GERM CELLS IN THE OVARIAS OF XO FEMALE INFANTS

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Individuals with a single X chromosome are phenotypic females. They may present in infancy with shortness of stature or after adolescence with additional signs and symptoms, including lack of breast development, primary amenorrhea, and infertility. Associated anomalies are common, especially involving the skeletal, urinary, and cardiovascular systems. If these anomalies follow a certain pattern, the condition is frequently called Ullrich-Turner's syndrome, after the authors who stressed the striking symptoms and signs.20,21 The most constant internal defects in patients with the XO chromosome anomaly affect the genital tract. The uterus, tubes, and vagina are hypoplastic and the ovaries are replaced by streaks of connective tissue. On histologic examination, these streaks often show a stromal arrangement similar to that seen in the normal ovary, but germ cells are rarely found. Pregnancy has been reported in only one XO female.1

We have had the opportunity to make a cytogenetic and autopsy study of an infant with a verified chromosome complement of 45, XO. There are few reports of neonates with this chromosome anomaly from whom the ovaries were available for study.

REPORT OF CASE

Infant Girl N. The propositus was the fourth child of normal parents, both of whom were 30 years of age and in excellent health. The previous pregnancies had been normal. During her present pregnancy, the mother was exposed to rubella at about the ninth week of gestation but did not develop any symptoms. The gestation period was 40 weeks. On admission to the West Haldimand Hospital, Hagersville, Ontario, the mother's temperature, pulse, and respirations were normal, hemoglobin was 12 Gm. per 100 ml., and urinalysis was not remarkable. After a labor of 17 hr., a small, full-term female infant was delivered spontaneously from the left occipitoanterior position. The baby weighed 2700 Gm. and the fetal heart rate ranged from 130 to 150 per min. The placenta was noted to be very small. The baby was moderately cyanotic and did not improve during the first 2 days of life. She was transferred to St. Joseph's Hospital, Hamilton, Ontario, on January 1, 1967.

On admission the baby's vital signs were: temperature 97.4 F.; pulse 160; respirations 88 per minute. The weight was 2520 Gm. and there was moderate cyanosis. The palate was higher than normal and arched. There was definite webbing of the neck and cubitus valgus. The sternum was shorter than normal. The external genitalia were of normal female infantile development. The legs were flexed. There was marked edema of both feet and lower legs (Fig. 1). The chest was clear, with adequate air entry. Pulmonary function studies revealed a \( \text{PO}_2 \) of 30 mm. Hg, \( \text{PCO}_2 \) of 28.5 mm. Hg, oxygen saturation 50%, blood pH 7.39, and bicarbonate of 17 mM per liter. After she had breathed 100% oxygen for 20 min., the \( \text{PO}_2 \) rose to only 40 mm. Hg, and the oxygen saturation to 74%. Other laboratory tests were within normal limits. A portable radiograph of the chest revealed a prominent vascular pattern of the lungs but the cardiac configuration could not be accurately assessed. An electrocardiograph was suggestive of right ventricular hypertrophy. Blood and stool specimens were obtained for viral studies. These were subsequently reported as negative.

The infant was placed in heat and 50% oxygen in the premature nursery, in isolation. Her temperature remained between 98 and 99 F. and the respirations were rapid. An intravenous pyelogram made on the day after admission was unsatisfactory. Feedings by gavage were well tolerated. The baby was continuously cyanotic and her condition
deteriorated steadily. She died in respiratory distress 6 days after admission, at the age of 8 days, on January 7, 1967.

**Autopsy Findings**

The postmortem examination was performed on a newborn, phenotypically female infant measuring 47 cm. from crown to heel and weighing 2740 Gm. There was symmetrical webbing of the neck, a moderate degree of cubitus valgus, and a short sternum with a depression of the lower chest. There was flexion of the limbs at the elbows, hips, and knees, and the legs could not be completely abducted. The skin of the hands and feet was loose and there was edema of both feet and lower legs as far as the knees. The internal examination revealed marked distention of the stomach with air, probably due to attempts at resuscitation.

The heart was grossly enlarged and the heart and lungs together weighed 100 Gm. The right atrium and ventricle were dilated and the ventricular myocardium was moderately hypertrophied. The foramen ovale was probe-patent and the tricuspid valve ring was dilated. The pulmonary artery divided into normal right and left branches and continued in a smooth curve with the aortic arch via a greatly dilated ductus arteriosus. The left atrium was dilated and the endocardium was yellow-gray and opaque. The mitral valve was of normal size; the left ventricle was hypoplastic with a small chamber and thickened wall. The interventricular septum was intact. The aortic valve was bicuspid and there was a marked degree of stenosis of the valve ring. There was no evidence of coarctation of the aorta. A marked degree of subendocardial fibroelastosis of the left atrium and ventricle, involving both the aortic and mitral valve cusps, was seen microscopically.

The lungs were blue-red, mottled, and firm, and marked medial hypertrophy of the medium-sized arteries and arterioles was seen microscopically. The sepal capillaries were engorged and focal anoxic hemorrhages were present throughout the parenchyma and beneath the pleura. There was focal atelectasis throughout all lobes. Death was attributed to cardiorespiratory failure as a result of congenital aortic stenosis.

The urogenital tract, including the vagina, uterus, and uterine tubes, showed normal infantile development. The ovaries were small and flat; the right one measured 1.5 by 0.3 by 0.2 cm. and the left 1.0 by 0.3 by 0.2 cm. The endometrium consisted of branching cleftlike spaces lined by a single layer of columnar epithelium when seen under the microscope. Longitudinal sections of each ovary revealed that the bulk of each organ consisted of vascular connective tissue that formed the hilus and was covered by a thin cortical layer about 1 mm. in thickness. Within this layer there were numerous closely packed, immature follicles composed of short columnar epithelial cells. No well-developed primary follicles were present; but many of the tubular structures contained degenerating ova. A tabulation of 1000 follicles was made according to whether they were empty, contained normal ova with a large vesicular nucleus and distinct intact nucleolus, or contained degenerating ova. There were 536 empty follicles, 14 with histologically “normal” ova, and 450 containing ova in various stages of degeneration. The earliest form of degeneration appeared to be loss of nucleoli and dissolution of the nuclear chromatin. In some, there was lysis of the nuclear membrane. Most of the degenerated ova consisted of an eosinophilic coagulum within the follicle. In the follicles that were empty, the assumption was made that there had been complete lysis of the ova, with resorption. Apparently normal primordial follicles are shown in Figure 2A. There were also several clusters of germ cells without true follicle formation (Fig. 2B). These were primarily found in the medulla of the ovary. Both cell types were found in blocks of tissue within 1 mm. or so of that used for tissue culture.

**Sex Chromatin and Chromosome Studies**

Sex chromatin was studied in buccal mucosal cells and in cells cultured from an ovarian biopsy. A blood smear was analyzed for “drumstick” appendages. Chromosome analysis was carried out on cells cultured from blood lymphocytes by a standard technic. Sterile pieces of fascia and ovary were removed at autopsy and cultured in Leighton tubes. One hundred cells each from buccal mucosa and tissue culture were
examined; all were chromatin-negative. Drumsticks were absent from 500 polymorphonuclear cells in a blood smear. The modal chromosome number in cultured cells was 45 and karyotype analysis showed an XO sex chromosome complex. There was no evidence of chromosome mosaicism in any of the cells from the three tissues cultured. Further details are given in Table 1.

**DISCUSSION**

In considering the effect of a chromosome anomaly on the phenotype, it is necessary to exclude examples of mosaicism. Therefore, only reports of subjects with a verified 45, XO chromosome complement can be validly compared.

The principal anomalies in the infant re-
TABLE 1

RESULTS OF CHROMOSOME ANALYSIS

<table>
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<th>Culture</th>
<th>Chromosome Counts</th>
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<td>Ovary culture</td>
<td>345 45 46</td>
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ported here involved the cardiovascular and genital systems. Congenital lymphedema of the extremities is common in "the XO syndrome." The most common defect of the heart and great vessels in subjects with a 45, XO genotype is usually said to be coarctation of the aorta. In Table 2 the incidence of this disorder is compared with that of aortic stenosis, the defect found in the subject of the present report. There were 27 subjects with cardiovascular anomalies among 114 XO individuals reported in four studies that included thorough clinical examination. There were few infants among these patients. Of the 27 individuals with cardiovascular anomalies, 15 had coarctation of the aorta and three had aortic stenosis. Detailed studies, including autopsy, are available on only six neonates with an XO sex chromosome complex. Of these, three had coarctation of the aorta, all of the infantile or preductal type. The other three had aortic valve stenosis or atresia associated with subendocardial fibroelastosis similar to that found in the infant who is the subject of the present report.

There have been few descriptions of ovarian pathology in infants with an XO sex chromosome complex. Conen and Glass described postmortem findings in two infants with a 45, XO sex chromosome pattern. In one infant the ovaries were thin and small, and serial sections showed an ovarian type of stroma without ova or follicles. In their second case, the right ovary was almost normal when seen grossly and microscopically. The left ovary was thin and contained fewer ova. Fröland and co-workers found a few primordial follicles in serial sections from the gonads of an XO infant who died at the age of 25 days. Court Brown and co-workers failed to find germ cells in the ovaries of two infants with XO sex chromosomes who died in the neonatal period. Similarly, Hodel and Egli examined the ovaries of a newborn infant with a proven XO karyotype and found no germ cells although there were islands of cells resembling primordial follicles. Although chromosome analysis failed in an infant reported by Shine and Corney, there was presumptive evidence of an XO sex chromosome complex on the basis of Xg blood group results. The child was a female Xg (a−) offspring of Xg (a+) parents and the buccal smear was chromatin-negative. The authors stated simply that "histological examination of the ovaries showed normal tissue."

Singh and Carr found that the ovaries of XO embryos appear to be normal when seen grossly and microscopically. In fetal life there is an increase in the size of connective tissue septa and a probable diminution in granulosa cells in comparison with those in normal fetuses of the same age. It seems likely that germ cells migrate to the gonadal ridge in XO embryos, but degenerate in late fetal life.

The ovarian picture at term is highly variable, as demonstrated by the present case and other reported XO infants who died in the neonatal period. The infant may have streak gonads, devoid of germ cells, which is the almost constant finding in adult females with only one X chromosome; on the other hand, the ovary of an XO infant may be normal. In the present case, the ova

<table>
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were greatly reduced and only 1.4% of follicles were judged to be normal. In very rare instances, the germ cells may persist into adult life, resulting in a fertile female.1

It has been suggested that the absence of gonocytes from the ovaries of XO individuals might be the result of failure of the germ cells to migrate to the gonadal ridge.10 On the basis of the present evidence, however, it seems likely that the XO anomaly affects germ cell maturation in females, just as the XXY anomaly affects spermatogenesis in males with Klinefelter's syndrome. In this condition, germ cells are usually found in the testes before puberty, although their number may be reduced.17 In general, the loss of germ cells from the ovary in the XO anomaly occurs much earlier than their loss from the testis in Klinefelter's syndrome. Normally, the population of germ cells in the ovary declines sharply between 5 months' gestation and term, with a slower decline after birth.2 On the other hand, there is a marked decrease in spermatogonia early in puberty.18 It is precisely at the time that germ cell degeneration normally occurs that the gonocyte population in XO ovaries and XXY testes also appears to decline.

Although gonadal failure is an almost regular finding in adults with XO gonadal dysgenesis, infertile patients are the ones likely to be studied. Because we know that the ovaries of XO infants may be normal and that an adult with this anomaly has conceived, it would be interesting to know whether there are normal, fertile women with only one X chromosome. Because many Papanicolaou smears are studied, chromatin-negative females of normal fertility may some day be discovered. It was by such a study that Close4 found double sex-chromatin masses in the cells of two normal fertile women. Chromosome study confirmed the presence of three X chromosomes in these patients and demonstrated that this anomaly is compatible with normal development.

**SUMMARY**

A case of an XO female, including chromosome analysis and autopsy study, is described. The infant had lymphedema of the extremities, aortic stenosis, a bicuspid aortic valve, and subendocardial fibroelastosis. Although the germ cells in the ovary were greatly reduced, some normal primordial follicles were found. A 45, XO chromosome complement was confirmed in lymphocytes and in fascial and ovarian cells. The literature concerning the ovarian pathology in neonates with an XO sex chromosome complement is reviewed. It is suggested that the sex cells degenerate in this condition because of the chromosome anomaly. The situation appears to be analogous to the one affecting the testes in Klinefelter's syndrome, although complete degeneration does not usually occur in the latter condition until puberty.

**REFERENCES**