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SHORT PAPER

Chromosomal anomalies that cause male sterility in the mouse also reduce ovary size

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SUMMARY

Two male-sterile chromosome anomalies, the insertion Is(7; 1)40H and the tertiary trisomy, Ts(5^{12})31H, were found to be associated with reduced ovarian volumes in immature females. Together with the reciprocal translocation, T(11; 19)42H, in which this effect was described previously, reduced ovaries have been found in all three male-sterile chromosome anomalies investigated so far, suggesting that ovarian involvement is likely to be common in these conditions. Assuming that the smaller ovarian size reflects a reduction in the number of oocytes, it is suggested that male-sterile chromosome anomalies may exert basically similar deleterious effects on meiotic germ cells in males and females, the difference in outcome being due to cell-physiological differences between spermatocytes and oocytes and to the small number of surviving oocytes required for fertility in females.

1. INTRODUCTION

Many chromosome anomalies are known that cause sterility in male but not in female mice (Searle, Beechey & Evans, 1978). They include a considerable number of translocations, involving not only sex chromosomes, but also those between autosomes, as was shown by Lyon & Meredith (1966); as well as tertiary trisomies derived from them (Searle, 1982). Apart from the sterility, the males appear to be normal. In heterozygous carriers of several chromosome rearrangements, spermatogenic arrest is complete, and testis size may be reduced to about 30% of the normal (Searle *et al.* 1978). Heterozygous females typically exhibit 'semisterility' owing to the production of chromosomally unbalanced zygotes that die *in utero* (Beechey, Kirk & Searle, 1980; Searle *et al.* 1983).

The question arises whether the altered chromosome constitution manifests itself exclusively in male gametogenesis or whether the female is also affected, albeit with less severe consequences. Following our finding that the ovaries of immature mice that were heterozygous for the reciprocal autosomal translocation T(11; 19)42H (henceforth called T42H) were smaller than those of their normal litter-mates (Mittwoch, Mahadevaiah & Olive, 1981), we have examined ovarian sizes in two other male-sterile chromosome anomalies, an autosomal non-reciprocal translocation (insertion) Is(7; 1)40H (henceforth called Is40H) and a tertiary trisomy Ts(5^{12})31H (henceforth called Ts31H). Both anomalies result in spermatogenic arrest and a reduction in testis size to about 30% of normal (Searle *et al.* 1978).

The chromosomal insertion Is40H originated from three breaks, one in chromosome

1 and two in chromosome 7, resulting in an inserted chromosome 1 and a deleted chromosome 7 (Searle *et al.* 1983) (Fig. 1). The inserted chromosome 1 appears as a long marker in preparation of mitotic chromosomes. Trisomy Ts31H is a derivative of the T(5; 12)31H reciprocal translocation (Fig. 2). The trisomic karyotype contains the small marker chromosome, 5^{12}, in addition to the normal mouse chromosome complement (Beechey *et al.* 1980).

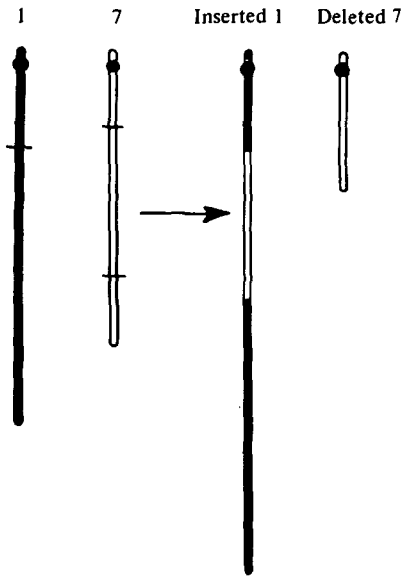

Fig. 1.

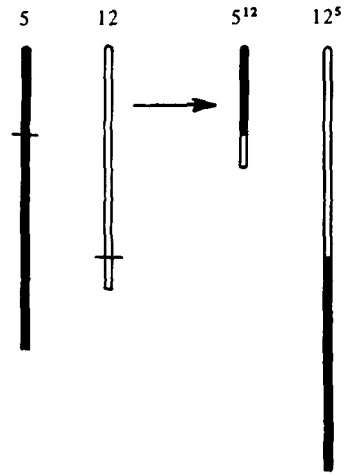

Fig. 2.

Fig. 1. Breakpoints leading to Is(7; 1) 40H insertion and deletion.

Fig. 2. Breakpoints leading to reciprocal translocation T(5; 12)31H. The tertiary trisomic derivative contains chromosomes 5^{12} in addition to the normal complement.

2. MATERIALS AND METHODS

Female mice heterozygous for the Is40H insertion and others carrying the Ts31H trisomy were obtained from the MRC Radiobiology Unit, Harwell.

The insertion stock was maintained by mating males with normal chromosomes but homozygous for alleles of ruby-eye-2 (*ru*-2) and of pink-eyed dilution, (*p*) to Is40H/+ females that carried the same alleles on their normal chromosome no. 7 and the normal alleles on the inserted chromosome. Since the loci are carried on the part of chromosome 7 that is involved in the insertion and since the mutant alleles are recessive, such matings give rise to translocation-carrying mice with black eyes and coats and to mice with normal chromosomes that have red eyes and light coat colour (Green, 1981).

Ts31H females were mated to C3H males. Presence or absence of the trisomic 5^{12} chromosome was assessed either from liver preparations made by a modification of the method of Eicher & Washburn (1978) or from fibroblast cultures, obtained from tail tips, processed by standard techniques.

Ovaries were collected from litters up to 7 days after birth containing at least one female with normal and one with abnormal chromosomes. The mice were weighed, killed by etherization and dissected. Both ovaries were fixed separately in Bouin's solution and prepared for serial sectioning at 7 μm; they were stained in haematoxylin and eosin.

Section areas were measured using a digitizer attached to a Spectrum microcomputer.

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The outlines of sections spanning the entire gonad were traced on the drawing plotter of the digitizer with the aid of a Leitz drawing tube, the areas being automatically calculated by the computer. Ovarian volumes were obtained by multiplying the sum of the areas by the effective thickness between measured sections.

Table 1. *Ovarian volumes and body weights in immature female mice from litters segregating for Is40H insertion*

Litter no.	Age (days)	Chromosomes	Mean ovarian vol. (mm^3)	Body weight (g)	Ovarian vol./body wt. (mm^3/g)
13	6	Is40H/+	0·071	5·38	0·013
		+/+	0·083	5·65	0·015
15	6	Is40H/+	0·063	5·96	0·011
		+/+	0·083	6·21	0·013
16	6	Is40H/+	0·065	5·00	0·013
		Is40H/+	0·066	4·75	0·014
		Is40H/+	0·078	5·34	0·015
		+/+	0·076	4·88	0·016
19	4	Is40H/+	0·065	4·65	0·014
		+/+	0·088	4·74	0·019
26	4	Is40H/+	0·062	3·77	0·017
		+/+	0·073	3·86	0·019
		+/+	0·066	3·69	0·018
27	6	Is40H/+	0·097	4·54	0·021
		Is40H/+	0·076	4·91	0·016
		Is40H/+	0·071	5·17	0·014
		+/+	0·081	4·98	0·016
		+/+	0·101	3·34	0·030
50	7	Ia40H/+	0·083	7·99	0·010
		Is40H/+	0·072	7·91	0·009
		+/+	0·095	7·56	0·013
52	6	Is40H/+	0·060	5·31	0·011
		+/+	0·069	5·41	0·013
		Weighted $\bar{d}$*	0·0125	−0·164	0·00331
		S.E.	0·0021	0·133	0·00066
		t_7	5·93	1·23	5·01
		P	<0·001	>0·2	<0·01

* For explanation see Material and Methods section.

To test the statistical significance of the differences between chromosomally normal and abnormal mice, a weighted t-test was used. For each litter segregating for a chromosomal anomaly, and for each variable, the mean for each chromosomal class (normal and abnormal) was calculated and the mean of the chromosomally abnormal class subtracted from that of the chromosomally normal class. The difference, d, thus obtained was assigned a weight, w, $= n_1 n_2/(n_1+n_2)$, where n_1 and n_2 are the numbers in each of the two chromosomal classes. The weighted mean difference, $\bar{d} = \Sigma wd/\Sigma w$, where Σ = summation over all litters segregating for the same chromosomal anomaly. The standard error, S.E., of the weighted mean was calculated according to the formula

$$\sqrt{\left[\frac{1}{(N-1)\Sigma w}\left(\Sigma wd^2 - \frac{(\Sigma wd)^2}{\Sigma w}\right)\right]},$$

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where N = number of litters. The weighted mean divided by its standard error, with $N-1$ degrees of freedom, was referred to in Student's t table.

3. RESULTS

Ovarian volumes and body weights of Is4OH/+ mice and normal litter mates are shown in Table 1. In all eight litters the mean ovarian volume of insertion carriers is less than that of their normal litter mates. The weighted t-test, with 7 degrees of freedom,

Table 2. *Ovarian volumes and body weights in immature female mice from litters segregating for Ts31H trisomy*

Litter no.	Age (days)	Chromosomes	Mean ovarian vol. (mm^3)	Body weight (g)	Ovarian vol./body wt. (mm^3/g)
17	4	Ts31H	0·038	3·35	0·011
		Ts31H	0·054	3·41	0·016
		Ts31H	0·047	3·34	0·014
		Normal	0·074	3·94	0·019
28	5	Ts31H	0·046	4·03	0·011
		Normal	0·081	4·34	0·019
29	3	Ts31H	0·048	3·15	0·015
		Ts31H	0·045	3·01	0·015
		Normal	0·068	3·10	0·022
31	4	Ts31H	0·073	4·43	0·016
		Normal	0·073	4·27	0·017
34	4	Ts31H	0·050	2·41	0·021
		Ts31H	0·049	3·08	0·016
		Normal	0·081	3·65	0·022
		Normal	0·073	3·21	0·023
35	3	Ts31H	0·028	2·36	0·012
		Normal	0·062	2·96	0·021
		Normal	0·049	2·67	0·018
		Normal	0·049	2·51	0·020
		Weighted $\bar{d}$	0·0238	0·0352	0·0055
		S.E.	0·00426	0·130	0·0010
		t_5	5·58	2·71	5·50
		P	<0·01	<0·05	<0·01

is 5·93, with a significance level below one in a thousand. There is no significant difference in body weight between the two classes of mice. Ratios of ovarian volumes to body weights are also lower in insertion carriers than in normal litter-mates. Estimates of the overall reduction of ovarian volumes and relative ovarian volumes, based on summed mean litter values of Is40H/+ and +/+ mice respectively, come to 16% and 19% respectively.

The corresponding data for Ts31H trisomic mice and their normal litter-mates are shown in Table 2. In five out of six litters, ovarian volumes are lower in trisomic mice than in their normal litter-mates, while in the sixth litter (no. 31) the values are equal. Relative ovarian sizes of trisomics are reduced in all six litters. The mean reduction in ovarian volumes of trisomic mice is 32%, while body weight is reduced by 8% and ovarian volume relative to body weight is reduced by 29%.

4. DISCUSSION

This investigation was undertaken to follow up our previous finding of a reduction in ovarian volume in immature mice heterozygous for the male-sterile reciprocal translocation T42H (Mittwoch *et al.* 1981). The mean ovarian volumes in 3- and 5-day-old mice were 30% less than those of their normal litter-mates, while relative ovarian volumes were reduced by 33%. This was the first indication of an abnormality in females carrying a male-sterile chromosomal rearrangement. The fact that two further male-sterile chromosome anomalies exhibit an essentially similar effect suggests that ovarian involvement is likely to be common in these conditions.

The Ts31H mice, which carry a small additional chromosome and hence have an unbalanced chromosome constitution, also show a slight reduction in body weight, as was described by Beechey *et al.* (1980) for weanlings. However, the reduction of ovarian size is greater than that of body weight (32% compared with 8%), suggesting that this male-sterile anomaly also exerts a specific effect on ovarian development.

Preliminary results obtained in T42H/+ females suggests that the smaller ovarian volumes are related to a reduction in oocyte number (Burgoyne, Mahadevaiah & Mittwoch, unpublished: Burgoyne & Baker, 1984). Additional data on this translocation are being accumulated, and this work will be followed by oocyte counts in Is40H/+ and Ts(5^{12})31H ovaries.

In all three chromosomal anomalies spermatogenic arrest is severe. Spermatocytes in Is40H/+ mice appear to die abruptly in pachytene (Searle *et al.* 1983); in Ts31H all meiotic stages are present but the number of spermatozoa is reduced to less than 1% and the majority are abnormal (Beechey *et al.* 1980); while T42H/+ spermatocytes reach pachytene only (Searle *et al.* 1978). The reason for spermatogenic failure is still problematical. In a study of meiotic prophase in three male-sterile autosomal translocations, Forejt & Gregorová (1977) observed frequent contact of the translocation configuration with the *XY* bivalent and postulated that the proximity of the autosomes interfered with the normal pattern of *X*-chromosome inactivation, a phenomenon that has been regarded as essential for successful spermatogenesis (Lifschytz & Lindsley, 1972). On this hypothesis ovarian development would be expected to be normal, since both *X* chromosomes are active during oogenesis (Epstein, 1969) and, therefore, any contact between *X*-chromosomes and autosomes would not be expected to be deleterious. Miklos (1970) has proposed the general hypothesis that reduction or absence of pairing in meiosis leads to gametogenic breakdown. Burgoyne & Baker (1984) have recently presented evidence in favour of this hypothesis based on data on XO female and sex-reversed mice.

It remains to be established whether the reduction in ovarian size in immature females carrying male-sterile chromosome anomalies is associated with a reduction in the number of oocytes. The likelihood of this is increased by the finding of Searle *et al.* (1983) of signs of a shortening in the reproductive life of females carrying the Is40H insertion. Furthermore, in male carriers of the T42H translocation, testicular size was normal prior to the onset of meiosis (Mittwoch, 1982), suggesting an effect that is confined to meiosis. As a working hypothesis we propose that these chromosome anomalies exert basically similar deleterious effects on meiotic cells of males and females and that different effects on fertility may be related to different physiological conditions prevailing in those cells. Furthermore a small number of surviving oocytes can ensure fertility in females, a situation that is in stark contrast to that in males. In view of the large difference in the numbers of gametes that need to be produced, meiotic requirements may be more stringent in males than in females. As a result, chromosome constitutions that lead to complete breakdown of spermatogenesis may allow a degree of oogenesis to proceed which, even if reduced, is sufficient for reproduction.

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