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Survival of XO mouse fetuses: effect of parental origin of the X chromosome or uterine environment?

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Summary

Using a recombinant product from the structurally abnormal Y chromosome, Y*, female mice with a single X of either maternal or paternal origin were generated. The two types of females were produced on the same genetic background and differ only in the origin of the X chromosome. Hence it has been possible to assess the effect of parental origin of the X on survival of females with a single X chromosome. A highly significant prenatal loss of females with a single X of paternal origin, but no comparable loss of females with a single X of maternal origin was observed. The reduced viability of females with a paternally derived X could be mediated by the parental origin of the X (i.e. X chromosome imprinting) or alternatively, since the mothers of females with a single paternally derived X have only a single X chromosome, the effect could be mediated by the genotype of the mother (i.e. maternal uterine effect).

Key words: XO, X chromosome, genomic imprinting, prenatal mortality.

Introduction

In humans 45,X conceptions occur in approximately 1–2 % of all clinically recognized pregnancies (Hassold, 1986). However, the XO condition is nearly always lethal and over 99 % of all such conceptions abort in the first trimester of pregnancy. The few 45,X females that survive to term have Turner syndrome, which is characterized by specific somatic abnormalities and sterility due to early germ cell loss. Because the phenotypic abnormalities are mild by comparison with other human aneuploidies, the lethality of monosomy X remains a genetic enigma.

The XO female mouse, reported in the same year as human Turner syndrome, also has been extensively studied. The only obvious somatic abnormality is a reduction in body weight, which reflects developmental retardation early in gestation (Burgoyne *et al.* 1983*a*; Burgoyne *et al.* 1983*b*). Like XO human females, XO mice suffer germ cell loss and, by birth, the germ cell population is reduced by approximately 50 %. However, the XO mouse is fertile, although with a reproductive lifespan that is approximately one-half as long as her normal XX sibs (Lyon and Hawker, 1973; Burgoyne and Baker, 1984).

The fertility of XO mice has made it possible to breed for and to study reproduction in XO females. A number of studies have reported that XO females produce fewer than expected XO offspring. However, the reason for this reduction remains an area of considerable controversy, with some studies suggesting that the X chromosome more frequently segregates to the egg than to the polar body, others finding evidence of reduced survival of XO offspring, and still others suggesting that both effects contribute (Morris, 1968; Kaufman, 1972; Luthardt, 1976; Russell, 1976; Brook, 1983).

We have used a recombinant product from a rearranged Y chromosome, Y* (Eicher *et al.* 1991), to generate female mice with a single X chromosome. These females also possess a small chromosome, Y^{*X}, that contains the centromere of the Y* chromosome and the pseudoautosomal region (Fig. 1). However, our analysis indicates that the presence of this chromosome does not affect reproductive performance in XY^{*X} females by comparison with XO females of the same strain background. Thus, XY^{*X} females can be used as a model for studying reproduction in females with a single X chromosome.

XY^{*X} offspring of XY* males have a maternally inherited X chromosome, but their XY^{*X} daughters have a paternally inherited X, thus this breeding scheme provides a mechanism of producing females with a single X chromosome of either maternal or paternal origin. Since previous breeding schemes have generated only XO females with a paternally derived X, the present strategy provides the first approach to assessing the effect of the parental origin of the X on survival of XO females. Our results suggest that, on the C57BL/6J background, there is a marked reduction in the survival of females with a single X chromosome when the X is paternally derived. However, this effect could be mediated by the parental origin of the X chromosome or by the genotype of the mother.

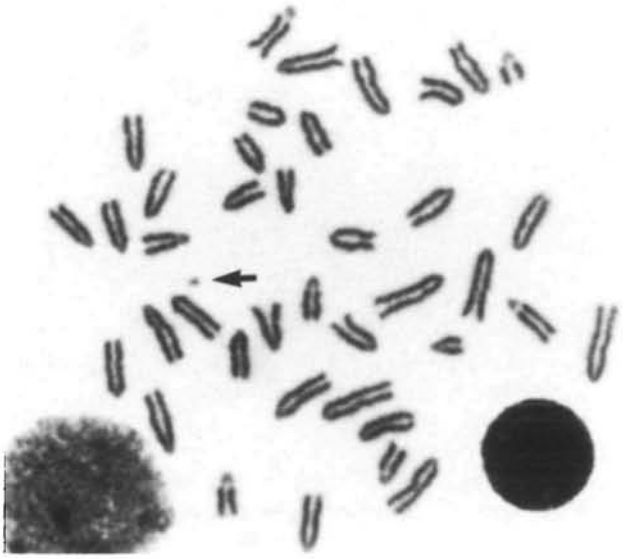

Fig. 1. Unbanded metaphase from an XY^{*X} female. The small Y^{*X} chromosome is indicated by the arrow.

Materials and methods

XY^{*X} females are obtained from matings of males carrying the structurally rearranged Y chromosome, Y* (T(HcX?;TpY)8Ei). The Y* mutation arose spontaneously and has been described previously (Eicher and Washburn, 1986). The rearrangement is hypothesized to involve the acquisition of a centromere at the distal end of the Y chromosome with concomitant loss/inactivation of the normal centromere at the proximal end of the chromosome. The origin of the acquired centromere remains unknown (Eicher *et al.* in preparation). Due to the fact that the pseudoautosomal region on the resulting Y* chromosome is near the centromere but proximal to the Y-linked genes involved in testis determination, recombination between an X and the Y* chromosome during male meiosis results in two recombinant products; X^{Y*}, in which almost the entire Y* chromosome is attached to the X at a point distal to the pseudoautosomal region and Y^{*X}, an extremely small chromosome that contains the Y* centromere and the pseudoautosomal region. XX^{Y*} mice are sterile males and XY^{*X} mice are fertile females.

Although the Y* mutation arose on the LT/SvEi background, it has been transferred to the C57BL/6JEi background by repeated backcrossing of XY* males to C57BL/6JEi females. Because of the high frequency of ovarian teratomas on the LT/SvEi background, the present study of XY^{*X} females was confined to females on the C57BL/6J background.

When XY* males are mated to normal females of the same strain, XX and XY^{*X} females are produced in approximately equal frequency (Eicher *et al.* 1991). In the present study, XY^{*X} females were identified at 5–7 weeks of age by chromosome analysis of peripheral blood cultures as described by Davisson and Akeson (1987). Additionally, during the course of the study, five XO females were identified. XO females are found in very low frequency (approximately 1%) among the liveborn offspring of normal females mated to XY* males and probably result from nondisjunction of the Y^{*X} chromosome before or during meiosis or at an early cleavage stage. The XO females were used as controls in the assessment of breeding performance of XY^{*X} females; although XO/XY^{*X} mosaicism cannot be excluded in these 5 females, no evidence of germline mosaicism was found since none of their offspring carried the Y^{*X} chromosome.

Fertility and reproductive lifespan of XY^{*X} females

Nine XY^{*X} and five XO females were mated with normal C57BL/6J males to assess fertility and reproductive lifespan. The females were monitored for pregnancies, and pregnant females were checked daily so that the birth date and number of offspring could be accurately recorded. A period of greater than two months without overt signs of pregnancy was defined as the end of a female's reproductive lifespan.

Cytogenetic analysis of offspring of XY^{*X} females

Liveborn offspring from five XY^{*X} females were karyotyped at 5–7 weeks of age, using the peripheral blood culture technique of Davisson and Akeson (1987). In addition, data were collected at late fetal stages from 17 pregnant XY^{*X} females. Fetal karyotyping was done at 13.5–18.5 days of gestation (day of vaginal plug counted as day 0) from fetal liver preparations (Eicher and Washburn, 1978). For each mouse, a minimum of 10 cells was scored for presence of the Y^{*X} chromosome.

For analysis of preimplantation embryos, 4–5 week old XY^{*X} females were superovulated with an intraperitoneal injection of 5 i.u. PMSG (Diosynth) followed 42 h later by an injection of 5 i.u. HCG (Sigma). After HCG injection females were placed with normal C57BL/6J males of proven fertility and examined the following morning for the presence of a vaginal plug. Two-cell embryos were recovered from the oviducts of mated females on the afternoon of day 1 (plug day=day 0) and cultured overnight in M16 media (Pratt, 1987) at 37°C in 5% CO_2 in air. Early on the morning of day 3, embryos that had cleaved beyond the 2-cell stage were scored for the number of blastomeres and the presence of pronuclear outlines, transferred to M16 media containing colchicine ($1\,\mu g\,ml^{-1}$ final concentration), and incubated. Seven to eight hours later chromosome preparations were made from individual embryos. Embryos were treated in a 0.56% KCl hypotonic solution for 5 min, transferred individually to a microscope slide and fixed *in situ* with 6–8 drops of fresh fixative (3 parts methanol:1 part acetic acid). The preparations were stained in 5% Giemsa stain and scored. Only cells in which the chromosomes could be easily counted were scored.

Other matings that produce XY^{*X} females

XY^{*X} females can be obtained from three different crosses. As described above, approximately 25% of the offspring of XY* males are XY^{*X} females, and all of the XY^{*X} females used in the study of fertility, reproductive lifespan and the offspring of XY^{*X} females were obtained from this cross. In addition, XY^{*X} females produce XY^{*X} daughters, although they are relatively infrequent and constitute only some 2% of liveborn offspring (see Results). Finally, XY^{*X} females also result from matings involving XYY^{*X} males; indeed XY^{*X} females constitute approximately 30% of the liveborn offspring from the mating of a normal female with an XYY^{*X} male (Hunt and Eicher, 1991). To assess survival of XY^{*X} offspring, data were collected at late fetal stages (13.5–18.5 days) as described above from 10 pregnant XX females mated to XY* males and 12 XX females mated to XYY^{*X} males.

Results

Fertility and reproductive lifespan of XY^{*X} females

The results of the study of breeding performance in nine XY^{*X} and five XO females of the C57BL/6J strain are shown in Table 1. Of the nine XY^{*X} females studied, one was infertile, one bred for less than two months, bearing 2 liveborn litters, two females bred for 4 months, producing 1 and 2 litters, and the remaining five females bred for a period of 4–6 months, bearing 2–4 litters each. In our laboratory it is not unusual for normal C57BL/6J females to breed for 8 months to one year, producing 8 litters or more. An XX sib of one of the XY^{*X} females in Table 1 was mated at the same time and retired after producing 7 litters during a breeding period that exceeded 8 months. The breeding performance of the five XO females is also presented in Table 1. One XO female was fertile for 3.5 months and produced 2 litters and the remaining four females were fertile for from 4 to less than 6 months, producing 3–4 litters each.

Offspring of XY^{*X} females

The offspring of XY^{*X} females were cytogenetically analyzed at three different developmental stages and these data are given in Table 2. 51 liveborn offspring were analyzed at 5–6 weeks of age. 37 (72.5%) were XX or XY, 13 (25.5%) were XXY^{*X} or XYY^{*X}, and one animal was XY^{*X}. 64 fetuses were analyzed; 40 (62.5%) were XX or XY, 19 (29.7%) were XXY^{*X} or XYY^{*X}, and 5 (7.8%) were either XO or XY^{*X}. Although fewer XO and XY^{*X} offspring were observed among liveborn than fetuses, the difference in frequency between the two stages was not statistically significant.

It is also apparent from Table 2 that the average litter size of XY^{*X} females at both late fetal stages and at term is low (3.8 and 4.75, respectively), and a high resorption rate (39%) is apparent at the late fetal stage. Litter size should be reduced in XY^{*X} females due to the loss of YO and YY^{*X} conceptions; however, these embryos would be lost at early cleavage stages and would not be observed as resorptions in late gestation. Thus, the 39% resorption frequency observed at the late fetal stage is consistent with a high rate of fetal loss in XY^{*X} females.

A striking increase in the frequency of XO and XY^{*X} offspring was observed among pre-implantation embryos (Table 2). Chromosome preparations were made from a total of 125 embryos from 4 different XY^{*X} females. 40 were discarded at analysis because the embryos had degenerated (11) or no dividing cells were present (29), and 42 did not yield divisions of sufficient quality for analysis. 43 embryos were successfully analyzed; 13 (30.2%) had a 40,XX or 40,XY constitution, 5 (11.6%) were either $41,XXY^{*X}$ or $41,XYY^{*X}$, 15 (34.9%) were either 39,X or $40,XY^{*X}$, and the remaining 10 embryos were either hyperploid (9.3%) or triploid (14%). YO and YY^{*X} embryos were not observed, but these are thought to die prior to the 4-cell stage (Morris, 1968; Burgoyne and Biggers, 1976), and hence would not have been identified in our analysis of 4-cell embryos.

Other matings that produce XY^{*X} females

XY^{*X} females can be obtained from three different crosses; approximately one quarter of the offspring of XY^{*} males are XY^{*X} females, XY^{*X} females produce XY^{*X} daughters, and 30% of the offspring of XYY^{*X} males are XY^{*X} females (Hunt and Eicher, 1991). Table 3 provides information on litter size, resorption frequency and incidence of XY^{*X} females for these three different crosses. All matings are on the C57BL/6J genetic background and analyses were made at comparable stages of fetal development. A direct comparison of the frequency of XY^{*X} females is not possible because the expected frequencies differ for the three different matings. However, examination of the average litter size and resorption frequency indicates that increased fetal loss is unique to matings involving XY^{*X} females.

Table 1. *Reproductive lifespan of XY^{*X} females*

Female	Average litter size	Infertile	Breeding period <2 mo.	2–4 mo.	4–6 mo.
XY^{*X}	4.5±1.9	1	1	2	5
XO	3.6±1.9	0	0	1	4

Discussion

XY^{*X} individuals are fertile females, demonstrating that the Y^{*X} chromosome does not contain the Y-linked testis determining gene (Eicher *et al.* 1991). Because the complete genetic composition of the Y^{*X} chromosome remains unknown, the first step in the study of XY^{*X} females was assessment of their reproductive performance, the aim being to determine

Table 2. *Offspring of XY^{*X} females*

Age at analysis	*n*	Sex chromosome constitution XX	XY	XY^{*X}	XO	XXY^{*X}	XYY^{*X}	Other	Avg. litter size	% resorption
Liveborn	51	23 (45%)	14 (27%)	1 (2%)	0 (–)	3 (6%)	10 (20%)	0 (–)	4.75	–
Late fetal	64	16 (25%)	24 (38%)	4 (6%)	1 (1.5%)	8 (12.5%)	11 (17%)	0 (–)	3.8	39%
Preimplantation	43	13 (30.2%)		15 (34.9%)		5 (11.6%)		10 (23.3%)	–	–

Table 3. *Comparison of average litter size and resorption frequency in 3 different matings producing XY^{*X} females*

Mating	No. of litters analyzed	Total no. of live fetuses	No. of XY^{*X} females	Avg litter size**	Resorption frequency
XX♀×XY*♂	10	88	17 (19%)	8.8±1.55	11%
XY^{*X}♀×XY♂	17	64	4 (6%)	3.8±2.39	39%
XX♀×XYY^{*X}♂	12	93	29 (31%)	7.8±3.19	10%

** Excluding resorptions.

if: (1) XY^{*X} females were reproductively impaired in comparison with XO females due to the presence of Y^{*X}-linked genes, or (2) at a reproductive advantage due either to Y^{*X}-linked genes or to the presence of a sex chromosome pairing partner for the X chromosome.

A comparison of the reproductive lifespan of nine XY^{*X} and five XO females revealed no obvious difference between the two groups with regard to breeding performance. The breeding period did not exceed 6 months for either type of female, while XX females on this background are fertile for 8 months to one year. Therefore, the presence of an additional, pseudoautosomal containing, marker chromosome in an otherwise XO individual does not appear to improve or impair reproductive performance.

In the analysis of offspring of XY^{*X} females, an extremely high frequency (35%) of XO and XY^{*X} females was observed among pre-implantation embryos, but virtually no such conceptuses were present among the liveborn offspring. Taken together with the high resorption rate observed in XY^{*X} females, this indicates that the viability of a specific category of offspring, namely females with a single X chromosome, is greatly reduced in XY^{*X} females. However, analyses of two other matings that generate XY^{*X} females (i.e. normal XX females mated to either XY* or XYY^{*X} males) revealed no evidence of reduced survival of XY^{*X} offspring. That is, neither a reduction in litter size nor an increase in resorption frequency at late fetal stages was apparent in matings involving XY* or XYY^{*X} males. Furthermore, in matings involving XY* males, 19% of the fetal offspring were XY^{*X} females; this is not significantly different from the expected value of 25% (calculated assuming an obligatory exchange between the X and Y* chromosomes and normal segregation), indicating that XY^{*X} females are not selectively eliminated in this mating.

There are at least two possible explanations for the reduction in viability of XY^{*X} (and XO) offspring in matings involving XY^{*X} females but not in matings involving XY* or XYY^{*X} males. First, XY^{*X} offspring of XY^{*X} mothers have a paternally derived X chromosome, whereas the X is of maternal origin in XY^{*X} daughters of both XY* and XYY^{*X} males. Hence parental origin of the X chromosome may affect the survival of females with a single X. Alternatively, the mating involving XY^{*X} females is the only mating of the three in which the uterine environment may be abnormal. That is, the XY^{*X} daughter of an XY^{*X} female develops in an 'effective' XO uterus, whereas in the other two matings the XY^{*X} offspring develops in an XX uterus. Hence the maternal environment may affect the survival of XY^{*X} females.

To date there is little basis for favoring one hypothesis over the other. Parental origin, or imprinting, effects involving the X chromosome clearly occur in the mouse, since the paternally inherited X chromosome is preferentially inactivated in extra-embryonic membranes (Takagi and Sasaki, 1975; West *et al.* 1977). If X chromosome imprinting influences the survival of females with a single X, the severity of the effect is likely modified by genetic background. That is, investigations of the lower-than-expected frequency of XO offspring in matings involving XO mothers have produced conflicting results, with some investigators finding evidence for nonrandom segregation of the X chromosome at first meiosis and others finding evidence of both nonrandom segregation and reduced survival of XO offspring (Morris, 1968; Kaufman, 1972; Luthardt, 1976; Russell, 1976; Brook, 1983). In all studies, the breeding schemes produced XO females with a paternally derived X chromosome, but the genetic background has varied widely and might be responsible for the observed survival difference. Therefore, it is possible that the C57BL/6J background represents one end of a broad spectrum, with a high rate of loss of XO conceptions occurring when the X chromosome is of paternal origin.

The alternative hypothesis suggests decreased survival of XY^{*X} offspring in an XY^{*X} uterus compared to an XX uterus. The present breeding scheme is the first that allows production of females with a single X chromosome of maternal or paternal origin on the same genetic background. However, even though the two types of females are genetically identical, those with a paternally inherited X chromosome can only be produced from mothers who themselves have a single X chromosome. It is possible that an XO or XY^{*X} mother provides a 'weaker' uterine environment than a normal XX female. The technique of embryo transfer has been used in a number of studies to evaluate the effect of embryonic and uterine genotype on survival (reviewed in Pomp *et al.* 1989). These studies have generally found a strong maternal uterine influence on the ability to support the overall survival and prenatal growth of transferred embryos. However, the prenatal mortality observed in the present study does not involve all

offspring, but preferentially affects the survival of XO and XY^{*X} offspring. It may be that these are the least viable embryos and therefore the most susceptible to influences of the uterine environment.

Data from the study of human XO females do not readily support either theory. Over 99% of human XO conceptions die *in utero* and, of those that survive to term, a high proportion are mosaics (Hook and Hammerton, 1977). Thus, it is possible that mosaicism, and not parental origin of the single X or maternal uterine environment, is the most important determinant of survival in human XO conceptuses. However, Burgoyne (1989) has recently suggested that the difference in survival between human and mouse XO females may reflect differences in dosage for the gene encoding a 'zinc finger' protein that is present on both the X and Y chromosome, ZFX and ZFY. Support for this theory comes from the fact that, in humans, but not in the mouse, the X-linked gene escapes X-inactivation. Therefore, deleterious effects associated with monosomy for ZFX/ZFY would be more pronounced in the human than in the mouse. Regardless of the correctness of this hypothesis, it is interesting to note that the genetic background on which we have observed a possible X chromosome imprinting effect, namely C57BL/6J, results in sex reversed XY females in the presence of certain Y chromosomes (Eicher *et al.* 1982). It may be that an X-linked gene present in the C57BL/6J background is responsible for both effects.

In summary, a breeding scheme that produces fertile female mice (XY^{*X}) with a single X chromosome of either maternal or paternal origin and a small marker chromosome containing the Y* centromere and the pseudoautosomal region has been devised. Analysis of reproductive performance in XY^{*X} females indicates no difference from that observed for XO mice of the same genetic background. However, cytogenetic studies of offspring of XY^{*X} mothers indicates extremely high *in utero* mortality of XY^{*X} and XO offspring, an effect that may be attributable to X chromosome imprinting or maternal environment influences.

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