Journal of Reproduction and Fertility (1999) **115**, 263–267

Developmental retardation of XO mouse embryos at mid-gestation

H. Ishikawa[1], M. Banzai[2] and T. Yamauchi[1]

[1]*Department of Public Health, School of Medicine, Mie University, Tsu, 514-8507, Japan; and* [2]*Department of Obstetrics and Gynecology, Saiseikai Yamagata Hospital, Yamagata, 990-8545, Japan*

It is not known why XO mouse embryos, which develop more slowly than XX embryos until early mid-gestation, reach the same stage in their growth and development as their XX littermates at the mid-gestation stage. It is hypothesized that there is an effect of 'litter size' that causes an acceleration of the development of XO embryos at mid-gestation. The present study was performed to determine whether the development of XO embryos is retarded compared with that of their XX littermates at early mid-gestation (day 8 of gestation), before reduction of litter size. The percentage of pre-somite stage XO embryos was greater than the percentage of pre-somite stage XX embryos, and the mean number of somites was greater in XX embryos than it was in XO embryos. These findings indicate that the development of XO embryos was retarded when compared with that of their XX littermates at early mid-gestation. This result is discussed with respect to the compensatory development of XO embryos at mid-gestation and the reduction of litter size shortly after early mid-gestation.

Introduction

In humans, about 99% of XO conceptuses die before birth, and XO individuals with Turner syndrome usually have a variety of morphological abnormalities and are sterile. However, XO mice do not have any external or visceral malformations, and are fertile (Cattanach, 1962; Epstein, 1986; Endo and Watanabe, 1989). Recent studies have revealed developmental problems of XO mice (Burgoyne *et al.*, 1983a,b; Hunt, 1991; Banzai *et al.*, 1995). The XO mouse embryos (having a paternally derived X chromosome) described by Burgoyne *et al.* (1983b) were already lagging behind their XX siblings in development by day 7.25 of gestation. This lag continued until day 10.5, and then there was evidence of catch-up growth between day 10.5 and day 12.25. However, this catch-up was not maintained, which is in keeping with the finding that XO mice are underweight at birth (Burgoyne *et al.*, 1983a). By contrast, the growth and development of XO mouse embryos (having a paternally derived X chromosome) of our colony progressed more slowly than those of their XX littermates at the pre-implantation stage (Banzai *et al.*, 1995), but afterwards, XO embryos were not retarded compared with their XX littermates at either day 10.0 of gestation (Omoe and Endo, 1993) or near term (Endo and Watanabe, 1989). There is little information about catch-up growth of XO embryos but an important factor may be the effect of litter size on development of XO embryos. The fact that there is an inverse relationship between fetal mass and litter size (number of live embryos per dam) in rodents is widely recognized (King, 1915; Barr *et al.*, 1970; Benson and Morris, 1971; Smart *et al.*, 1972; Cowley *et al.*, 1989; Leamy, 1992; Romero *et al.*, 1992). In previous observations at days 10.0 and 17.0 of gestation, the litter size of XO females was about 7.0 (Endo and Watanabe, 1989; Omoe and Endo, 1993), whereas the litter size at day 8 of gestation was about 13.0, which was almost the same as that at the preimplantation stage (Banzai *et al.*, 1995; Ishikawa and Endo, 1998).

The present study was undertaken to examine whether, at early mid-gestation before the reduction of litter size, XO mouse embryos with a paternally derived X chromosome have the potential to catch up with XX littermates.

Materials and Methods

All mice used in this study were obtained from our XO mouse colony, established 13–16 generations ago from XO offspring of Jcl/ICR females exposed to X-rays after copulation (Endo and Watanabe, 1989). The XX and XO karyotypes of the female offspring were diagnosed by cytogenetic analysis of peripheral lymphocytes from the tail vein (Endo and Watanabe, 1988). The animals were maintained at a room temperature of 23 ± 2°C at 50 ± 10% relative humidity with a 12 h light:12 h dark cycle (lighting 16:00–04:00 h). Virgin XO and XX mice (3–6 months old) were mated with males of the same colony for 1 h (13:00–14:00 h). Short-time mating (1 h) was used to minimize interlitter variability. Matings were confirmed by the presence of a vaginal plug at the end of the mating period (plug positive = day 0 of gestation), and the middle of the mating period was considered the point of fertilization.

Mice were killed by cervical dislocation at 192 h (day 8 of gestation) after mating and the reproductive tracts were removed. Conceptuses were dissected from each uterus and transferred to a watch glass containing Hanks' solution

Received 3 June 1998.

© 1999 Journals of Reproduction and Fertility Ltd
0022–4251/99 $15.00

Downloaded from Bioscientifica.com at 03/13/2022 08:25:42PM
via free access

(Nissui Inc., Tokyo). Embryos were dissected from the decidual tissue at ×20 magnification under a dissecting microscope, and the presence or absence of somites was recorded for each embryo.

Embryos were then prepared cytogenetically according to the method of Evans *et al.* (1972) with minor modifications. Embryos were incubated in Hanks' solution containing 0.1 μg colcemid ml^{-1} (Gibco BRL Products, Tokyo) for 120 min at 37°C, transferred to individual tubes of pre-warmed (37°C) 1% (w/v) sodium citrate solution, and then kept in this hypotonic solution for 7 min and fixed in 3:1 methanol:glacial acetic acid. The cells were dissociated in 50% acetic acid. Two slides were made from each embryo. Slides were air-dried overnight and stained with 5% (v/v) Giemsa.

For each embryo, the number of somites was first counted, after which the chromosomes in metaphase were counted. Twenty well-spread metaphases from each embryo were examined for diagnosis of chromosome constitution. When the chromosome number was 40, sex was diagnosed by the presence or absence of the Y chromosome. In our XO colony (Jcl/ICR strain), the Y chromosome is identified easily by its morphological characteristics using conventional Giemsa staining. XO embryos were diagnosed when the chromosome number of embryos was 39 and all metaphase sets were found to be Y chromosome negative. Data were compared by either unpaired Student's *t* test, Mann–Whitney U test, Wilcoxon signed ranks test or chi-squared test, as appropriate. Correlation analysis was performed using Pearson's correlation test.

Results

The reproductive parameters in the XO and XX groups were compared (Table 1). The number of corpora lutea was significantly higher in XO mice compared with XX mice ($P < 0.01$) and the number of live embryos per dam in XO mice was significantly different from that of XX mice ($P < 0.05$).

The morphological stages of counterpart embryos from XO and XX dams, or among XY, XX and XO embryos from XO dams, were compared (Table 2). In XO mice, the percentages of pre-somite (underdeveloped) XY and XX embryos were significantly greater than those of the XX group ($P < 0.001$), and the mean number of somites for XY and XX embryos in XO mice was significantly lower than those of their counterparts in the XX mice group ($P < 0.001$). In the XO mice group, the proportion of pre-somite XO embryos was greater than it was in the group XY and XX embryos, and the mean number of somites in XO embryos (3.0) was significantly lower than it was in XY (4.3) and XX (4.0) embryos. Furthermore, in both the XX and XO mice groups, the mean number of somites in XX embryos tended to be lower than it was in XY embryos.

Table 1. Reproductive characteristics in XX and XO mice

	XX mice	XO mice
Number of dams examined	17	40
Mean ± SD number of corpora lutea*	16.2 ± 1.3	18.8 ± 3.2[a]
Mean ± SD number of live embryos*	14.4 ± 1.5	11.7 ± 4.1[b]

*Litter basis.
[a]$P < 0.01$, [b]$P < 0.05$ compared with each counterpart in the XX mice group by Mann–Whitney U test.

The differences between the mean number of somites of XX and XO embryos within the same litter were calculated and compared (Table 3). In almost all litters, the average number of somites in XX embryos exceeded the average number of somites in XO embryos. Again, the mean number of somites in XO embryos was significantly lower than it was in XX embryos.

The regression line between the mean somite numbers of embryos of the various genotypes and the litter size is shown (Fig. 1). The slope of the line is –0.344 (95% confidence interval ranging from –0.655 to –0.032) and there was a significant correlation ($r = -0.57$; $P = 0.033$). There was no significant relationship between litter size and the proportion of XO embryos ($r = 0.13$, $P = 0.67$) (Fig. 2).

Table 2. Comparison of developmental difference of the XX, XO and XY embryos between XX and XO dams

Group	XX mice	XO mice
Number of embryos examined	230	420
Mean ± SD number of somites*		
40,XY	6.3 ± 1.8[a] ($n = 105$)	4.3 ± 2.0[bc] ($n = 92$)
40,XX	5.8 ± 1.7 ($n = 94$)	4.0 ± 1.7[bd] ($n = 97$)
39,XO		3.0 ± 1.5 ($n = 23$)
Percentage of embryos with pre-somite		
40,XY	12.5 ($n = 15$)	42.5[ef] ($n = 68$)
40,XX	14.6 ($n = 16$)	52.0[e] ($n = 105$)
39,XO		60.3 ($n = 35$)

*Fetal basis, excluding pre-somite.
[a]$P < 0.05$ compared with XX embryos in the XX mice group by unpaired *t* test.
[b]$P < 0.001$ compared with each counterpart in the XX mice group by unpaired *t* test.
[c]$P < 0.01$, [d]$P < 0.05$ compared with XO embryos in the XO mice group by unpaired *t* test.
[e]$P < 0.0001$ compared with each counterpart in the XX mice group by chi-squared test.
[f]$P < 0.05$ compared with XO embryos in the XO mice group by chi-squared test.

Downloaded from Bioscientifica.com at 03/13/2022 08:25:42PM
via free access

Table 3. Comparison of mean number of somites of XX and XO embryos at day 8 of gestation in XO dams

	XX embryos		XO embryos		
Dam* number	Number	Mean number of somites	Number	Mean number of somites	d[a]
1	1	2.0	2	3.5	−1.5
2	2	3.5	1	3.0	0.5
3	2	2.5	1	1.0	1.5
4	5	6.6	1	4.0	2.6
5	6	3.8	2	3.5	0.3
6	3	5.7	3	4.3	1.4
7	5	3.4	1	1.0	2.4
8	4	2.0	4	2.0	0
9	2	4.0	1	2.0	2.0
10	1	5.0	1	2.0	3.0
11	3	2.0	1	1.0	1.0
12	4	5.0	2	3.5	1.5
13	2	4.0	1	1.0	3.0
14	4	4.3	1	3.0	1.3
	Mean ± SD	3.84 ± 1.42	Mean ± SD	2.49 ± 1.20[b]	

d: difference between XX and XO groups; [a]$P < 0.01$ by Wilcoxon signed ranks test.
[b]$P < 0.05$ compared with XX embryos group by Mann–Whitney U test.
*These XO dams had XX, XO and XY embryos at somite stage and were selected for this analysis from all dams studied.

Discussion

In the present experiment, XO mouse embryos were retarded compared with their XX littermates at day 8 of gestation. This finding is in agreement with the results of Burgoyne *et al.* (1983b) who compared the growth and development of XO and XX embryos from day 7 to day 18 of gestation by measuring either egg cylinder volume or body weight, and concluded that, at day 7.25 of gestation, XO egg cylinders were retarded in development compared with XX egg cylinders, and that this lag in development remained until day 10.5. By day 12.25 of gestation, there was a considerable degree of 'catch-up', but this was not fully maintained. The present authors examined the growth and development of XO embryos by several different parameters from the parameters used by Burgoyne *et al.* (1983b) (Omoe and Endo, 1993) and stated that "... no delay in development was detected in XO embryos". However, more detailed analysis of the data in that paper revealed that some parameters of growth and development tended to indicate less development in XO embryos than in XX embryos, although the difference was not significant, and there was still some degree of 'delay' at day 10 of gestation. In addition to accelerated growth, two alternative explanations for the

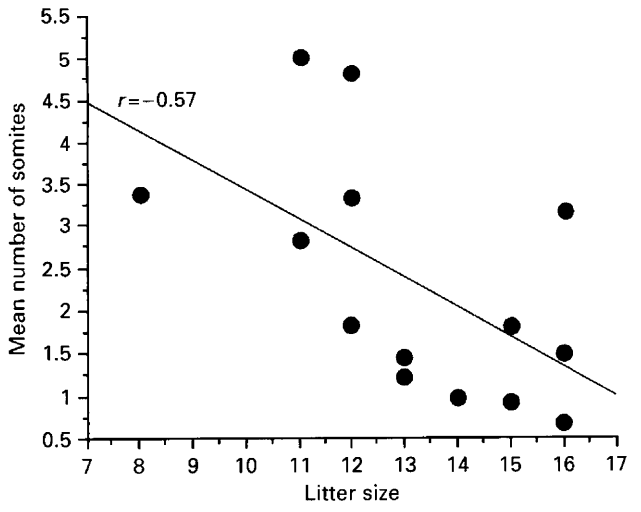

Fig. 1. Correlation of the mean number of somites and litter size in XO dams at day 8 of gestation. These XO dams had XX, XO and XY embryos at somite stage and were selected for this analysis from all dams.

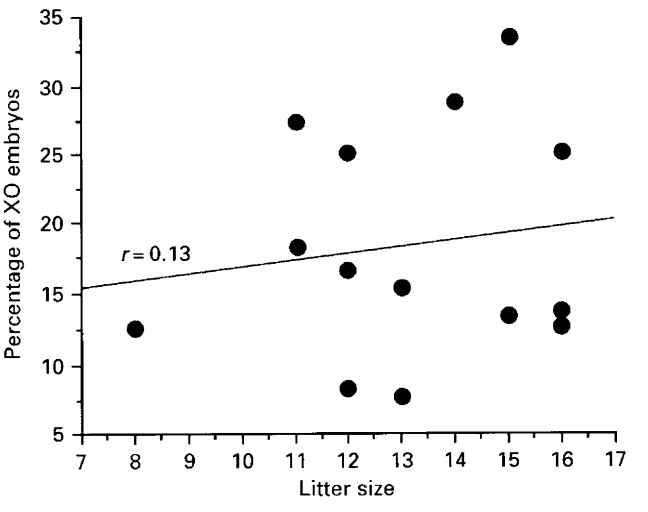

Fig. 2. Correlation of the percentages of XO embryos and litter size in XO dams at day 8 of gestation. These XO dams had XX, XO and XY embryos at somite stage and were selected for this analysis from all dams.

Downloaded from Bioscientifica.com at 03/13/2022 08:25:42PM
via free access

observation that XO embryos showed little delay at day 10 of gestation are: (1) that the XO embryos are less delayed in our XO colony and a small delay is more difficult to identify at day 10 than at day 8; and (2) that the most retarded XO embryos are eliminated so that they are no longer found at day 10. There was not a significant relationship between litter size and the proportion of XO embryos at day 8 of gestation in the present study. Therefore, there is no evidence that the retarded development of large litters is a consequence of a higher proportion of XO embryos. Our previous (Omoe and Endo, 1993) and present study showed that the litter size of XO dams at day 10 of gestation was about 7 while, at day 8, it was about 12. Furthermore, the percentage of XO embryos was 16% at day 10 and 14% at day 8 of gestation. These findings imply that litter sizes decrease during mid-gestation because XX and XY embryos are lost rather than XO embryos, whereas there is no evidence of preferential XO embryo mortality from day 8 to day 10 of gestation.

The development of XO embryos is retarded compared with that of XX embryos at the preimplantation stage (day 3 of gestation) (Banzai *et al.*, 1995). These findings and the present results indicate that XO monosomy may have a retarding effect on development from day 3 of gestation to the mid-gestation stage, as shown by Burgoyne *et al.* (1983b). As Burgoyne *et al.* (1983b) suggested, this may occur because the X and Y chromosomes share some genes that are required in double dose in the early embryo. However, XO embryos in the study of Burgoyne *et al.* (1983b) and in the present study differ from XY embryos in having a paternally, rather than a maternally, derived X chromosome. Thornhill and Burgoyne (1993) showed that XO fetuses having maternally derived X chromosomes are larger than XX fetuses and are equivalent in size to XY fetuses, and suggested that a paternally derived X chromosome has a retarding effect on development.

The development of XO embryos at mid-gestation reaches almost the same stage as that of their XX littermates (Omoe and Endo, 1993). A similar phenomenon with respect to body weight in normal mice was described by Monteiro and Falconer (1966) and Gall and Kyle (1968). Biggers and Papaioannou (1991) confirmed that 'compensatory growth', as defined by Monteiro and Falconer (1966), occurs in the genetically identical offspring of an F_1 cross. In addition to confirming the occurrence of compensatory growth in body weight and tail length, Biggers and Papaioannou (1991) produced direct evidence that, in animals that become relatively large during early growth, growth rate is slower compared with that of small animals as they approach maximum size, and that the reverse occurs with small animals. Furthermore, during early organogenesis, mouse embryos have a remarkable capacity for recovery from severe damage and developmental retardation (Snow and Tam, 1979; Tam, 1981). The enhanced development in XO embryos may be regarded as a type of 'compensatory growth' after the developmental retardation during early pregnancy. In addition to 'compensatory growth', several reports have shown that there was an inverse relationship between fetal development and litter size in mice (Cowley *et al.*, 1989; Leamy, 1992). In the present study, there was a statistical correlation between the mean number of somites of the embryos (when data included all three genotypes) and the litter size in XO mothers. However, when XO, XX and XY embryos were considered separately, each showed a negative correlation between litter size and mean number of somites but the correlation coefficients varied and the correlation was only significant for XX embryos (XO, $r = -0.39$, $P = 0.16$; XX, $r = -0.66$, $P = 0.01$; XY, $r = -0.23$, $P = 0.38$). The XO:XX:XY ratio was 1.0:2.7:2.5 at day 10 of gestation (Omoe and Endo, 1993) while, at day 8, it was 1.0:3.5:2.8 (present study). These findings suggest that the effects of litter size reduction on viability act more on XX embryos than on XO and XY embryos. In any case, it seems likely that the litter size has an effect on the development of XO embryos during mid-gestation. Litter size at mid-gestation (day 10 of gestation) (Omoe and Endo, 1993) and at near-term (day 17) (Endo and Watanabe, 1989) was about 7 for XO dams, while the litter size at preimplantation (day 3) was 12.7 (Banzai *et al.*, 1995). In the present study, at day 8 of gestation, the litter size was 11.7. These findings indicate that about half of the embryos from XO mothers must have been eliminated during the mid-gestation period. The elimination period of embryos from XO mice corresponds to the period when the development of XO embryos could 'catch up' with that of XX embryos. Therefore, these 'effects of reduced litter size' may cause an acceleration of development of XO mouse embryos at mid-gestation as compensation for developmental retardation during early pregnancy, allowing the development of XO embryos to catch up with that of XX embryos.

The development of XX and XY embryos from XO mice was delayed compared with that of their counterparts from XX mice. The retarded development of all embryos from XO mice has been shown at the preimplantation stage (Banzai *et al.*, 1995), at day 8 of gestation (present study) and near-term (Endo and Watanabe, 1989). Taken together, these findings indicate that embryos from XO mice grow later than their counterparts from XX mice, and that a retarded development at the preimplantation stage results in a subsequent relative delay in development that persists throughout gestation. This conclusion is supported by a study (Burgoyne and Biggers, 1976) that showed, using *in vitro* culture techniques, that the development of embryos from XO dams was retarded compared with that of embryos from XX dams at the preimplantation stage. Burgoyne and Biggers (1976) suggested that the mechanism of this developmental retardation of the embryos from XO dams was that maternal X dosage deficiency in the maternal germ line causes a deficiency in the materials stored in the oocyte, and this deficiency affects the embryos of the various genotypes indiscriminately. In addition, precocious or premature ageing (Lyon and Hawker, 1973), the only known characteristic feature of XO mice, may be related. In the original strain (Jcl/ICR) from which this XO mouse colony was derived, the percentage of pre-somite embryos at day 8 of gestation from middle-aged females (9–11 months) is 50%, almost equal to that found in our study of XO dams (Ishikawa and Endo, 1995). The developmental retardation of the embryos obtained from young XO females in the XO colony may be a manifestation of such a feature in reproductive functions.

Downloaded from Bioscientifica.com at 03/13/2022 08:25:42PM
via free access

The authors appreciate the invaluable advice of anonymous reviewers for improving the manuscript.

References

Banzai M, Omoe K, Ishikawa H and Endo A (1995) Viability, development and incidence of chromosome anomalies of preimplantation embryos from XO mice *Cytogenetics and Cell Genetics* **70** 273–277

Barr M, Jr, Jensh RP and Brent RL (1970) Prenatal growth in the albino rat: effect of number, intrauterine position and resorptions *American Journal of Anatomy* **128** 413–428

Benson GK and Morris LR (1971) Foetal growth and lactation in rats exposed to high temperatures during pregnancy *Journal of Reproduction and Fertility* **27** 369–384

Biggers JD and Papaioannou VE (1991) Postnatal compensatory growth of manipulated mouse embryos *Human Reproduction* **6** 36–44

Burgoyne PS and Biggers JD (1976) The consequences of X-dosage deficiency in the germ line: impaired development *in vitro* of preimplantation embryos from XO mice *Developmental Biology* **51** 109–117

Burgoyne PS, Evans EP and Holland K (1983a) XO monosomy is associated with reduced birthweight and lowered weight gain in the mouse *Journal of Reproduction and Fertility* **68** 381–385

Burgoyne PS, Tam PPL and Evans EP (1983b) Retarded development of XO conceptuses during early pregnancy in the mouse *Journal of Reproduction and Fertility* **68** 387–393

Cattanach BM (1962) XO mice *Genetical Research* **3** 487–490

Cowley DE, Pomp D, Atchley WR, Eisen EJ and Hawkins-Brown D (1989) The impact of maternal uterine genotype on postnatal growth and adult body size in mice *Genetics* **122** 193–203

Endo A and Watanabe T (1988) Individual and strain differences in patterns of long-term persistence of urethane-induced sister chromatid exchanges (SCEs) in mouse lymphocytes and their relation to carcinogen susceptibility *Environmental Molecular Mutagenesis* **12** 375–383

Endo A and Watanabe T (1989) Teratogen susceptibility of XO mothers and XO embryos in mice *Teratology* **40** 525–529

Epstein CJ (1986) Monosomy X (Turner syndrome, gonadal dysgenesis). In *The Consequences of Chromosome Imbalance: Principles, Mechanisms and Models* pp 324–343 Eds PW Barlow, PB Green and CC Wylie. Cambridge University Press, New York

Evans EP, Burtenshaw MD and Ford CE (1972) Chromosomes of mouse embryos and newborn young: preparations from membranes and tail tips *Stain Technology* **47** 229–234

Gall GAE and Kyle WH (1968) Growth of the laboratory mouse *Theoretical and Applied Genetics* **38** 304–308

Hunt PA (1991) Survival of XO mouse fetuses: effect of parental origin of the X chromosome or uterine environment? *Development* **111** 1137–1141

Ishikawa H and Endo A (1995) Combined effects of maternal age and delayed fertilization on the frequency of chromosome anomalies in mice *Human Reproduction* **10** 883–886

Ishikawa H and Endo A (1998) Analysis of colchicine-induced chromosomal aberrations in embryos obtained from XO mice *Reproductive Toxicology* **12** 115–118

King HD (1915) On the weight of the albino rat at birth and the factors that influence it *Anatomical Record* **9** 213–231

Leamy L (1992) Morphometric studies in bred and hybrid house mice *Growth, Development and Aging* **56** 35–43

Lyon MF and Hawker SG (1973) Reproductive lifespan in irradiated and unirradiated chromosomally XO mice *Genetical Research* **21** 185–194

Monteiro LS and Falconer DS (1966) Compensatory growth and sexual maturity in mice *Animal Production* **8** 179–182

Omoe K and Endo A (1993) Growth and development of 39,X mouse embryos at mid-gestation *Cytogenetics and Cell Genetics* **63** 50–53

Romero A, Villamayor F, Grau MT, Sacristan A and Ortiz JA (1992) Relationship between fetal weight and litter size in rats: application to reproductive toxicology studies *Reproductive Toxicology* **6** 453–456

Smart JL, Adlar PF and Dobbing J (1972) Effect of maternal undernutrition and other factors on birth weight in the rat *Biology of Neonate* **20** 236–244

Snow MHL and Tam PPL (1979) Is compensatory growth a complicating factor in mouse teratology? *Nature* **279** 555–557

Tam PPL (1981) The control of somitogenesis in mouse embryos *Journal of Embryology and Experimental Morphology* **65** (Supplement) 103–128

Thornhill AR and Burgoyne PS (1993) A paternally imprinted X chromosome retards the development of the early mouse embryo *Development* **118** 171–174

Downloaded from Bioscientifica.com at 03/13/2022 08:25:42PM
via free access