PRENATAL DIAGNOSIS OF SEX USING CELLS FROM THE AMNIOTIC FLUID

(A Preliminary Report)

by

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There has always been a natural curiosity of people, specially of the parents, to know the sex of an expected child. For many years attempts have been made to develop a reliable method for antenatal sex determination by different approaches.

Many have professed their ability to predict this by various criteria, e.g. whether the baby is carried "all at the back" or "all at the front", whether a needle suspended over the abdomen rotates clockwise or widdershines, and when in the menstrual cycle, the child is supposed to have been conceived. There is also mention in Egyptian Papyrus: "Another test for a woman who will bear or a woman that will not bear. Wheat and spelt: Let the woman water them daily with her urine.... If they both grow, it will bear; if the wheat grows, it will be a girl. If neither grows, she will not bear."

The more scientific methods have been based on the foetal heart rate, X-ray for foetal scrotal shadow, radiography of the foetal lumbar vertebra, which shows a coronal cleft only in male children, the excretion of 17-ketosteroids in the urine, the changes in the saliva or cytology of vagina of the mother. None of them, however, has stood the test of time and all failed to receive sufficient confirmation for general acceptance.

It has been known that sex determination is related to a pair of chromosomes that differ according to the individual's sex. When the early work on insects was extended to other animal forms and human beings, it was found that the sex determining mechanism depends on females having the XX and males the XY sex-chromosome-complex.

Barr and Bertram (1949) first demonstrated sexual differences in the morphology of intermitotic nuclei in the nerve cells of the female cat and it has come to be known as the "sex chromatin". The sex chromatin has

certain characteristics that make its identification possible. It is a sharply defined plano-convex body, usually lying against the inner surface of the nuclear membrane, and consists of desoxy-ribo-nucleic acid. The size of the sex chromatin is about 0.7 μ x 1.2 μ. The sex chromatin is believed to be formed as a result of the fusion of heterochromatic portions of the two X-chromosomes of female intermitotic cells to form one conspicuous mass of chromatin. It is present in about 75% of female cells and in the male a similar or rather smaller chromatin nodule may be found under 10% of the nuclei. Further studies have shown that sex chromatin may be demonstrated in the various tissues of human and some animals. This subject has recently been reviewed (Baruah, 1960).

In man, the sex chromatin was first observed in the skin biopsies (Moore et al, 1953) and since then most human tissues have been investigated. More recently, it has been found in the smears from the buccal mucosa of newborn infants (Moore and Barr, 1955; Marberger et al, 1955; Dixon and Torr, 1956) and from the vaginal wall (Carpentier et al, 1955) and this has brought nuclear sexing into the field of exfoliative cytology. It has also been demonstrated in the cell nuclei of the amniotic membrane in cats (Graham, 1954) and in early human embryo and foetus (Glenister, 1956).

The recognition of definite sex chromatin mass in the nuclei of epithelial cells in oral and vaginal smears in every female and the accuracy with which the sex can be ascertained cytologically prompted the study of the nuclear morphology of cells in human amniotic fluid in relation to sex of the offspring. The technical concept appears to be directly applicable to the desquamated cellular debris in amniotic fluid and since all such cells are of foetal origin, it should be possible, therefore, to establish the genetic sex of the foetus 'in utero' by this method. Since the original report by Sachs et al (1955), various workers, e.g. Shettles (1956) in New York, Fuchs and Riis (1956) in Copenhagen, Makowski et al (1956) in Minneapolis, James (1956) in Amsterdam and Dewhurst (1956) in Sheffield, have found independently that a diagnosis of foetal sex can be correctly made from examination of amniotic fluid cells.

Using the same technique in our laboratory, we have been able to diagnose accurately the sex of the foetus before birth.

Method

Liquor amnii was obtained (R.K.B.) by artificial high rupture of the membranes with Drew Smythe catheter during induction of labour in some selected cases of prolonged pregnancy. About 10 c.c. of fluid was collected. Care was taken to avoid admixture of maternal cells. The fluid was centrifuged, the vernix and the supernatant fluid were discarded. The sediment cells were spread on a slide, previously coated with egg albumin and fixed at once in Papanicolaou's fixative (equal parts of 95% ethyl alcohol and ether) for 2 to 24 hours, while still moist. After fixation the slides were stained by haematoxylin and eosin,

Papanicolaou and Giemsa stains and all gave good results.

The smears were examined by one of us (B.D.B.) under oil immersion objective without any prior knowledge of the foetal sex at the time of counting. About 50 to 100 cells were counted. The presence of a dark staining plano-convex mass in contact with the nuclear membrane was taken as indicating the presence of sex chromatin and its percentage was determined. The diagnosis of sex was based on determination of the percentage of cells: in the male, it is less than 5% and in the female, it is 30-50% of the nuclei counted. The sex of the new-born infant was established on the basis of external physical examination.

Observations

Amniotic fluid smears from 12 cases were examined. It has been found that many of the nuclei in the amniotic fluid debris were not suitable for identification of the sex chromatin body. Some were so markedly pyknotic and shrunken, too densely stained, or those overlapped by other structures that nuclear detail could not be discerned. While others were degenerated, distorted, took the stain poorly or were badly fragmented. In every smear, however, there were sufficient nuclei, in which the nuclear detail was clear to allow a definite distinction between male and female cells by the absence or the presence of the typical sex chromatin mass in the cell nuclei as in other tissues.

In all these 12 cases (4 male and 8 female), the sex of the foetus was predicted correctly. In smears from cases where females were subsequently born, typical sex chromatin was found in 25-40.8% of the nuclei suitable for study (Fig. 1). While

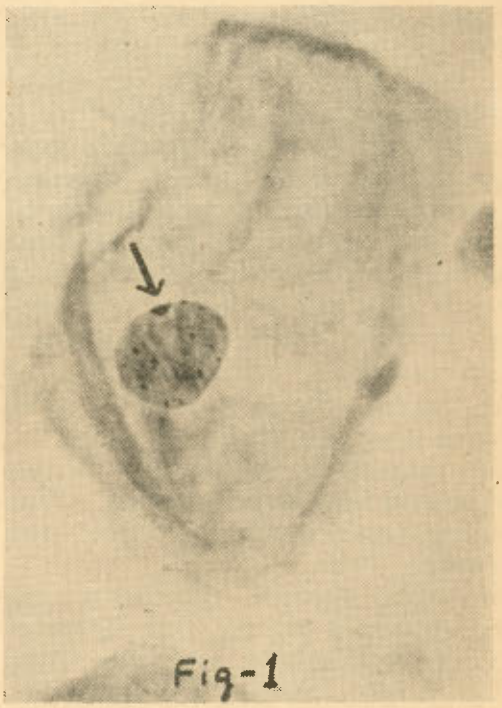

Fig. 1
Photomicrograph of a cell from the amniotic fluid smear. Note the compact mass in contact with the nuclear membrane, indicating female sex. H.E. x 1500.

in smears from cases where males were subsequently born, only 4.2-7.8% showed such an arrangement. Fig. 2 shows the incidence of the sex chromatin in the amniotic cells in these cases. It may be seen that the mean incidence of sex chromatin in the nuclei found in the fluid of female foetuses (32%) is several times that found in male foetuses (4%) and there is no overlap between the maximum counts on male foetuses and the minimum on

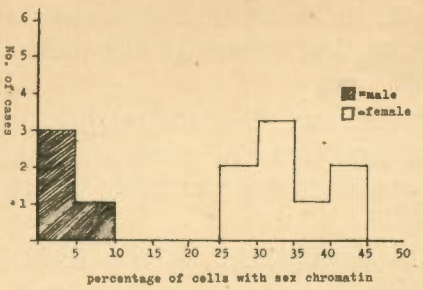

Fig. 2
Histogram showing percentage of cells containing sex chromatin in the amniotic fluid from 12 cases. The figures have been grouped at intervals of 5.

females. Since there is no erroneous diagnosis, these observations are highly significant.

Comment

It seems clear from these results that with careful study of a properly prepared and stained specimen from the amniotic fluid, a correct forecast of the sex of the foetus is possible. Difficulties in interpretation are due to cell shrinkage simulating sex chromatin mass and to debris partly obscuring nuclear detail. In all cases there have been a sufficient number of diagnosable cells, but the search for sufficient number of nuclei with good definition is very arduous and time consuming.

The possible sources of origin of the cells in the amniotic fluid include the skin, gastro-intestinal, respiratory and genito-urinary tracts, umbilical cord and amnion. Tissues and smears from each of these sources have been found to contain in females the typical sex chromatin mass (Marberger et al, 1955; Moore and Barr, 1954, 1955). The degree of variation in morphology of the cells in the amniotic fluid is in agreement with the possible multiple sources of their origin, however, the nuclear chromosomal constitution is identical.

Sachs et al (1956) using Papanicolaou stain found 3 main types of epithelial cells in the amniotic fluid, which they classified as basal, precornified, and cornified and keratinized cells. The basal and precornified cells with green staining cytoplasm are the most suitable for sex diagnosis and are derived principally from the oral and vaginal mucosa and epithelium of the amniotic membrane. The cornified and keratinized cells with pink and orange staining cytoplasm probably come from the skin surface and are unsuitable, since their nuclei show different kinds of degeneration, such as pyknosis, loss of stainability and karyorrhexis. According to Sachs et al (1956), the percentage of diagnosable cells in the amniotic fluid smear is about 12% for both males and females.

Most workers have relied chiefly on the amniotic fluid obtained just prior to delivery. Others obtained liquor amnii by paracentesis uteri between the 32nd and 36th weeks of pregnancy (Dewhurst, 1956), or at caesarean section. Aspiration of a small volume of amniotic fluid from a pregnant uterus can also be performed by abdominal or vaginal puncture of the membranes through the uterine wall. A diagnosis can be made from the 3rd month and possibly earlier. Even a diagnosis has been made on the cells in the fluid obtained by abdominal puncture of

an 8 weeks' old aborted embryo (Sachs et al 1956).

It therefore indicates that this method is quite reliable and the only possible source of error would seem to be, and this can be ignored for practical purposes, is the rare case of an intersex, in which the sexual phenotype does not correspond to the sex chromosome constitution.

For this test to have any practical value, it is necessary for the liquor amnii to be obtained with perfect safety sometime before term. But uptil now no method of doing this by other than paracentesis uteri seems possible. Although this has been carried out often for therapeutic and experimental reasons without accidents, mere curiosity does not justify the procedure for widespread general use, to discover a child's sex, which would soon be known for certain, because of the risk of inducing premature labour or causing foetal injury. Thus its practical value is limited in the human at present. It is a subject, which may excite some popular interest and may have applications beyond the mere satisfaction of parental curiosity in future. However, it has clinical value in dealing with certain rare sex-linked and blood-group-linked hereditary disease. In such cases, it is important to know the correct sex of the foetus in order to determine its chances of manifesting the disease at a stage, when pregnancy can safely be interrupted. It is also possible to apply this method in veterinary practice.

Summary

A reliable prenatal diagnosis can be made from the study of the sex chromatin in the nuclei of exfoliated cells of the foetus in the amniotic fluid.

Specimens of amniotic fluid were examined from 12 cases and the sex of the foetus was correctly diagnosed in all.

The application of this technique in clinical medicine is discussed.

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