J. Embryol. exp. Morph. 88, 327–332 (1985)
Printed in Great Britain © *The Company of Biologists Limited 1985*

Absence of a sex vesicle in meiotic foetal germ cells is consistent with an XY sex chromosome constitution

HEATHER HOGG AND ANNE MCLAREN

MRC Mammalian Development Unit, Wolfson House (University College London), 4 Stephenson Way, London NW1 2HE, U.K.

SUMMARY

Sex vesicles were not seen in meiotic germ cells isolated from male and female foetal adrenals, although they were readily identified in adult male meiotic germ cells prepared by the same air-drying method. It is suggested that the failure of the XY germ cells from the male adrenals to develop a sex vesicle is due to their embarking on oogenesis rather than spermatogenesis, and that the absence of a sex vesicle does not necessarily indicate lack of a Y chromosome.

INTRODUCTION

In normal male mice, XY germ cells in the testis enter meiosis only after birth, in the course of spermatogenesis. Meiotic prophase in such spermatogenic germ cells is characterized from the late zygotene stage onwards by the presence at the periphery of the nucleus of a large sex vesicle (Sachs, 1954), in which are located the X and Y chromosomes (Solari, 1964, 1974). No such sex vesicle is found in XX germ cells entering meiosis prenatally in the mouse ovary.

Aggregation chimaeras containing both an XX and an XY cell population usually develop as males (McLaren, 1975). These chimaeric males were found often to possess a few germ cells in meiotic prophase before birth, in the foetal testis (Mystkowska & Tarkowski, 1970). There was no indication as to whether these meiotic germ cells were all from the XX population, programmed to enter meiosis prenatally, or whether they also included XY cells, perhaps induced to enter meiosis before birth by the presence of patches of XX somatic cells in the gonad. McLaren, Chandley & Kofman-Alfaro (1972), having confirmed the earlier findings, made air-dried preparations of the meiotic germ cells from foetal testes, in order to address this question. The meiotic cells all proved to lack a sex vesicle, and after exposure to [^{3}H] thymidine did not show the pattern of incorporation characteristic of XY germ cells. McLaren *et al.* (1972) interpreted these findings as giving strong support to the view that the meiotic germ cells were from the XX germ cell population only.

However, as pointed out by Burgoyne (1978), this conclusion rests on the assumption that XY germ cells in meiotic prophase would exhibit a sex vesicle at whatever stage of development they entered meiosis. An opportunity to test this

Key words: sex vesicles, germ cells, meiosis, oogenesis, adrenals, mouse embryo.

assumption arose when Upadhyay & Zamboni (1982) reported that the adrenal glands of mouse foetuses contained some germ cells, and that these germ cells entered meiotic prophase at the same stage of foetal development as did ovarian germ cells, irrespective of whether the adrenals belonged to a female or a male foetus.

We therefore decided to isolate meiotic germ cells from the adrenals of normal male foetuses presumed to be XY in chromosome constitution, and to examine them for the presence of a sex vesicle. While this study was in progress, a preliminary report appeared (Kundu, Winking & Gropp, 1983), stating that no sex vesicles could be found in foetal oocytes of XY sex-reversed mice, developing as females rather than as males because their Y chromosome was introduced from a very distantly related source (Eicher, Washburn, Whitney & Morrow, 1982).

MATERIALS AND METHODS

Air-dried primary spermatocytes from adult testes were prepared according to the procedure of Meredith (1969) or Evans, Breckon & Ford (1964). Sex vesicles were more prominent with the procedure of Evans *et al.* and this technique was therefore modified so that it could be used with very small amounts of tissue.

Mice were from the MF1 (Olac) randomly bred strain. Male and female adrenals, and ovaries to provide negative controls, were dissected from foetuses 16½ days *post coitum*. Tissues of the same type were pooled within a litter. A small piece of adult testis tubule provided positive controls. All tissues were dissected into Hepes-buffered medium (M2; Whittingham, 1971), and treated as follows.

After rinsing in 2·2 % sodium citrate, the tissues were transferred to 0·02 % EDTA solution (De Felici & McLaren, 1982) for 10 min, then in about 0·3 ml 2·2 % sodium citrate the tissues were pricked and agitated with a needle to release as many cells as possible. The cell suspension was centrifuged at 170 *g* for 5 min, and the pellet resuspended in 1 % sodium citrate for 12 min. The cells were again centrifuged, and resuspended in fixative (3:1 ethanol: acetic acid with up to 1 part in 40 of chloroform added). After 5 min this procedure was repeated, and after a further 10 min the cells were spun down and resuspended in the small amount of fixative left after decanting the supernatant. After air drying on a clean slide, the cells were stained in 1 % Giemsa for 15 min. Siliconized pipettes were used throughout.

RESULTS

The results are shown in Table 1. In the preparations of adult testis, most of the germ cells in meiotic prophase showed a clear sex vesicle from zygotene onwards (Fig. 1). Comparable meiotic stages in preparations of foetal ovary did not show a sex vesicle. In the preparations of foetal adrenals, germ cells in meiotic prophase (mostly zygotene and pachytene stages) could readily be identified. As can be seen from Table 1, these meiotic germ cells lacked sex vesicles, whether the foetal adrenals were derived from female (Fig. 2) or from male (Fig. 3) foetuses.

DISCUSSION

We have shown that XY germ cells entering meiosis before birth in male foetal adrenals do not develop a sex vesicle. It follows that absence of a sex vesicle can no longer be adduced as evidence that the meiotic germ cells in XX↔XY chimaeric foetal testes do not include XY germ cells (McLaren *et al.* 1972).

Mystkowska & Tarkowski (1970) and Tarkowski (1978) originally proposed that XY and XX germ cells contribute indifferently to the population of meiotic germ cells in chimaeric foetal testes. The following lines of evidence suggest that this view is correct:

(1) The number of germ cells entering meiosis is small, so could in any case represent only a subset of XX germ cells. (2) The distribution of meiotic germ cells, adjacent to the mesonephric region of the urogenital ridge, is similar to that seen in the foetal testis of sex-reversed (*Sxr*) XX mice, which have a piece of Y chromosome attached to one X chromosome (McLaren, 1981): this spatial localization suggests that entry into meiosis is associated with some environmental factor, rather than with the chromosome constitution of the germ cell itself. (3) XY germ cells are known to be capable of entering meiosis before birth, not only in the male adrenal (Upadhyay & Zamboni, 1982), but also in the foetal testis if its structure is disrupted by transplantation (Ożdżenski, 1972) or explantation (Byskov & Saxen, 1976). (4) Meiotic germ cells can still be detected in the foetal testis of XX↔XY chimaeras, even when the XX component is homozygous for the white-spotting gene (*W*) that eliminates all or almost all its germ cells from the gonad, leaving only the XY germ cell population (McLaren & Buehr, unpublished observations).

It is possible that the sex chromosome constitution of meiotic germ cells could be determined directly, using *in situ* hybridization of a DNA probe that hybridizes preferentially to Y chromosome sequences (for review, see Stewart, 1983). Sequences homologous to the snake DNA probe Bkm have been shown to be concentrated in the Y chromosome, and *in situ* hybridization shows a concentration of labelling over the sex vesicle when meiotic germ cells from adult mouse testis are hybridized with Bkm (Singh & Jones, 1982).

Why should XY germ cells entering meiotic prophase before birth fail to develop a sex vesicle? The suggestion that it is due merely to their immaturity (Burgoyne, 1978) has at present no evidence to support it. Absence of a sex vesicle

Table 1. *Presence of a sex vesicle in germ cells in meiotic prophase in mouse gonads and adrenals*

Tissue of origin	No. of meiotic germ cells examined	Sex vesicle Present	Absent	Unscorable
Adult testis	112	76	26	10
Foetal ovary	98	2	91	5
Foetal adrenal:				
Female	41	0	35	6
Male	51	0	45	6

Slides were scanned, and the location of all nuclei in meiotic prophase recorded. The nuclei were then examined by two independent observers, and the presence or absence of a sex vesicle was noted in those nuclei suitable for scoring (i.e. in zygotene, pachytene or diplotene, well spread, and not obscured by other bodies). A small number of nuclei on which the observers disagreed on suitability for scoring have been omitted.

has been reported in two cases of abnormal spermatogenesis, an azoospermic man (Hulten, Solari & Skakkebaek, 1974), and a sterile hybrid between two species of muntjac (Liming & Pathak, 1981). In both, the spermatocytes were degenerating, and synaptonemal complex formation was defective. It seems unlikely that the failure of XY meiotic cells to develop sex vesicles in the foetal adrenal is a sign of degeneration: Zamboni & Upadhyay (1983) report that though some of the meiotic germ cells were degenerating, most showed normal synaptonemal complexes, and the germ cells subsequently differentiated into growing oocytes, each surrounded by a zona pellucida. It therefore seems more likely that the absence of sex vesicle development in germ cells entering meiosis prenatally stems from the fact that they are thereby committed to oogenesis rather than to spermatogenesis.

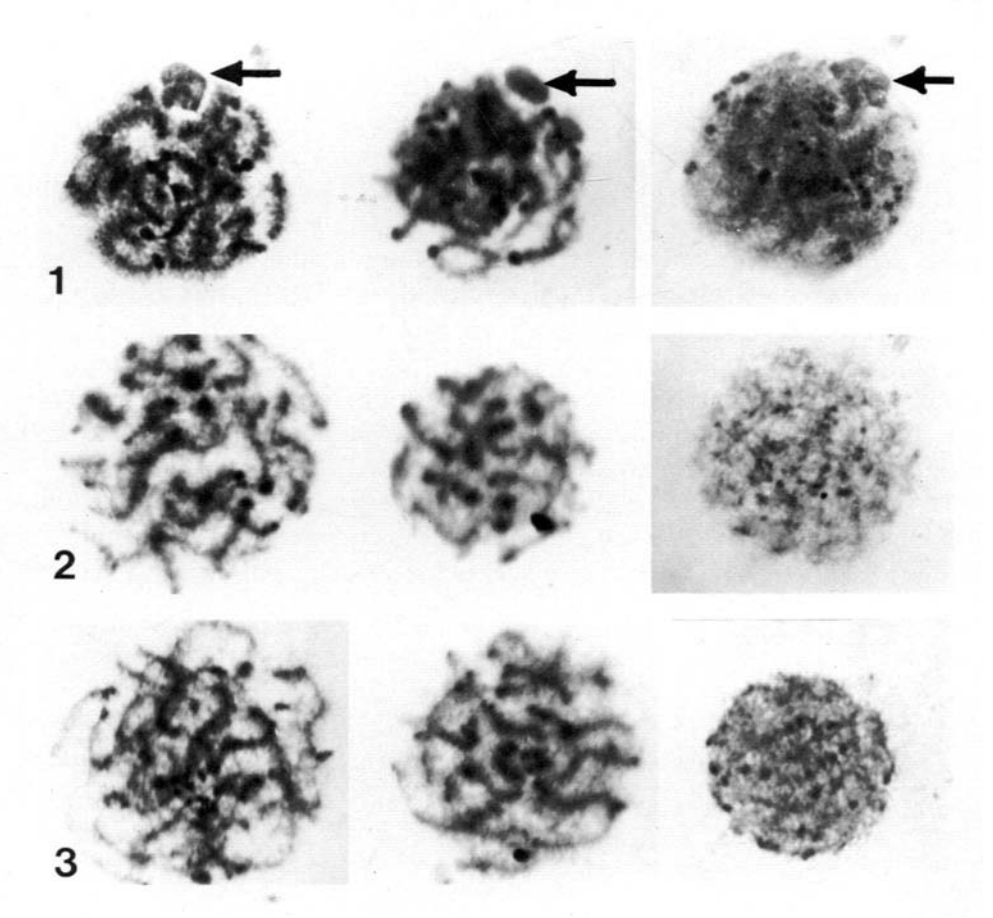

Fig. 1. Germ cell nuclei in meiotic prophase, from adult testes, showing sex vesicles (arrowed).

Fig. 2. Germ cell nuclei in meiotic prophase, from female foetal adrenals. No sex vesicles are apparent.

Fig. 3. Germ cell nuclei in meiotic prophase, from male foetal adrenals. No sex vesicles are apparent.

In all the known instances in which XY germ cells, or any germ cells in the testis, enter meiosis before birth, growing oocytes have been detected after birth. This is so for the adrenal (Upadhyay & Zamboni, 1982; Zamboni & Upadhyay, 1983), for the transplanted testis (Ożdżenski, 1972), for male XX↔XY chimaeras (Mystkowska & Tarkowski, 1970), for XX *Sxr* mice (McLaren, 1980), and for XY sex-reversed mice (Eicher *et al.* 1982). We may therefore presume that prenatal onset of meiosis is an integral part of the process of oogenesis in the mouse, and any germ cell entering meiosis before birth goes on to develop as an oocyte.

The suggestion that the development of a sex vesicle is associated with spermatogenesis as such, rather than with the presence of an entire Y chromosome, is supported by the observation that XO *Sxr* spermatocytes possess a characteristic sex vesicle, though they lack all but a fragment of the Y chromosome (Levy & Burgoyne, unpublished observations), as well as by the reported absence of a sex vesicle in XY oocytes of sex-reversed XY female mice (Kundu, Winking & Gropp, 1983). Why should a sex vesicle develop during spermatogenesis and not during oogenesis? Solari (1974) points out that the sex vesicle has many features in common with the sex chromatin or Barr body in XX cells, and that both contain an inactivated X chromosome. Indeed, it is argued that inactivation of the X chromosome is a critical control step in spermatogenesis (Lifschytz & Lindsley, 1972). Since during oogenesis both X chromosomes are active, it is perhaps not surprising that no sex vesicle develops, even when the germ cell contains a Y chromosome.

We are grateful to Ann Chandley (MRC Clinical and Population Cytogenetics Unit) for checking some of our preparations and for helpful comments, and to Elaine Levy for technical advice.

REFERENCES

Burgoyne, P. S. (1978). The role of the sex chromosomes in mammalian germ cell differentiation. *Ann. Biol. anim. Bioch. Biophys.* **18**, (2B), 317–325.

Byskov, A. G. & Saxen, L. (1976). Induction of meiosis in fetal mouse testis *in vitro*. *Devl Biol.* **52**, 193–200.

De Felici, M. & McLaren, A. (1982). Isolation of mouse primordial germ cells. *Expl Cell Res.* **142**, 476–482.

Eicher, E. M., Washburn, L. L., Whitney, T. B. & Morrow, K. W. (1982). *Mus poschiavinus* Y chromosome in the C57BL/6J murine genome causes sex reversal. *Science* **217**, 535–537.

Evans, E. P., Breckon, G. & Ford, C. E. (1964). An air-drying method for preparations from mammalian testes. *Cytogenetics* **3**, 289–294.

Hulten, M., Solari, A. J. & Skakkabaek, N. E. (1974). Abnormal synaptonemal complex in an oligochiasmatic man with spermatogenic arrest. *Hereditas* **78**, 105–116.

Kofman-Alfaro, S. & Chandley, A. (1970). Meiosis in the male mouse. An autoradiographic investigation. *Chromosoma (Berl.)* **31**, 404–420.

Kundu, S. C., Winking, H. & Gropp, A. (1983). Meiosis in XY sex-reversed female mice. *Abstr. No. 271, Proc. XV Intl Congr. Genet.*, New Delhi.

Lifschytz, E. & Lindsley, D. L. (1972). The role of X-chromosome inactivation during spermatogenesis. *Proc. natn. Acad. Sci., U.S.A.* **69**, 182–186.

Liming, S. & Pathak, S. (1981). Gametogenesis in a male Indian muntjac × Chinese muntjac hybrid. *Cytogenet. Cell Genet.* **30**, 152–156.

McLaren, A. (1975). *Mammalian Chimaeras*. Cambridge: Cambridge University Press.

McLaren, A. (1980). Oocytes in the testis. *Nature* **283**, 688–689.

McLaren, A. (1981). The fate of germ cells in the testis of fetal sex-reversed mice. *J. Reprod. Fert.* **61**, 461–467.

McLaren, A., Chandley, A. C. & Kofman-Alfaro, S. (1972). A study of meiotic germ cells in the gonads of foetal mouse chimaeras. *J. Embryol. exp. Morph.* **27**, 515–524.

Meredith, R. (1969). A simple method for preparing meiotic chromosomes from mammalian testis. *Chromosoma (Berl.)* **26**, 254–258.

Mystkowska, E. T. & Tarkowski, A. K. (1970). Behaviour of germ cells and sexual differentiation in late embryonic and early postnatal mouse chimaeras. *J. Embryol. exp. Morph.* **23**, 395–405.

Ożdżenski, W. (1972). Differentiation of the genital ridges of mouse embryos in the kidney of adult mice. *Archs Anat. Microsc.* **61**, 267–278.

Sachs, L. (1954). Sex-linkage and the sex chromosomes in man. *Ann. Eugenics (Lond.)* **18**, 255–261.

Singh, L. & Jones, K. W. (1982). Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent nonreciprocal crossover involving the X and an aberrant Y chromosome. *Cell* **28**, 205–216.

Solari, A. J. (1964). The morphology and ultrastructure of the sex vesicle in the mouse. *Expl Cell Res.* **36**, 160–168.

Solari, A. J. (1974). The behaviour of the XY pair in mammals. *Int. Rev. Cytol.* **38**, 273–317.

Stewart, A. D. (1983). The role of the Y-chromosome in mammalian sexual differentiation. In *Development in Mammals*, Vol. **5** (ed. M. H. Johnson), pp. 321–367. Elsevier Science Publications, B.V.

Tarkowski, A. K. (1978). Germ cells in XX↔XY mouse chimeras. In *Genetic Mosaics and Chimeras in Mammals* (ed. L. B. Russell), pp. 135–142. New York and London: Plenum Press.

Upadhyay, S. & Zamboni, L. (1982). Ectopic germ cells: Natural model for the study of germ cell sexual differentiation. *Proc. natn. Acad. Sci., U.S.A.* **79**, 6584–6588.

Whittingham, D. W. (1971). Culture of mouse ova. *J. Reprod. Fert. Suppl.* **14**, 7–21.

Zamboni, L. & Upadhyay, S. (1983). Germ cell differentiation in mouse adrenal glands. *J. exp. Zool.* **228**, 173–193.

(*Accepted 16 April 1985*)