Prenatal diagnosis and gonadal findings in X/XXX mosaicism¹

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SUMMARY Prenatal diagnosis of a case of X/XXX mosaicism is presented. In spite of the fact that over 50% of the cells cultured from both ovaries were trisomic for the X chromosome, fetal oocytes were rarely found. This case illustrates that the presence of a triple-X cell line, even in a relatively high percentage of ovarian cells, does not necessarily protect the ovary from 'aöogenesis'. This observation might prove useful in the counselling of future cases involving the prenatal detection of sex chromosome mosaicism.

The observation of chromosomal mosaicism in amniotic fluid cell cultures has repeatedly presented interpretational difficulties (Hsu *et al.*, 1973; Cox *et al.*, 1974; Kohn *et al.*, 1975). Moreover, prediction of the phenotype in cases of sex chromosome mosaicism is extremely hazardous because of the wide clinical variability often associated with such conditions. We wish to report the first prenatal detection of X/XXX mosaicism and to describe the anatomical findings in the subsequently aborted fetus and placenta.

Case report and cytogenetic findings

A 41-year-old woman was referred to the Department of Human Genetics for amniocentesis, because of advanced maternal age. She was the mother of 4 healthy children but had previously experienced 2 first trimester spontaneous abortions. Trans-abdominal amniocentesis was performed during the 19th week of gestation and two 20 ml aliquots of amniotic fluid were withdrawn. From each aliquot, one culture from 3 ml unspun fluid, and 2 cultures from the centrifuged cells were initiated. Cultures initiated from each aliquot were propagated in separate laboratories. All cells were cultured in Ham's F-10 nutrient medium, containing 30% fetal calf serum (GIBCO), supplemented with penicillin and streptomycin and maintained at 37°C in an atmosphere of 5% CO₂ in air.

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Chromosome analysis of primary cell cultures from separate culture flasks and different amniotic fluid aliquots, examined 10 to 14 days after amniocentesis, all yielded the same mosaic condition. From a total of 50 cells, 23 had a chromosome of group C missing (2N=45) while 19 had an extra C-group element (2N=47) (Table). Those cells with 44 chromosomes showed random loss. Though the cells with 46 chromosomes might have been normal, detailed analysis revealed that they were actually 2N=47 cells which had undergone random chromosome loss. Giemsa banding (Patil *et al.*, 1971) showed that in each case the missing and extra element was an X chromosome, indicating X/XXX mosaicism. This finding was discussed with the patient who elected to terminate the pregnancy. Intra-amniotic instillation of hypertonic urea was performed during the 23rd week of gestation.

The resultant fetus, a female, measured 36 cm crown heel (CH) length, and weighed 700 g (corresponding to 23 to 24 weeks of gestation). Though

Table Chromosome analysis of various tissues cultured

Cells studied	Karyotype					Total
	44,X	45,X	46,XXX	47,XXX	48,XXX,+C	
Amniocentesis	2	23	6	19		50
Fetal cultures						
Skin	1	9	2	13		25
Lung	1	10	5	33	1	50
R. ovary		9	2	14		25
L. ovary	1	12	1	10		24
Total	5	63	16	89	1	174

Fig. (a) Microphotograph of a human fetal ovary at the beginning of the 7th month of gestation. Note numerous oocytes surrounded by follicular cells. (H and E $\times 290$.) (b) Microphotograph of the ovary of the X/XXX fetus at the end of the sixth month of gestation. Note hypercellular stroma with only few oocytes (arrows), the majority being in various phases of degeneration. (H and E $\times 290$).

oedematous, as often occurs after pregnancy interruption by hypertonic solutions, the fetus was without external anomalies. No gross anomalies were found except that the ovaries, uterus, and Fallopian tubes proved to be small for gestational age. Microscopical examination of serial sections showed the uterus and Fallopian tubes to be histologically normal while the ovaries showed an increase of both stroma and the number of blood vessels. Only very few 'normal' oocytes were found. However, oocytes in different phases of degeneration, sometimes surrounded by follicular cells, were found in approximately half of the sections (Fig.). These observations were compared with those of 5 normal female fetuses (gestational age 4 to 7 months) whose ovaries showed a large number of oocytes, some surrounded by follicular cells, and to one 45,X fetus (17 to 18 weeks) with bilateral cystic hygroma of the neck, whose ovaries contained only a few oocytes.

The placenta (160 g) showed subchorionic haemorrhage, subchorionic fibrin thrombi, desquamation of the amniotic epithelium, and necrosis of subchorionic villi. All these changes are commonly observed after pregnancy interruption by hypertonic urea (Segal *et al.*, 1976).

Cell cultures were established from skin, lungs, and both ovaries and chromosome analyses of these tissues are presented in the Table.

Discussion

Sex chromosome mosaicism is often associated with highly variable clinical manifestations in living individuals. Therefore, when observed in cultured amniotic fluid cells, this phenomenon poses a particularly difficult and vexing problem. In mosaic gonadal dysgenesis (Turner's syndrome), the most frequent finding is a monosomic 45,X cell line, whose influence may or may not be expressed (Hecht and MacFarlane, 1969). The phenotype in such mosaics may vary from classic Turner's syndrome to an almost normal, fertile female (German, 1971), probably as determined by both type and degree of mosaicism in various tissues and organs.

X/XXX mosaicism has been only infrequently described in living individuals (Hsu and Hirschhorn, 1971). To the best of our knowledge, the present case represents the first instance in which the prenatal detection of this type of sex chromosome mosaicism has been achieved. Several fetal tissues were examined and no evidence of a 46,XX cell line was present. Therefore, the most likely explanation of the observed mosaicism is a non-disjunctional event occurring at the first cleavage division of a normal 46,XX zygote.

Since the triple-X female is usually not grossly

abnormal (Kohn *et al.*, 1968) and has no consistent or well-defined phenotype, one might assume that the clinical picture and prognosis of an X/XXX mosaic would not significantly differ from that of an X/XX individual. Therefore, counselling indicated the possibility that the fetus might express the full Turner's syndrome phenotype or might be a fertile female, the ultimate outcome depending on the distribution of the two cell lines. The patient was also apprised of the possible increased risk of mild mental retardation. On this basis she chose to interrupt the pregnancy.

Gonads, derived from X fetuses, have shown a normal number of germ cells up to the third month of gestation (Singh and Carr, 1966; Weiss, 1971). However, as pregnancy progresses, these germ cells degenerate and the internal ovarian architecture is gradually replaced by streaks of connective tissue. In some X/XX individuals examined, the ovaries may be normal (Leas *et al.*, 1966). However, it is of interest to note that though over 50% of the cells derived from the amniotic fluid, skin, lung, and both ovaries were triple-X, the fetal ovaries were histologically abnormal. In this fetus, most oocytes were already degenerating and the ovaries were fibrotic by the sixth month of fetal life. The uterus and Fallopian tubes were also small for gestational age.

In most cases of X/XX mosaicism short stature is evident, but many additional anomalies commonly associated with 45,X monosomy are frequently absent. In the present case, abnormal morphological findings were confined to the gonads alone. However, minor features of X/XX or X/XXX mosaicism might have escaped attention because of the gestational age of the fetus.

Although about 5% of all spontaneous abortions have an X karyotype, only 3 out of 39 have been mosaics (Carr, 1965, 1971). This observation infers that mosaicism, particularly of the X/XX or X/XXX, types, asserts a protective and/or ameliorating influence on the fetus which is subsequently carried to term. None the less, the assertion that the degree of mosaicism, as reflected in tissue culture, may be indicative of the phenotype is probably erroneous since selection pressures may be operative against either cell line of the mosaic individual (Taysi *et al.*, 1970). Certainly, the present case shows that even a high percentage of triple-X cells in various cultures should not be used as an encouraging sign regarding normal or near-normal ovarian development.

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References

- Carr, D. H. (1965). Chromosome studies in spontaneous abortions. *Obstetrics and Gynecology*, **26**, 308-326.
- Carr, D. H. (1971). Chromosomes and abortion. In *Advances in Human Genetics*, pp. 201-257. Ed by H. Harris and K. Kirschhorn. Plenum Press, London and New York.
- Cox, D. M., Niewczas-Late, V., Riffell, M. I., and Hamerton, J. L. (1974). Chromosomal mosaicism in diagnostic amniotic fluid cell cultures. *Pediatric Research*, **8**, 679-683.
- German, J. (1971). Sex chromosome abnormalities. *Southern Medical Journal*, **64**, 73-80.
- Hecht, F., and MacFarlane, J. P. (1969). Mosaicism in Turner's syndrome reflects the lethality of XO. *Lancet*, **2**, 1197-1198.
- Hsu, L. Y. F., Dubin, E. C., Kerenyi, T., and Hirschhorn, K. (1973). Results and pitfalls in prenatal cytogenetic diagnosis. *Journal of Medical Genetics*, **10**, 112-119.
- Hsu, L. Y. F., and Hirschhorn, K. (1971). Unusual Turner mosaicism (45,X/47,XXX; 45,X/46,XXqi; 45,X/46,XXr): detection through declaration from normal linear or secondary amenorrhea. *Journal of Pediatrics*, **79**, 276-281.
- Kohn, G., Mennuti, M. T., Kaback, M., Schwarz, R. M., Chemke, J., Goldman, B., and Mellman, W. J. (1975). Chromosomal mosaicism in amniotic cell culture. *Israel Journal of Medical Sciences*, **11**, 476-481.
- Kohn, G., Winter, J. S. D., and Mellman, W. J. (1968). Trisomy X in three children. *Journal of Pediatrics*, **72**, 248-252.
- Leas, J. C., Voorhess, M. L., Schlegel, J. R., and Gardner, L. I. (1966). XX/XO mosaicism in nine preadolescent girls. Short stature as presenting complaint. *Pediatrics*, **38**, 972-981.
- Patil, S. R., Merrick, S., and Lubs, H. A. (1971). Identification of each human chromosome with a modified Giemsa stain. *Science*, **173**, 821-822.
- Segal, S., Ornoy, A., Bercovici, B., Antebi, S. O., and Polishuk, W. Z. (1976). Placental pathology in midtrimester pregnancies interrupted by intramniotic injection of hypertonic urea. *British Journal of Obstetrics and Gynaecology*, **83**, 156-159.
- Singh, R. P., and Carr, D. H. (1966). The anatomy and histology of XO human embryos and fetuses. *Anatomical Record*, **155**, 369-384.
- Taysi, K., Kohn, G., and Mellman, W. J. (1970). Mosaic mongolism. II. Cytogenetic studies. *Journal of Pediatrics*, **76**, 880-885.
- Weiss, L. (1971). Additional evidence of gradual loss of germ cells in the pathogenesis of streak ovaries in Turner's syndrome. *Journal of Medical Genetics*, **8**, 540-544.