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# The development of XO gynogenetic mouse embryos

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## Summary

**Diploid gynogenetic embryos, which have two sets of maternal and no paternal chromosomes, die at or soon after implantation. Since normal female embryos preferentially inactivate the paternally derived X chromosome in certain extraembryonic membranes, the inviability of diploid gynogenetic embryos might be due to difficulties in achieving an equivalent inactivation of one of their two maternally derived X chromosomes. In order to investigate this possibility, we constructed XO gynogenetic embryos by nuclear transplantation at the 1-cell stage. These XO gynogenones showed the same mortality around the time of implantation as did their XX gynogenetic counterparts. This shows that the lack of a paternally derived autosome set is sufficient to cause gynogenetic inviability at this stage. Autosomal imprinting and its possible relation to X-chromosome imprinting is discussed.**

Key words: gynogenetic, parthenogenetic, X-inactivation, X chromosome, imprinting, mouse embryo.

## Introduction

Mouse eggs activated to develop parthenogenetically, i.e. without fertilization, undergo morphologically normal preimplantation development and implant in the uterus at a high frequency. They usually cease development at or very soon after implantation, but occasionally develop to somite stages (Witkowska, 1973*a*,*b*; Kaufman & Gardner, 1974; Kaufman, 1983*a*). Nuclear transplantation experiments at the 1-cell stage have shown that the developmental failure is not associated with the cytoplasm of the parthenogenetic egg, which is functionally normal, but with the presence of two maternally derived pronuclei (Mann & Lovell-Badge, 1984; Surani, Barton & Norris, 1984). Diploid gynogenetic embryos, which can be produced by the experimental removal of the paternal pronucleus, are in this way equivalent to diploid parthenogenetic embryos, and their potential for development is the same (Surani & Barton, 1983). Embryos containing only two paternally derived pronuclei also fail to develop, and it is the combination of maternal and paternal pronuclei that is necessary for normal development (McGrath & Solter, 1984; Barton, Surani & Norris, 1984).

How the maternally and paternally derived pronuclei complement one another to achieve normal development is uncertain, although in parthenogenones and gynogenones it is possible that the lack of a paternally derived X chromosome, $X^P$, may be involved. In female embryos, $X^P$ is preferentially inactivated in certain extraembryonic membranes, i.e. the trophectoderm and primitive endoderm and their derivatives, as distinct from random X-inactivation in the remaining membranes and embryo proper (Papaioannou & West, 1981; Harper, Fosten & Monk, 1982). Parthenogenones and gynogenones possess two maternally derived X chromosomes, $X^M$, and therefore may have difficulties in activating an X in the extraembryonic membranes (Kaufman, 1983*b*). This possibility is consistent with the fact that their inviability usually occurs at around the time that these X-inactivation events take place (Takagi, Wake & Sasaki, 1978; Harper *et al.* 1982). Furthermore, a relatively poor development of extraembryonic membranes has been reported in parthenogenetic embryos that develop to somite stages (Endo & Takagi, 1981; Surani *et al.* 1984). In these membranes, inactive X chromosomes have been observed cytologically (Endo & Takagi, 1981; Rastan, Kaufman, Handyside & Lyon, 1980). However, these observations cannot

show if the process is entirely normal. Parthenogenetic cells may only survive if they manage to inactivate an X chromosome.

We have constructed by nuclear transplantation gynogenetic embryos with a single X chromosome, i.e. $X^MO$, in which X-chromosome dosage-related functions should be normal. If disturbance of these functions is the cause of the inviability of $X^MX^M$ diploid gynogenones and parthenogenones, then these $X^MO$ gynogenetic embryos should be viable, as are $X^MO$ embryos derived from fertilized eggs (Russell, 1976).

## Materials and methods

Although XO mice can be used as a source of eggs lacking an X chromosome, these eggs are of poor quality due to X-dosage deficiency in the maternal germ line (Burgoyne & Biggers, 1976). Mice heterozygous for the large X inversion In(X)1H (Evans & Phillips, 1975) produce litters in which a significant proportion of the female offspring are XO (Phillips, Hawker & Moseley, 1973). Crossing over within the inversion frequently generates a dicentric bridge which prevents normal disjunction at the first meiotic division, with the result that about 22 % of ovulated eggs lack an X chromosome (P. S. Burgoyne & E. P. Evans, unpublished data; Phillips & Kaufman, 1974). These eggs are of good quality because there is no X-dosage deficiency in the germ line prior to extrusion of the first polar body.

Females heterozygous for the inversion [In(X)/X females] were produced by mating In(X)/Y males (MRC Mammalian Development Unit colony) to outbred MF1 females (OLAC). In(X)/X females were superovulated by intraperitoneal injection of 5 i.u. pregnant mare serum gonadotrophin (Folligon; Intervet) followed 48 h later by 5 i.u. human chorionic gonadotrophin, hCG (Chorulon; Intervet), and mated to males carrying the X-linked phosphoglycerate kinase-1 electrophoretic variant allele *a*, *Pgk-1*$^a$. In(X)/X females were homozygous *Pgk-1*$^b$. Approximately 22 h after injection of hCG, the females were killed, the oviducts excised, and the cumulus masses containing 1-cell embryos released into 1 % (w/v) hyaluronidase in medium M2 (Fulton & Whittingham, 1978) to remove the cumulus cells, then washed in medium M2.

Nuclear transplantation was carried out according to McGrath & Solter (1983). This method involves transplanting pronuclei, surrounded by a small portion of cytoplasm and plasma membrane, utilizing Sendai virus membrane fusion. Gynogenetic and control embryos were constructed according to the procedure illustrated in Fig. 1. This procedure, involving three embryos, was successful in the majority of attempts. Sometimes plasma membrane breakage or fusion failure occurred. In initial experiments a mixture of embryos from three to five mice, i.e. mixed clutches, was used. In later experiments, the procedure was performed only with embryos from the same mouse, i.e. a single clutch. The maternal pronucleus could usually be distinguished from the paternal by its smaller size and closer proximity to the second polar body. Embryos in which this distinction was unclear were not used. Transplantation was carried out with a Leitz Laborlux 2 microscope, phase-contrast optics and Leitz mechanical micromanipulators, and in medium M2 containing 5·0 $\mu g\,ml^{-1}$ cytochalasin B (Sigma) and 0·3 $\mu g\,ml^{-1}$ nocodazole (Sigma). Embryos were present in these cytoskeletal inhibitors for a total of about 1·5 h. The inhibitors were removed by passing the embryos through 2 ml of medium M2 for 5 min, then four drops of medium M16 (Whittingham, 1971) under paraffin oil. Enucleation pipettes were rinsed in 1·25 % (v/v)

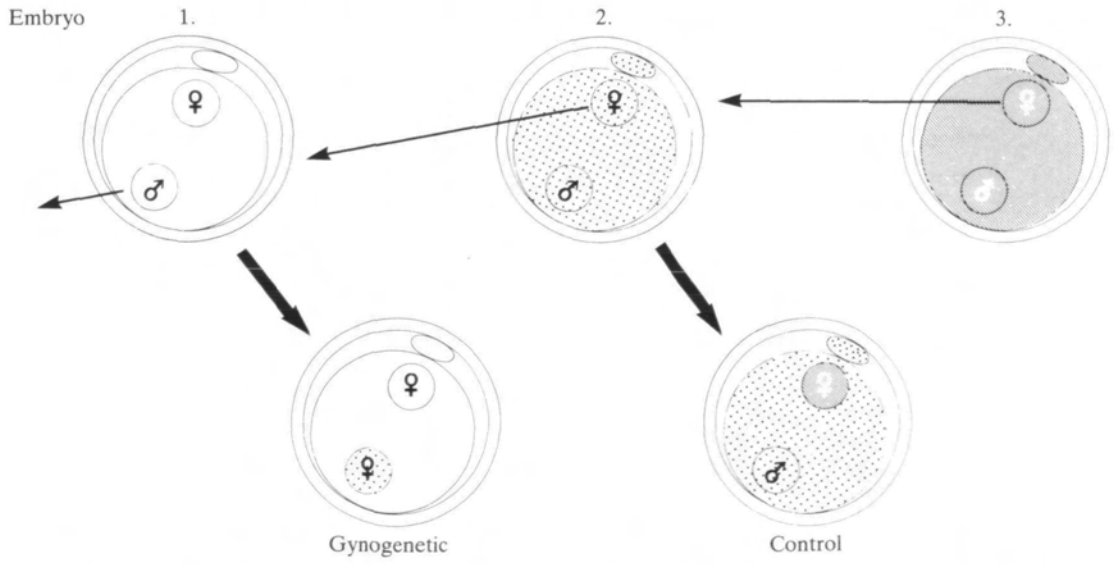


**Fig. 1.** Construction of gynogenetic and control embryos. 1-cell embryos were from matings of In(X)/X *Pgk-1*$^b$/*Pgk-1*$^b$ females to *Pgk-1*$^a$/*Y* males. Gynogenetic embryos: the paternal pronucleus (♂) was removed from one embryo and a maternal pronucleus (♀) from a second embryo was transplanted to it. Control embryos: a maternal pronucleus from a third embryo was transplanted into the second embryo that had been left with a paternal pronucleus.

Tween-80 (Sigma) to prevent sticking of the plasma membrane. Sendai virus was grown in embryonated chicken eggs and inactivated with 0·1 % (v/v) β-propiolactone (Sigma) (Neff & Enders, 1968; Giles & Ruddle, 1973).

Embryos were cultured overnight in drops of medium M16 under paraffin oil at 37 °C in 5 % $CO_2$ in air to cleave to two cells, then replaced into recipients on day ½ of pseudopregnancy (day of vaginal plug after mating to vasectomized males of proven sterility). Usually four gynogenetic and four control embryos were put into each oviduct of a (CBA×C57BL/6)$F_1$ recipient. Recipients were killed at 13½ days of gestation and the sex (determined by gonad morphology), PGK-1 isozyme type (Bücher, Bender, Fundele, Hofner & Linke, 1980) and chromosome number (Burgoyne, Tam & Evans, 1983) of each embryo determined. All possible types of embryos, gynogenetic and control, could be distinguished using these parameters. This also acted as a safeguard for any error in distinguishing the maternal and paternal pronuclei during micromanipulation.

Eggs of In(X)/X females have a 22 % chance of being nullo-X, a 72 % chance of carrying an X [or In(X)] and a 6 % chance of carrying an X-chromosome dicentric, which causes death soon after implantation due to the generation of polyploidy (P. S. Burgoyne & E. P. Evans, unpublished data, based on the karyotypic examination of 127 two- and 66 four-cell embryos). Thus, the expected proportion of embryo types constructed would be as follows. Controls: 11 % XO, 36 % XX and 36 % XY (all potentially viable), 11 % OY (preimplantation lethal), and 6 % X dicentrics (peri-implantation lethal). Gynogenetic: 32 % XO (viability?), 52 % XX (invariably lethal well before 13½ days), 5 % OO (preimplantation lethal) and 11 % dicentrics (presumably peri-implantation lethal). The nuclear transplantation procedure would therefore be expected to produce three times as many gynogenetic XO embryos as control XO embryos.

## Results

Fusion occurred in 233 out of 235 micromanipulated embryos. All but one of these cleaved to two cells. These were replaced into 16 recipients of which 3 did not become pregnant. Rates of implantation and development of the micromanipulated embryos are shown in Table 1. In every case, implantations were either resorbing decidua containing no discernible embryonic material, or normal 13½-day embryos. Decidua that were resorbing could have resulted from the implantation of gynogenetic or control embryos, hence only the total number of implantations is shown. Control OY and gynogenetic OO embryos would not have implanted, these make up an expected proportion of 8 % of all embryos replaced. The 91 % implantation rate of embryos in the single clutch group therefore indicates that gynogenetic XO embryos were able to develop to the blastocyst stage. More embryos implanted and developed when nuclear transplantation was done with embryos from a single clutch than with embryos from mixed clutches. As fertilization of eggs within a single clutch would have occurred over a narrower time span than in eggs of mixed clutches, this result supports the suggestion of McGrath & Solter (1984), that synchrony between the resident and introduced pronuclei is important in determining embryo viability.

**Table 1.** *Rates of implantation and development of gynogenetic and control embryos*

| Embryo constructs | Number of embryos | | |
|---|---|---|---|
| | Replaced | Implanted | Developed post-implantation |
| *Mixed clutches* | | | |
| Control | 31 | 37 (57 %) | 14 (45 %) |
| Gynogenetic | 34 | | 0 |
| *Single clutch* | | | |
| Control | 64 | 116 (91 %) | 43 (67 %) |
| Gynogenetic | 64 | | 1 |

Classification of all embryos obtained at 13½ days according to sex, PGK-1 type and chromosome number is shown at Table 2. As four XO control embryos were obtained, approximately 12 XO gynogenetic embryos would have been expected had they been equally viable (see Materials and methods section). However, only one XO embryo of the PGK-1 isozyme type expected for a gynogenetic embryo was obtained. In fact, it is possible that this embryo was a control, in which a sex chromosome was absent in, or lost from, the paternal pronucleus. The spontaneous occurrence of $X^MO$ mice ranges from 0·2 to 1 % of females (Russell, 1976).

## Discussion

These results show that postimplantation development usually fails in diploid embryos with two maternally derived genomes, even when X-chromosome status is such that it should not impair development, i.e. $X^MOA^MA^M$ embryos (where A refers to autosomes). As $X^MOA^MA^P$ embryos are viable, the lack of paternally derived autosomes, or perhaps some other specific paternal pronuclear component apart from an X chromosome, seems responsible for this mortality at or very soon after implantation. This could also be sufficient to explain the equivalent mortality of $X^MX^MA^MA^M$ embryos, but does not preclude the possibility that the presence of two $X^M$ chromosomes may also exert a deleterious effect. Paternal autosomes are not essential for preimplantation development, since most gynogenetic and parthenogenetic embryos can develop into blastocysts.

**Table 2.** *Classification of embryos obtained at 13½ days*

| Sex | PGK-1 phenotype | Number of chromosomes | Embryo classification | Number of embryos |
|---|---|---|---|---|
| ♀ | A | 39 | XO control | 4 |
| ♀ | AB | 40 | XX control | 25 |
| ♂ | B | 40 | XY control | 28 |
| ♀ | B | 39 | XO gynogenetic | 1 |

However, as suggested by Graham, "The death of parthenogenones at and immediately after implantation is consistent with a requirement for orderly gene expression at this time" (Graham, 1974). The present results would suggest that this orderly gene expression is dependent on the presence of paternal autosomes. Indeed, it is likely that it depends on the combination of paternal and maternal autosomes, as diploid androgenetic embryos can also develop to the blastocyst stage and usually die at implantation.

Paternal and maternal autosomes must be distinguished by some form of differential modification or 'imprint' (Chandra & Brown, 1975), presumably imparted during gametogenesis. That this can determine differential activity of maternally and paternally derived genes is evident in the anomalous and opposite phenotypes observed in mice with maternal duplication/paternal deficiency and the reciprocal (paternal duplication/maternal deficiency) of certain autosome regions (Cattanach & Kirk, 1985). Such mice arise by the union of unbalanced gametes in crosses between reciprocal translocation heterozygotes. In some crosses, certain duplication-deficiency offspring are never observed (Searle & Beechey, 1978; Cattanach & Kirk, 1985), and these may delineate regions of the autosomes where imprinting is important for normal embryonic development. However, in some instances, offspring might not be observed due to abnormal haploid expression acting in an unbalanced gamete to prevent fertilization. That the expression of a certain gene in the haploid phase of spermatogenesis is necessary for fertilization is suggested in a transgenic line of mice reported by Palmiter, Wilkie, Chen & Brinster (1984). Males heterozygous for a foreign DNA insert cannot transmit this to the following generation, even though litters sired by these males are of normal size. However, for the majority of autosomal regions investigated, mice receiving both copies from one parent only develop normally. This indicates that a large proportion of homologus genes are not differentially regulated in a way important for development.

Does imprinting of the autosomes effect differential homologous gene expression in a similar way as does imprinting of the X? X-inactivation is apparently initiated at one site (possibly the X-controlling element locus, *Xce* (Cattanach, 1975)), and imprinting of a site such as this may be all that is required for differential expression of the X in the trophectoderm and primitive endoderm (Lyon & Rastan, 1984). If this were the case, then many homologous X-linked genes would be differentially expressed even though they themselves were not differentially modified or imprinted during gametogenesis. Whether an analogous mechanism may operate in certain autosome regions, or whether all differentially expressed autosomal loci are also imprinted, remains to be determined. The molecular nature of X and autosomal imprinting may or may not be similar, but both must be such that they can be stably maintained and clonally inherited throughout some periods of development. Possible candidates therefore could be one or more of the molecular mechanisms hypothesized for the stable maintenance and clonal inheritance of X-inactivation (reviewed by Graves, 1983 and Gartler & Riggs, 1983). Consistent with the idea of methylation of cytosine residues being an imprinting mechanism is the finding that oocytes are relatively less methylated than sperm on certain sequences (Sanford, Forrester, Chapman, Chandley & Hastie, 1984) and on a genomic scale (Marilyn Monk, personal communication).

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