Oocyte depletion in XO mice and their XX sibs from 12 to 200 days *post partum*

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Summary. The total number of oocytes (healthy and atretic) in XO and XX mice at ages ranging from 12 to 200 days after birth was determined using a quantitative histological technique. For each oocyte the stage of the enveloping follicle was recorded. The results demonstrated that throughout the period studied XO mice had approximately half the number of oocytes found in XX mice. No increase in the incidence of atresia was detected in XO ovaries. In XO mice at 12 days of age the establishment of the population of growing follicles was clearly retarded.

Introduction

Ohno (1969) has presented evidence that the genetic content of the X chromosome has been conserved during the evolution of mammals. One consequence of such genetic conservation should be that X chromosome monosomy will be associated with the same primary defects in all mammalian species. In apparent contradiction to this was the discovery that adult XO mice have histologically normal ovaries and are fertile (Welshons & Russell, 1959; Cattanach, 1962) whereas XO (Turner's syndrome) women are almost always infertile with the ovaries represented by connective tissue streaks (Simpson, 1976). This seemingly major disparity between the XO phenotype of the two species was reduced when Singh & Carr (1966) reported that oocytes are found in the ovaries of XO human abortuses (germ cell loss occurring in the second half of pregnancy), and Lyon & Hawker (1973) showed that XO mice become sterile earlier than their XX sibs. Lyon & Hawker (1973) suggested that the premature sterility of XO mice was due to the exhaustion of a reduced oocyte pool. The present study investigated this suggestion and the extent and timing of oocyte loss.

Materials and Methods

The material consisted of serial sections of 40 ovaries from XO mice and 40 ovaries from XX mice. The mice comprised 4 sib pairs at each of the ages 12, 25, 50, 100 and 200 days. The mice were derived as follows. Firstly, stud males carrying the X inversion In(X)1H (Evans & Phillips, 1975) were mated to outbred Schofield albino females (S. Schofield & Co., Delph, nr Oldham, Lancs). The heterozygous In(X)/X female offspring were subsequently mated to Schofield albino males to provide litters in which about 25% of the females are XO (Phillips, Hawker & Moseley,

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1973). Males were culled from the litters at birth. The females of each litter were kept caged together until they were weighed, killed and karyotyped (using bone marrow cells). One XO (if present) and one XX female were randomly selected from within each litter; the ovaries were fixed in aqueous Bouin, processed for histology, serially sectioned at 6 μ m, and stained with haematoxylin and eosin. All the sectioned material was coded and randomized before counting. The procedures for counting oocytes, classifying follicles and assessing atresia were essentially those of Mandl & Zuckerman (1950) and have been described by Baker, Challoner & Burgoyne (1980). For the present work a section interval of 1 in 10 was used.

Ovaries were also obtained and processed from some XO and XX mice aged 400 days to provide material for a qualitative assessment of longer term changes in the XO ovary.

Table 1. Total numbers of oocytes, together with their distribution between pool (stage I) and growing (stages II–VI) follicles, for XO and XX mice at 12, 25, 50, 100 and 200 days after birth

Age (days)	Sib pair	XX mice			XO mice		
		Pool (I)	Growing (II–VI)	Total	Pool (I)	Growing (II–VI)	Total
12	1	23 040	2500	25 540	15 150	2210	17 360
	2	19 460	1530	20 990	6170	1710	7880
	3	20 550	2170	22 720	9850	1780	11 630
	4	15 740	2450	18 190	9050	2070	11 120
Mean		19 698	2162	21 860	10 055	1942	11 998
$\pm$ s.e.m.		± 1517	± 223	± 1541	± 1873	± 118	± 1971
25	1	14 950	2500	17 450	12 960	1580	14 540
	2	10 240	1710	11 950	8290	1410	9700
	3	15 410	2620	18 030	8000	1720	9720
	4	9110	1940	11 050	9880	2120	12 000
Mean		12 428	2192	14 620	9782	1708	11 490
$\pm$ s.e.m.		± 1608	± 219	± 1814	± 1137	± 151	± 1151
50	1	8060	2770	10 830	6120	2500	8620
	2	9080	2520	11 600	2040	1540	3580
	3	7860	3150	11 010	3050	3000	6050
	4	15 200	2660	17 860	3050	1510	4560
Mean		10 050	2775	12 825	3565	2138	5702
$\pm$ s.e.m.		± 1737	± 135	± 1686	± 884	± 368	± 1097
100	1	12 200	2700	14 900	4720	1860	6580
	2	9260	3120	12 380	2210	2090	4300
	3	13 760	2690	16 450	3380	1980	5360
	4	6490	1800	8290	4310	2250	6560
Mean		10 428	2578	13 005	3655	2045	5700
$\pm$ s.e.m.		± 1610	± 278	± 1781	± 557	± 83	± 547
200	1	2170	1530	3700	1*	3*	4*
	2	2970	1280	4250	2670	850	3520
	3	3280	2000	5280	990	920	1910
	4	6270	1760	8030	1430	1060	2490
Mean		3672	1642	5315	1272	708	1981
$\pm$ s.e.m.		± 897	± 154	± 962	± 553	± 229	± 738

* These are total counts instead of estimates based on counts of every 10th section.

Results

Our observations on ovarian histology fully accord with those of Lyon & Hawker (1973), the ovaries of XO mice being abnormal only in that they have the appearance of older XX ovaries.

The data obtained on oocyte numbers are summarized in Table 1. With the exception of sib pair 4 at 25 days, all ovaries from XO mice had fewer oocytes than did those from their XX sibs.

The large differences between females of the same genotype within an age group preclude any accurate estimates of mean oocyte numbers at specific ages. Examination of XO–XX sib pair differences revealed similar variability so that mean differences are also poorly estimated. However, it has been shown previously (Jones & Krohn, 1961) that the oocyte depletion curve gives a good fit to a straight line if oocyte numbers are expressed as logarithms. Accordingly the present XX and XO data were log-transformed and regression lines fitted (Text-fig. 1). The fit to a straight line was satisfactory for both genotypes (XX, $r = 0.85$; XO, $r = 0.84$). Comparison of the regression lines (Snedecor & Cochran, 1967) revealed that (i) the two slopes did not differ, and (ii) the XX regression line was significantly higher than the XO regression line ($P < 0.005$). Thus the analysis demonstrates that XO mice have fewer oocytes than XX mice, but during the period studied the rate of oocyte loss is the same as for XX mice on a proportional basis. The latter observation is supported by our finding that the rates of atresia were the same for the two genotypes (Table 2). From the difference in the heights of the regression lines it can be calculated that the XO mice had only 53% of the oocyte population of their XX sibs. However, the extent of the reduction varied widely from one XO mouse to another (Table 3).

Text-fig. 1. The relationship between age and log total numbers of oocytes for XO (●) and XX (□) mice. One point (0.6, 200 days) has been excluded because it was an outlier ($P < 0.01$; see p. 157 Snedecor & Cochran, 1967). The regression equations were (a) XO, $y = -0.00340x + 4.07$, and (b) XX, $y = -0.00286x + 4.30$.

Since oocytes were classified with respect to the stage of development of the enveloping follicles, the relative proportions of the different follicular stages could be compared for the two genotypes. The data on pool sizes (stage I follicles) and on the numbers of growing follicles (stages II–VI) were unremarkable in the light of the observations on total oocyte numbers, both being reduced in XO mice (Table 1). The reduction was proportionately greater for the pool than

Table 2. The mean percentage atresia ($\pm$ s.e.m.) for pool (stage I) and growing (stages II–VI) follicles in XO and XX mice

Age (days)	Follicular stages			
	Pool (I)		Growing (II–VI)	
	XX	XO	XX	XO
12	43.3 $\pm$ 0.5	42.8 $\pm$ 0.8	22.6 $\pm$ 2.8	23.2 $\pm$ 1.8
25	39.0 $\pm$ 0.5	40.4 $\pm$ 1.3	26.0 $\pm$ 1.6	28.6 $\pm$ 4.6
50	38.8 $\pm$ 0.6	38.6 $\pm$ 1.0	30.5 $\pm$ 1.3	36.3 $\pm$ 3.8
100	40.0 $\pm$ 1.1	40.4 $\pm$ 1.4	30.6 $\pm$ 2.7	30.2 $\pm$ 1.6
200	38.4 $\pm$ 1.0	33.6 $\pm$ 3.5	33.3 $\pm$ 2.0	27.6 $\pm$ 1.9

Table 3. The number of oocytes in individual XO mice as a percentage of the mean number of oocytes in XX mice of the same age

Age (days)	No. of oocytes (XO) Mean no. of oocytes (XX) $\times$ 100 (%)			
12	79.4	36.0	53.2	50.9
25	99.5	66.3	66.5	82.1
50	67.2	27.9	47.2	35.6
100	50.6	33.1	41.2	50.4
200	(0.1)*	66.2	35.9	46.8

* This mouse should perhaps be excluded on statistical grounds. See Text-fig. 1.

for growing follicles. Comparisons (using the original counts) of the distribution of oocytes among the follicular stages II–VI revealed some important differences between XO and XX mice. At 12 days the χ^2 value was highly significant ($P < 0.0005$), due to major differences in the proportions of follicles at stages II, IV and V + VI (pooled because of small numbers). These differences represent a retardation of follicular development in XO mice (Table 4). The χ^2 values were also significant ($0.001 < P < 0.005$) for the 25- and 100-day groups, and in both this was due to an increased proportion of stage V follicles in the XO individuals (Table 4).

Table 4. The mean number of oocytes in follicular classes II–VI in XO and XX mice aged 12–200 days

Age (days)	Genotype	Follicular class				
		II	III	IV	V	VI
12	XO	1330.0	477.5	117.5	17.5	0.0
	XX	1160.0	522.5	357.5	107.5	1.5
25	XO	927.5	325.0	125.0	277.5	52.5
	XX	1310.0	425.0	182.5	205.0	72.5
50	XO	1125.0	430.0	252.5	245.0	85.0
	XX	1607.5	460.0	305.0	315.0	87.5
100	XO	1230.0	357.5	152.5	207.5	97.5
	XX	1677.5	385.0	225.0	150.0	140.0
200	XO	430.0	120.0	35.0	77.5	47.5
	XX	1132.5	205.0	107.5	127.5	70.0

Discussion

The results obtained in the present study demonstrate that the oocyte population is markedly reduced (approximately halved) in XO mice, thus confirming the suggestion made by Lyon & Hawker (1973). This reduction has already occurred by 12 days *post partum* (the earliest age studied). At 12 days *post partum* follicular development is retarded, but in all other respects follicular relationships appear to be normal in XO mice. Thus follicular atresia is not increased in XO mice and oocytes are depleted at the same rate as in controls (i.e. the same constant proportion of the total is lost per unit time). Furthermore, the finding that the reduction in pool size in XO mice is not paralleled by an equivalent reduction in the number of growing follicles is in line with the compensation that occurs as pool size decreases with age in control mice. The same phenomenon is also observed following unilateral ovariectomy (Baker *et al.*, 1980).

It is useful to compare the extent and timing of oocyte depletion in the XO mouse with what is known for the XO human. The ovaries are of normal appearance in XO human embryos, a decline in germ cell numbers occurring in the second half of pregnancy (Singh & Carr, 1966; Carr, 1972). The extent of oocyte loss is variable, some XO infants having histologically normal ovaries while others have ovarian 'streaks' which are devoid of oocytes (Carr, Haggard & Hart, 1968). In XO women, primary amenorrhoea is usual (97% of cases: Simpson, 1976) but menstruation and pregnancy have been reported (King, Magenis & Bennett, 1978).

Clearly, the reduction in oocyte numbers is much more severe in XO women than in XO mice. Nevertheless, a few XO women have functional ovaries containing oocytes. It is invariably assumed that the surviving oocytes in these cases are XX. Thus a paper describing a fertile XO woman (Nakashima & Robinson, 1971) is followed by the editorial comment: "The theoretical explanation is simple . . . Such a patient must be an example of undetected mosaicism with a normal cell line of 46,XX in the germ cells." An alternative explanation follows from the current finding that there is considerable variation in the extent of loss (Table 3) and that those oocytes which survive until 12 days *post partum* are depleted at the normal rate. For the human XO variability in the extent of loss is suggested by the variable ovarian phenotype of XO infants, and if normal depletion rates occur subsequently there may occasionally be XO oocytes remaining at puberty. It is perhaps relevant that the prognosis for pregnancy outcome in XO or XO mosaic mothers is very poor, 33 out of 48 reported pregnancies ending in spontaneous abortion, stillbirth, neonatal death or congenital abnormality (King *et al.*, 1978). This poor prognosis is consistent with that expected for XO oocytes, since any XO fetuses produced should almost always abort, and further pregnancy losses should accrue as a result of the poor quality of XO oocytes (Burgoyne & Biggers, 1976).

The loss of XO germ cells in the human XO is said to occur throughout the period when oogonia are entering meiosis (Carr, 1972). The present data for the mouse demonstrate that the loss occurs before 12 days *post partum*. Furthermore, the retardation of follicular development at 12 days might indicate some delay in the meiotic process since follicular growth must await the completion of the first stages of meiosis. A qualitative assessment of XO and XX fetal mouse ovaries (A. McLaren & P. S. Burgoyne, unpublished) has revealed that meiosis is indeed retarded in XO fetuses.

In conclusion, the present study has confirmed that oocyte loss is a consequence of X chromosome monosomy in the mouse, just as it is in man. We believe that studies of XO mouse ovaries from the fetal meiotic period, which are now in progress, will provide further insights into the aetiology of XO gonadal dysgenesis in man.

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