an aneuploid chromosome constitution. The majority of these aneuploid oocytes had 19 or 21 chromosomes with about an equal frequency. The one aneuploid oocyte in the F_1LAC series had 19 chromosomes (Table 2). These results have been summarized elsewhere (Kaufman & Sachs, 1975).

(*c*) *An assessment of embryonic loss when T6/+ and control females were mated to non-translocation-bearing males*

All females in which a vaginal plug was observed were considered to have mated, and were killed either on day 13·5 or 14·5 of gestation (day of vaginal plug = day 1 of pregnancy). In both series all mated females were found to be pregnant when

dissections were carried out. In the experimental series 17 T6/+ females and in the control series 14 F_1LAC females were mated to non-translocation-bearing males. The total corpora lutea counts, numbers of resorptions, dead and live embryos for both series are presented in Table 3. The proportionate incidence of preimplantation and early and later postimplantation embryonic deaths (Table 3, section *b*)

Table 2. *The chromosome complement of oocytes at ovulation*

F_1 hybrid	Stage of meiosis	Total oocytes examined	Chromosome number 19	20	21	22	Aneuploid (%)
F_1T6	Metaphase II (recently ovulated oocytes)	74	15	46	12	1	37·8
F_1LAC	Metaphase II (recently ovulated oocytes)	29	1	28	0	0	3.4

Table 3. *Embryonic survival at 13·5–14·5 days gestation in crosses of T6/+♀ with +/+♂ mice*

	T6/+♀ × +/+♂ experimental	+/+♀ × +/+♂ (control)
(*a*) Total females mated	17	14
Total females pregnant	17	14
Total corpora lutea	173	124
Total resorptions	81	2
Total dead embryos	7	0
Total live embryos	67	116
(*b*) Preimplantation loss (%)	18/173 (10·4%)	6/124 (4·8%)
Early postimplantation loss (resorptions, %)	81/173 (46·8%)	2/124 (1·6%)
Later postimplantation loss (dead embryos, %)	7/173 (4·0%)	0/124 (0%)
Viable implantations (live embryos, %)	67/173 (38·7%)	116/124 (93·6%)

have been obtained from the figures presented in Table 3, section (*a*). In contrast to the very low incidence of early postimplantation embryonic loss in the control series (1·6%), 46·8% of the total zygotes produced in the F_1T6 series died at this stage of gestation. The proportion of live embryos observed on days 13·5–14·5 of gestation was 38·7% and 93·6% of the total zygotes produced in the F_1T6 and control series, respectively. The estimated preimplantation embryonic losses were 10·4% and 4·8% in the F_1T6 and control series, respectively.

4. DISCUSSION

In order to be able to assess what proportions of eggs ovulated by T6/+ mice have the normal haploid number of chromosomes ($n = 20$), but are genetically unbalanced, two distinct approaches may be employed. Embryos at various stages of development may be karyotyped using giemsa or fluorescent banding techniques. This was the approach taken by Oshimura & Takagi (1975), but has the

disadvantage that isolation of embryos at the egg cylinder stage, in the very early post-implantation period when maximum embryonic losses would be expected, is technically very difficult. A second approach based on the dominant lethal assay was employed in the present study. This approach gives information of the incidence and developmental potential of unbalanced gametes, but is unable to distinguish between gametes with different genotypes. In this respect, the two approaches are complementary. In extrapolating from the results obtained in the present experiments, where translocation-bearing T6/+ and genotypically similar but non-translocation-bearing +/+ control mice were used, certain assumptions have to be made. It has generally been accepted that in mice the corpora lutea count represents the total number of ova ovulated, there being an extremely low incidence of polyovular follicles in this species. Second, that all the eggs ovulated by non-induced females have an equal chance of becoming fertilized, even those with an abnormal genetic constitution. Third, that the F_1T6 and F_1LAC mice used in the present experiments were genotypically identical the only difference being that the F_1T6 were heterozygotes for the T6 translation (T6/+). No rejection was observed when skin grafts were made between the two male parental strains used to produce the F_1T6 and F_1LAC mice, namely CBA-T6T6 and CBA/Lac. While the experimental and control females may not have been identical at all genetic loci, the graft data suggests that the differences between them were probably very small. Fourth, that nearly all the zygotes with 39, and over one half of those with 41 chromosomes would die at some stage during embryonic development. One estimate of viability in embryos with 41 chromosomes was that reported by Evans & Meredith (cited in Cattanach, 1967) who noted that approximately 6 % of the total 9-day embryonic population in their series was of this type. This proportion was similar to the post-natal incidence observed by Cattanach (1967). In a more recent study Oshimura & Takagi (1975) noted that approximately 13 % of the viable embryos examined on days 16·5–18·5 of gestation had 41 chromosomes. The post-natal incidence of translocation trisomics in Eicher's series (Eicher, 1973) was about 16 %.

When in the present series of experiments, the uterine contents and ovaries of pregnant T6/+ and +/+ females previously mated to non-translocation-bearing males (+/+) were examined on days 13·5–14·5 of pregnancy, certain obvious differences between these two groups were observed. The incidence of preimplantation and early and later postimplantation embryonic losses were higher in the T6/+ series than in the +/+ control females. The 4·8 % preimplantation embryonic mortality observed in the control series could either have been due to the failure of oocytes to become fertilized, or to the loss of zygotes at any stage between fertilization and implantation. The embryonic loss in this group must have been due to factors unrelated to the T6 condition. The comparable preimplantation loss in the T6/+ series was 10·4 %. The preimplantation embryonic loss in the T6/+ was presumably made up of two components, the first due to factors unrelated to the T6 condition, and the second resulting directly from the T6 state. By extrapolation from the control data it seems likely that the second component might account for

approximately 5–6 % of the total embryonic loss in the T6/+ females. This is considerably lower than might have been expected if all zygotes with 39 chromosomes die prior to implantation. This therefore suggests that, at least in the present series, a high proportion of genetically unbalanced fertilized embryos, which may have deletions or duplications of genetic material, were capable of development beyond implantation. It is not clear whether these embryos were only capable of evoking a decidual response or of developing for a limited period beyond implantation. If all oocytes with 19 or 21 chromosomes were capable of fertilization, they would account for approximately 36 % of the initial zygote population. Assuming all embryos with 39 chromosomes were inviable as some stage during the prenatal period, this alone would account for approximately 18 % of the expected total embryonic loss observed in the T6/+ females. In the Evans & Meredith series (cited in Cattanach, 1967) and that of Cattanach (1967), about 50 % of the embryos with 41 chromosomes died prenatally. More recently Oshimura & Takagi (1975) observed that 10–15 % of oocytes from T6/+ mice had either 19 or 21 chromosomes. Karyotypes carried out on day 6·5 of gestation demonstrated that a high proportion of the embryos with 41 chromosomes were still viable, in contrast to the very low incidence of embryos with 39 chromosomes. The 13 % incidence of viable embryos with 41 chromosomes present on days 16·5–18·5 of gestation reported by these authors would seem to suggest that in their series very few embryos of this type died during the prenatal period. It is obviously impossible to extrapolate from the present findings what proportion of embryos with 41 chromosomes were viable at the time of dissection as karyotyping was not carried out.

The expected embryonic mortality in the T6/+ females due to aneuploidy would be about 23–35 %. This estimate accounts for the incidental preimplantation losses unrelated to the T6 state, and assumes that all embryos with 39 chromosomes were inviable, and that 40–100 % of embryos with 41 chromosomes were viable. As the overall embryonic viability in the T6/+ series was about 39 %, the observed embryonic loss was nearer to 61 % of the estimated original zygote population. If one assumes that about 50 % of the embryos with 40 chromosomes or, by extension almost 50 % of the oocytes ovulated with 20 chromosomes were genetically unbalanced this figure fits well with expectation. This is consistent with previous observations where it has been shown that genetically unbalanced embryos are more likely to die in the early postimplantation period than at other times prenatally (Snell, 1946).

These data support the findings of Oshimura & Takagi (1975), and suggest that the incidence of adjacent segregation in oocytes which undergo normal disjunction at meiosis I in the T6/+ females employed in the present study was about 50 %. Further, that the incidence of non-disjunction was not less than 36 % in these mice. It should also be noted that the overall viability in T6/+♀× +/+♂ matings has been remarkably consistent, ranging from 36–39 % in three series (Carter *et al.* 1955; Oshimura & Takagi, 1975; present series), despite the fact that the genetic backgrounds of the non-translocation-bearing strains have varied widely.

It is unclear whether the increase in the incidence of univalent-trivalent configurations which appeared to be related to meiotic maturation in the T6/+ oocytes, may also be observed in male translocation heterozygotes. A detailed re-evaluation of the preparations examined by E. P. Evans (cited in Eicher & Green, 1972) and Oshimura & Takagi (1975) of meiotic configurations from T6/+ males, where the incidence of quadrivalents was 63% and 54% and univalent-trivalents was 37% and 46% respectively, might help to clarify this point. In both these series, the univalent was always the T6M chromosome. The present findings on the configurations at first meiosis may be relevant to the difference in fertility observed between male and female translocation-bearing mice (Lyon & Meredith, 1966), and to the production of translocation trisomics by female but not male translocation carriers (Eicher, 1973).

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REFERENCES

CARTER, T. C., LYON, M. F. & PHILLIPS, R. J. S. (1955). Gene-tagged chromosome translocations in eleven stocks of mice. *Journal of Genetics* **53**, 154–166.

CATTANACH, B. M. (1967). A test of distributive pairing between two specific non-homologous chromosomes of the mouse. *Cytogenetics* **6**, 67–77.

EICHER, E. M. (1973). Translocation trisomic mice: Production by female but not male translocation carriers. *Science* **180**, 81.

EICHER, E. M. & GREEN, M. C. (1972). The T6 translocation in the mouse: its use in trosomy mapping, centromere localization and cytological identification of linkage group III. *Genetics* **71**, 621-632.

FOREJT, J. (1974). Nonrandom association between a specific autosome and the *X* chromosome in meiosis of the male mouse: possible consequence of the homologous centromeres' separation. *Cytogenetics and Cell Genetics* **13**, 369–383.

KAUFMAN, M. H. & SACHS, L. (1975). The early development of haploid and aneuploid parthenogenetic embryos. *Journal of Embryology and Experimental Morphology* **34**, 645–655.

LYON, M. F. & MEREDITH, R. (1966). Autosomal translocation causing male sterility and viable aneuploidy in the mouse. *Cytogenitics* **5**, 335–354.

MILLER, O. J., MILLER, D. A., KOURI, R. E., ALLDERDICE, P. W., DEV, V. G., GREWAL, M. S. & HUTTON, J. J. (1971). Identification of the mouse karyotype by quinacrine fluorescence and tentative assignment of seven linkage groups. *Proceedings of the National Academy of Sciences* **68**, 1530–1533.

NESBITT, M. N. & FRANCKE, U. (1971). Analysis of the T (3;?)6 Ca and T (14;17)264 Ca translocations in the mouse by quinacrine mustard staining. *Genetics* **69**, 517–522.

OSHIMURA, M. & TAKAGI, N. (1975). Meiotic disjunction in T(14:15)6 Ca heterozygotes and fate of chromasomally unbalanced gametes in embryonic development. *Cytogenetics and Cell Genetics* **15**, 1–16.

SNELL, G. D. (1946). An analysis of translocations in the mouse. *Genetics* **31**, 157–180.

SNELL, G. D., BODEMANN, E. & HOLLANDER, W. (1934). A translocation in the house mouse and its effect on development. *Journal of Experimental Zoology* **67**, 93–104.

TARKOWSKI, A. K. (1966). An air-drying method for chromosome preparations from mouse eggs. *Cytogenetics* **5**, 394–400.