A reciprocal autosomal translocation which causes male sterility in the mouse also impairs oogenesis

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Summary. A quantitative histological analysis of ovaries from 3- and 5-day-old female mice heterozygous for the male-sterile reciprocal autosomal translocation, T(11;19)42H, revealed a marked reduction (by 65%) in the number of oocytes as compared to controls. These findings call into question the widely held view that chromosomal anomalies causing spermatogenic failure have no effect on oogenesis. It is suggested that during meiosis in males and females there is a mechanism operating which tends to eliminate cells which had incomplete chromosome pairing at the pachytene stage.

Introduction

Heterozygosity for certain reciprocal autosomal translocations is one of a number of chromosomal causes of male sterility in mice (Lyon & Meredith, 1966; Searle, 1982). Females carrying these male-sterile translocations typically have reduced fertility due to death *in utero* of chromosomally unbalanced embryos (de Boer & de Maar, 1976; Beechey, Kirk & Searle, 1980). Although Beechey, de Boer & van der Hoeven (1976) found evidence of premature ovarian failure in a tertiary trisomic, it has generally been assumed that oogenesis is unaffected.

Thus, Forejt (1982) asks “Why is only male fertility affected, while oogenesis is not impaired in the presence of chromosome rearrangement?” However, Mittwoch, Mahadevaiah & Olive (1981) reported that at 3 and 5 days after birth the ovaries of female mice heterozygous for the male-sterile reciprocal autosomal translocation, T(11;19) 42H (hereafter abbreviated T42H), were 30% smaller than those of their chromosomally normal sisters. Subsequently, Mittwoch, Mahadevaiah & Setterfield (1984) have shown that ovarian size is also reduced in 3–7-day-old females carrying two other male-sterile chromosomal anomalies (an insertion and a tertiary trisomy). This has led these authors to question whether oogenesis is unaffected by chromosomal anomalies which cause male sterility. Burgoyne & Baker (1984) also concluded that chromosomal anomalies causing male sterility affect oogenesis, after realizing the parallels between the spermatogenic impairment in sterile XO *Sxr* male mice (Cattanach, Pollard & Hawkes, 1971) and the oogenic impairment in XO female mice (Burgoyne & Baker, 1985).

In the present paper we show that the reduced ovarian size in T42H/+ female mice is associated with a marked reduction in the number of oocytes.

Materials and Methods

The ovaries analysed in this study were T42H/+ and +/+ ovaries for which ovarian volume data had been collected by Mittwoch *et al.* (1981) together with ovaries from an additional 3-day-old T42H/+ with a +/+ sib pair. All ovaries had been fixed in Bouin’s fixative, embedded in paraffin

Table 1. Ovarian volumes, tissue volumes and numbers of oocytes for +/+ and T42H/+ ovaries of mice at 3 and 5 days *post partum*

Litter no.	Age (days)	Mouse no.	Genotype	Independent* estimates	Distributed point count estimates			
				Ovarian vol. (mm ³ × 10 ⁻⁵)	Ovarian vol. (mm ³ × 10 ⁻⁵)	Somatic tissue vol. (mm ³ × 10 ⁻⁵)	Germinal tissue vol. (mm ³ × 10 ⁻⁵)	No. of oocytes
4	5	16	+/+	7000	7044	5863	1181	6779
		15	T42H/+	5140	5046	4521	525	1990
7	5	26	+/+	6530	6825	5119	1706	10689
		25	T42H/+	4720	4638	3967	671	3622
10	5	33	+/+	8630	8006	6912	1094	7732
		34	+/+	10590	10806	8837	1969	10428
		32	T42H/+	6750	7284	6168	1116	1728
12	5	38	+/+	15280	15444	12644	2800	11310
		39	T42H/+	7810	7350	6606	744	2426
13	3	41	+/+	4980	5469	3544	1925	10623
		40	T42H/+	3950	4091	3282	809	3261
		43	T42H/+	4890	5228	4287	941	4404
24	3	72	+/+	5440	5753	4769	984	4768
		73	T42H/+	5030	5403	4353	1050	(5476)† 4966 (5460)†
112	3	277	+/+	5340	5228	4178	1050	5657
		278	T42H/+	3270	3675	3252	423	2219
Mean +/+‡				7740	7881	6284	1597	8415
Mean T42H/+‡				5306	5437	4664	772	2977
Mean weighted difference (<i>d</i>)				2370	2358	1541	817	5581
s.e. <i>d</i>				874	944	756	243	1118
<i>d</i> /s.e. <i>d</i> = <i>t</i> ₆				2.71	2.50	2.04	3.36	4.99
<i>P</i> (two-tailed)				0.05-0.01	0.05-0.01	0.1-0.05	0.05-0.01	0.005-0.001

* Independent estimates of ovarian volume derived from section area measurements made using a modified Whipple eyepiece (Mittwoch *et al.*, 1981) or a digitiser table (Mice 277 and 278).

† Oocyte numbers estimated by reconstruction.

‡ Means of litter means.

wax, serially sectioned at 7 µm and stained with haematoxylin and eosin. Since differences in ovarian volume between mice and between genotypes greatly exceed differences within mice (left ovary versus right ovary) it was decided to analyse only one ovary from each mouse.

The methods used for the estimation of tissue volumes and numbers of oocytes for each ovary were those described by Burgoyne & Baker (1985). Briefly, the volume of somatic and of germinal tissue was estimated using an evenly distributed point count. During this sampling procedure an estimate of the oocyte size distribution was obtained, enabling a mean oocyte volume to be calculated. Dividing the germinal volume by the mean oocyte volume estimates the number of oocytes in that ovary.

In addition, the oocyte numbers for two ovaries (from Mice 72 and 73) were estimated by reconstruction. All sections were photographed and oocyte outlines traced from the prints on acetate sheets. By superimposing acetate sheets, oocytes appearing in more than one section could be identified.

Because there is considerable variation between litters, comparisons were made between genotypes within litters. For each litter the difference (*d*) between the +/+ and T42H/+ means

Text-fig. 1. Oocyte size distributions for +/+ and T42H/+ ovaries at 5 and 3 days *post partum* as estimated by the distributed point count method of Burgoyne & Baker (1985).

was calculated, and then assigned a weight according to the formula $w = n_1 n_2 / (n_1 + n_2)$ where n_1 and n_2 are the numbers of +/+ and T42H/+ mice in the litter. The mean weighted difference, $\bar{d} = \Sigma wd / \Sigma w$, and its standard error, $\text{s.e. } \bar{d} = \sqrt{\left[\frac{1}{(N-1)\Sigma w} \left(\Sigma wd^2 - \frac{(\Sigma wd)^2}{\Sigma w} \right) \right]}$, where N is the number of litters.

Results

Table 1 summarizes the results from the quantitative histological analysis of T42H/+ and +/+ ovaries. Also included in the table are the oocyte counts obtained by reconstruction for Mice 72 and 73, and independent estimates of ovarian volume obtained (except for those for litter 112) in the course of the study by Mittwoch *et al.* (1981). The ovarian volume estimates obtained by the

'distributed point-count method' described by Burgoyne & Baker (1985) are in good agreement with the independent estimates. The T42H/+ ovaries are estimated to be 31% smaller than +/+ ovaries by both methods.

The histological analysis reveals that the smaller size of T42H/+ ovaries is due to a reduction in both somatic tissue volume (by 26%) and germinal tissue volume (by 52%) although the reduction in somatic tissue is not quite significant. When the amount of germinal tissue is expressed as the number of oocytes, the reduction is even greater (65%) because the mean oocyte size is greater in T42H/+ than in +/+ ovaries. The reason for this increased mean oocyte size is apparent in Text-fig. 1, which gives the estimated distribution of oocyte size in T42H/+ and +/+ ovaries at 3 and 5 days. Although the pool of small oocytes is severely reduced in T42H/+ ovaries, the population of growing oocytes is normal with respect to both the size distribution and number of oocytes. At 5 days after birth, when the growing oocytes are clearly demarcated (diameter > 20 µm), there is an average of 587 growing oocytes in T42H/+ ovaries and 599 in +/+ ovaries.

The oocyte counts obtained by reconstruction for the ovaries from Mice 72 and 73 are higher than the estimates obtained from the histological analysis. It is not clear which figures are closer to the true values, since reconstruction can overestimate oocyte numbers when no allowance is made for section distortion (see Burgoyne & Baker, 1985). This pair of ovaries is unusual in not showing the otherwise consistently large difference in oocyte numbers between +/+ and T42H/+. This is mainly due to a particularly low oocyte count in the control ovary.

Discussion

The ovaries of mice increase in size rapidly in the days after birth (Burgoyne & Baker, 1985) due to the growth of a subset of the oocytes and the proliferation of the follicle cells which surround them. This establishes the 'growing population' of oocytes, which is continuously supplemented by growth of oocytes from the 'pool', and is depleted by atresia and eventually by ovulation.

The reduced ovarian volume seen postnatally in XO mice (Burgoyne & Baker, 1985) is due in part to a delay in the establishment of the 'growing population', which is a reflection of the overall retardation of XO mice, and in part to a deficiency of 'pool' oocytes. The results obtained here show that in T42H/+ mice the establishment of the growing population of oocytes is normal, and that the reduced ovarian volume is due entirely to a marked deficiency of 'pool' oocytes and their enveloping follicle cells. We have therefore established that heterozygosity for T42H, which results in spermatogenic arrest in males, leads to a 65% reduction in the number of oocytes in females aged 3–5 days. From work in progress it is clear that the reduced volume of Ts(5¹²)31H/+ ovaries (Mittwoch *et al.*, 1984) is also associated with oocyte deficiency (L. A. Setterfield & U. Mittwoch, unpublished), so we are confident that impaired oogenesis will prove to be a feature of female carriers of other male-sterile chromosomal anomalies.

Although females affected the gametogenic failure is less severe than in males. In considering why this should be we will compare XO female with XO *Sxr* male mice, and T42H/+ females with T42H/+ males.

In XO females and XO *Sxr* males the initial phases of gametogenic impairment are similar in that, despite considerable losses at the pachytene stage, many cells reach diplotene. In XO females, those oocytes that reach the diplotene stage are subsequently depleted at normal rates (Burgoyne & Baker, 1981), can complete meiosis, and produce normal numbers of eggs per cycle (Morris, 1968), although the shortage of oocytes does result in a shortened reproductive lifespan (Lyon & Hawker, 1973). However in XO *Sxr* males the second meiotic division is frequently omitted so that as many as 92% of spermatids may be diploid (Burgoyne & Baker, 1984; E. R. Levy & P. S. Burgoyne, unpublished data). Many of these spermatids degenerate and spermiogenesis generates only a few very abnormal spermatozoa with consequent sterility. As discussed by Burgoyne & Baker (1984) a mechanism which removes meiotic cells carrying chromosomes that were unpaired or incompletely

paired during pachytene (Miklos, 1974) may explain the gametogenic impairment in these XO male and female mice with unpaired X chromosomes. The more complete failure in the male might be due to the complexities of spermiogenesis, or perhaps the meiotic arrest at diplotene during oogenesis allows time for damage to be repaired.

Although studied in less detail, T42H/+ females are essentially similar to XO females in that a proportion (35% of normal) of oocytes reach diplotene and these diplotene oocytes function normally. However, T42H/+ males, in contrast to XO *Sxr* males, show a very abrupt and complete arrest in mid-pachytene, no diplotene spermatocytes being formed (de Rooij, 1980). This suggests that, in addition to the losses which may be a consequence of the incomplete pairing in the translocation quadrivalent, another factor must be operating which causes further problems at the pachytene stage. Forejt (1982) has been a strong proponent of the idea that inactivation of the X chromosome in early pachytene is a prerequisite for spermatogenesis (Lifschytz & Lindsley, 1972) and that rearranged autosomes by association with the unpaired part of the X can interfere with this process. In XO *Sxr* males there is nothing to interfere with the inactivation of the X chromosome, but in T42H/+ males 88% of pachytene spermatocytes show univalent or multivalent contact with the XY body ('sex vesicle') (de Boer, Searle, van der Hoeven & Beechey, 1980).

In conclusion, we have shown that oogenesis is affected by chromosomal anomalies which cause male sterility. We suggest that in males and females a mechanism is operating which removes cells carrying chromosomes that were unpaired or incompletely paired at the pachytene stage. In males, chromosomal anomalies may cause additional damage during pachytene by interfering with the normal silencing of the X chromosome.

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