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# The role of the sex chromosomes in mammalian germ cell differentiation

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**Summary.** An analysis is presented of the relationship between abnormalities of mammalian germ cell differentiation and their sex-chromosomal make-up. It is concluded that germ cells in an ovary require two functional X chromosomes for optimal development. The effects of X-dosage deficiency are most severe in species where there is a long period of time between the formation and utilisation of the oocytes. Thus the ovaries of XO (Turner's syndrome) and XY women are almost invariably devoid of oocytes. Germ cells in the testis on the other hand, must have only one X chromosome if they are to survive. Consequently adult XX and XXY males have sterile testes.

Ohno (1969) has presented evidence that the genetic content of the sex chromosomes has been conserved during the evolution of the mammals. Students of sex chromosome function are therefore in the enviable position of being able to expect essentially similar sex chromosomal effects in all mammalian species. It is the aim of the present paper to draw some general conclusions about the consequences of sex chromosome activity in the germ cells of eutherian mammals.

In 1961 Lyon put forward convincing arguments for there being only one functional X chromosome in the cells of female mammals. However, Ohno and coworkers found that both X chromosomes were euchromatic (indicating functional capacity) in oogonia and oocytes from fetal ovaries of the rat, hamster, human and mouse (Ohno, Kaplan and Kinosita, 1961 ; Ohno and Weiler, 1961 ; Ohno, Klinger and Atkin, 1962 ; Ohno, 1963). Recent biochemical observations have confirmed that both Xs are functional in oocytes (Epstein, 1969 ; 1972 ; Gartler *et al.*, 1972 ; Gartler, Liskay and Gant, 1973 ; Kozak, McLean and Eicher, 1974 ; Gartler, Andina and Gant, 1975 ; Mangia, Abbo-Halbach and Epstein, 1975) and probably also in oogonia (Migeon and Jelalian, 1977). Thus, female germ cells do not conform to Lyon's hypothesis of X-dosage compensation, both X chromosomes being functional throughout most of their lifespan. As will become evident later, both Xs are also functional in XX germ cells which have colonised a testis.

In the following sections, the role of the sex chromosomes in germ cell differentiation will be assessed by following the fate of XO and XY germ cells in an ovary, and of XX, XO and XXY germ cells in a testis.

## The sex chromosomes and germ cell fate in an ovary

*XO germ cells in an ovary.*

It is well known that XO women are typically sterile, their ovaries lacking both oocytes and follicles. Germ cells are, however, present in the ovaries of XO fetuses (Singh and Carr, 1966). Germ cell loss begins late in fetal life, and few oocytes remain by the time of birth (Carr, Haggar and Hart, 1968). Jirásek (1977) has observed that there is incomplete enclosure of the oocytes by the developing follicles in XO fetuses, and suggests that this is the cause of the subsequent oocyte loss. Burgoyne (1978) on the other hand, has argued that the genetic basis for XO gonadal dysgenesis is the X-dosage deficiency in the oocytes. These two points of view are not mutually exclusive : X-dosage deficient oocytes may induce poor follicular development, and this in turn may accelerate the processes leading to oocyte death.

In contrast to XO women, XO mice are fertile. Nonetheless, two studies have shown that they also are subject to considerable reproductive impairment. Thus Lyon and Hawker (1973) found that the reproductive lifespan of XO mice was markedly reduced due to premature exhaustion of the supply of oocytes ; while Burgoyne and Biggers (1976) have shown that the development of preimplantation embryos from XO mice *in vitro* is severely impaired. Apparently, X-dosage deficiency impairs the health of the oocytes, shortening their lifespan and reducing the viability of the embryos they generate.

The authors of both these studies went on to suggest that the important difference between XO mice and women may be one of timescale ; XO mice reaching puberty before X-dosage deficiency effects in the oocytes become severe, XO women reaching puberty after all oocytes have degenerated. The available data on ovarian morphology and fertility for other mammalian XOs is consistent with this hypothesis ; XOs from species with a short generation time being fertile, while those from species with a much longer generation time are sterile (table 1).

TABLE 1

*Fertility status and ovarian morphology of mammalian* XOs

| Species | Fertility status | Ovarian morphology | References |
|---|---|---|---|
| Human<br>Rhesus monkey<br>Horse | Sterile | Streak ovaries | Review. Simpson, 1976<br>Weiss *et al.*, 1973<br>Chandley *et al.*, 1975 ;<br>Hughes *et al.*, 1975 |
| Cat (4 day old) | — | Oocytes and follicles present | Norby *et al.*, 1974 |
| Black rat<br>Mole rat<br>Wood lemming<br>Field mouse<br>House mouse | Fertile or presumptively fertile | Oocytes and follicles present | Yong, 1971<br>Sharma and Raman, 1971<br>Gropp *et al.*, 1976<br>Bianchi and Contreras, 1967<br>Cattanach, 1962 |

*XY germ cells in an ovary.*

XY germ cells in the fetal testis can enter a « female-type » meiosis in response to the meiotic inducer produced by the fetal ovary (Byskov and Saxén, 1976 ; O and Baker, 1976), but these meiotic cells soon genenerate. The question to be discussed here, is whether such meiotic XY germ cells will survive and form functional oocytes if they are present in an ovary.

In the human there is an inherited condition in which genetic males differentiate as females (Goldstein and Wilson, 1974). These XY women have streak ovaries just like XO women, and it can again be argued that this is a consequence of X-dosage deficiency in the oocytes (Burgoyne, 1978). In the mouse, X-dosage deficiency does not lead to sterility (XO mice are fertile), so that in contrast to the human, XY germ cells in mice should be able to form functional oocytes. As yet no female XY mice have been identified, but some important information has been obtained from two adult XX ↔ XY chimaeric female mice. (NB Most XX ↔ XY mice develop as males — see section on XX germ cells in a testis.) Thus Ford *et al.* (1975) obtained a male mouse which had received its Y chromosome from its XX ↔ XY mother ; while Evans, Ford and Lyon (1977) identified an XY oocyte from the ovary of another XX ↔ XY female. At first sight these observations seem to confirm the expectation that XY oocytes will be functional. However, the male described by Ford *et al.* (1975) had

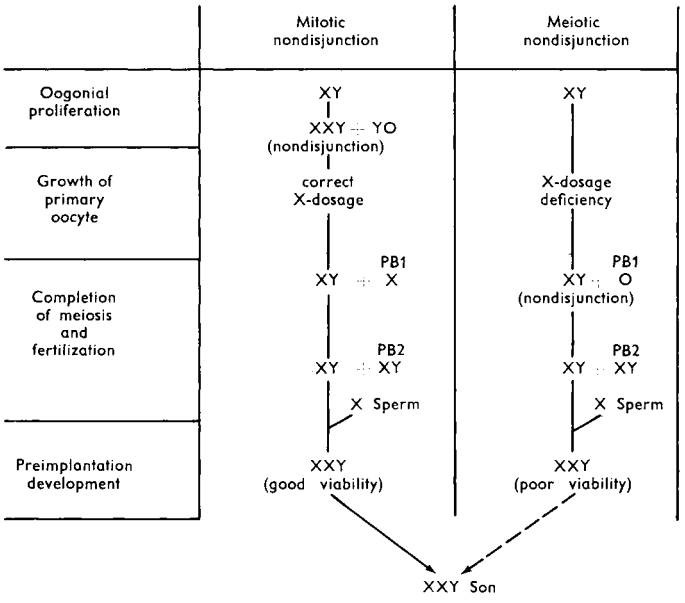


FIG. 1. — *Mitotic and meiotic nondisjunction as alternatives for generating an* XXY *son from an* XX ↔ XY *mother.* PB1, first polar body ; PB2, second polar body. Note that mitotic nondisjunction restores the X-dosage to normal before the growth phase of the oocyte, so that a normally viable XXY embryo should result. In contrast, the XXY embryo produced following meiotic nondisjunction will probably be at a disadvantage when in competition with embryos from XX oocytes.

the rare karyotype XXY, which raises some important questions. If, as seems certain, this male arose by the fertilization of an XY egg by an X sperm, then the XY egg could have arisen by mitotic (oogonial) or meiotic nondisjunction (fig. 1). Burgoyne and Biggers (1976) favoured nondisjunction at an oogonial stage because the resulting XXY oocyte would have the correct X-dosage. This would increase the chances for survival of the XXY embryo, which must compete with embryos from healthy XX oocytes. The alternative possibility of a meiotic error is tentatively supported by Evans, Ford and Lyon (1977) because the XY oocyte they identified had the X and Y as univalents at metaphase 1. (This would facilitate a meiotic error.) However, the observed X-Y separation could be a technical artefact, since this is not an uncommon feature of « air dried » first metaphase preparations from spermatocytes.

*Germ cells in an ovary — conclusions.*

It is concluded that germ cells in an ovary require two functional X chromosomes for optimal growth and development. Germ cells with only a single X are lost from the human ovary long before puberty ; but in the mouse, with its short generation time, X-dosage deficiency leads only to reduced fertility and a shortened reproductive lifespan. Despite unresolved questions, it can also be concluded that a Y chromosome does not prevent a germ cell from developing into a functional oocyte.

## The sex chromosomes and germ cell fate in a testis

*XX germ cells in a testis.*

In the early 1960s techniques were developed which enabled the aggregation of pairs of preimplantation mouse embryos and their subsequent development into single live « chimaeric » mice (Tarkowski, 1961 ; Mintz, 1962). Half of the pairs are XX ↔ XY combinations, and most of these develop as normal fertile males (McLaren, 1976). What is the fate of the XX germ cells in these XX ↔ XY males ? Extensive breeding (996 offspring) from chimaeras in which the XX and XY cell lines carried cell markers, has demonstrated that there are no progeny from the XX cell line (Mystkowska and Tarkowski, 1968 ; Ford *et al.*, 1974 ; McLaren, 1975). In an XX ↔ XY chimaera in which the strain combination allowed the identification of the source of the sperm using morphological criteria, no sperm from the XX cell line were detected (Burgoyne, 1976). Furthermore, all spermatogonia, primary spermatocytes and secondary spermatocytes examined using suitable cytogenetic markers (a total of 855 spermatogenic cells) were derived from the XY cell line (Mystkowska and Tarkowski, 1968 ; Ford *et al.*, 1974). Clearly, XX germ cells do not survive into the adult testis.

There is a considerable body of evidence supporting the generality of this conclusion. Thus adult XX males (resulting from sex-reversal) in the mouse, goat and human (Cattanach, Pollard and Hawkes, 1971 ; Short, 1972 ; De La Chapelle, 1972) almost invariably lack germ cells in their testes. The only reports of XX spermatogenic cells

in adult testes (Benirshke and Brownhill, 1963 ; Hampton, 1973) have recently been questioned by Ford and Evans (1977).

The time course for XX germ cell loss has been studied in sex-reversed mice and goats. Cattanach, Pollard and Hawkes (1971) found that 16 day mouse fetuses had an apparently normal complement of germ cells, but by the time of birth most were degenerating. A few underwent spermatogonial divisions, but by day 10 after birth, germ cell loss was complete. In the XX male goat germ cell loss also begins late in gestation, and seems to be complete by the time of birth (Short, 1972).

XX germ cells differ from XY germ cells in possessing an extra X chromosome and in lacking a Y chromosome. It must be one or other of these differences which is responsible for the death of XX germ cells in a testis. Occasionally, small pockets of meiotic cells are found in the testes of adult XX male mice, and cytogenetic analysis has shown that these meiotic cells are XO (Lyon, 1974). Thus, the loss of the second X by a disjunctional accident allows the germ cells to survive into the adult testis and enter meiosis. The death of testicular XX germ cells must therefore be due to their having two X chromosomes ; implying that both Xs are functional, just as they are in oocytes.

XO *germ cells in a testis.*

Cattanach, Pollard and Hawkes (1971) have obtained XO males by introducing the sex-reversing mutation $S \times r$ into XO mice. These XO males proved to be sterile. Histological examination revealed that although there was spermatogenic activity throughout their testes, many of the spermatogenic cells degenerated during the meiotic stages, and few sperm were produced. However, these observations do not provide unequivocal answers as to the developmental potential of testicular XO germ cells, because the germ cells are XO, $S \times r$, raising the possibility that the $S \times r$ mutation is responsible for their partial success, or even their eventual failure. An XO $\leftrightarrow$ XY chimaera would be a better model for testing the functional capacity of XO germ cells in a testis, but attempts to produce these chimaeras have not been fruitful.

XXY *germ cells in a testis.*

If, as argued earlier, the presence of two X chromosomes in a testicular germ cell leads to perinatal germ cell death, then XXY individuals should be sterile. This is indeed the case for all mammalian species in which XXY individuals have been identified (table 2). Some data on the timing of XXY germ cell loss is available for the human. In three boys aged 3, 4 and 12 months, Mikamo *et al.* (1968) found the number of spermatogonia to be only 24, 18 and 0.1 p. 100 of controls. In prepubertal boys aged 7-12 years (Ferguson-Smith, 1959) testicular biopsies revealed two types of tubules. Most lacked spermatogonia, but very occasionally fertile tubules were encountered. Fertile tubules are also occasionally found in Klinefelter men, especially when there is overt XY/XXY mosaicism (Paulsen *et al.*, 1968). The present author is of the opinion that the fertile tubules in XXY boys and XXY men are the result of localised repopulation by XY germ cells, these having originated from XXY

germ cells by disjunctional accidents (cf. XO germ cells in the testes of XX male mice). Contrary to this is the finding of XY and XXY spermatocytes in an XXY man by Skakkebaek, Philip and Hammen (1969) ; but the XXY spermatocyte illustrated was unconvincing and, as the authors admitted, the preparations were of poor quality. Meiotic studies of XXY men (with fertile tubules) using modern banding methods may eventually resolve this question.

TABLE 2

*Mammalian* XXYs

| Species | Testicular morphology (Adult) | References |
|---|---|---|
| Man | Small testes lacking spermatogenic cells [1] | Review. Simpson, 1976 |
| Cow | | Rieck, 1970 |
| Sheep | | Bruere, Marshall and Ward, 1969 |
| Pig | | Breeuwsma, 1968 |
| Dog | | Clough *et al.*, 1970 |
| Cat | | Centerwall and Benirschke, 1975 |
| Mouse | | Cattanach, 1961 |
| Wood lemming | | Gropp *et al.*, 1976 |

[1] Occasionally small pockets of spermatogenic cells are present.

*Meiotic germ cells in the testes of fetal mouse chimaeras.*

This discussion would be incomplete without mention of the meiotic cells which have been found in the fetal testes of XX ↔ XY mouse chimaeras (Mystkowska and Tarkowski, 1970). It should be pointed out that meiotic cells do not appear in normal testes until about two weeks after birth. The appearance of these fetal meiotic cells follows the time course for meiotic cells in the fetal ovary, and they lack the features (sex vesicle, late-labelling sex chromosomes) of meiotic germ cells in the normal adult testis (McLaren, Chandley and Kofman-Alfaro, 1972). It was therefore considered likely that these meiotic cells were XX and not XY. More recent work (Byskov and Saxén, 1976) has nevertheless shown that XY germ cells in the fetal mouse testis can enter meiosis in response to a meiotic inducer produced by contiguous fetal ovarian grafts. This inducer probably comes from the ovarian rete (Byskov, 1974). In the light of this it would be reasonable to suppose that in XX ↔ XY males there is localised production of the meiotic inducer by XX (rete ?) cells, and that this causes germ cells in the vicinity to enter meiosis. This would explain why the germ cells follow the female schedule for entry into meiosis, but this interpretation would also predict that XX and XY germ cells would enter meiosis. This is not necessarily at variance with the observations of McLaren, Chandley and Kofman-Alfaro (1972) because the absence of a sex vesicle and late-labelling sex chromosomes may reflect the immaturity of the meiotic cells, rather than their genotype.

*Germ cells in a testis — conclusions.*

It is concluded that the correct X-dosage (1X) is essential for germ cells to survive in a testis. The question as to whether a germ cell must have a Y chromosome in order to generate functional spermatozoa is still unresolved.

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**Résumé.** Une analyse est présentée sur les relations entre les anomalies de la différenciation des cellules germinales selon leur équipement en chromosomes sexuels. On doit conclure que les cellules germinales dans un ovaire nécessitent la présence de deux chromosomes X actifs, pour que leur développement soit optimal. Les effets des déficiences dans le dosage de l'X sont plus sévères chez les espèces où se déroule une longue période de temps entre la formation et l'utilisation des ovocytes. Ainsi, les ovaires des XO (Turner) et des femmes XY sont invariablement vides d'ovocytes. Au contraire, les cellules germinales dans le testicule doivent seulement avoir un X pour survivre. C'est pourquoi les mâles XX ou XXY ont des testicules stériles.

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