ANTENATAL SEX DETERMINATION IN SECOND TRIMESTER BY STUDY OF SEX CHROMATIN IN AMNIOTIC FLUID†

by

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Introduction

It was twenty years ago that an attempt was made to solve one of the most challenging and intriguing problems of determining the sex of baby before birth. Towards the end of 1955 and early 1956 within a period of 5 weeks papers were submitted in various journals by a group of four investigators on the antenatal determination of foetal sex (Fuchs and Riis, 1956; Makowshi *et al* 1956; Ser *et al*, 1955; Shettles, 1956). Samples of the amniotic fluid were obtained from full term pregnant women at the time of rupture of membranes by puncture of the bulging membranes in the vagina for induction of labour, by needle puncture of membranes during caesarean section, or by needle puncture through the uterine wall in cases requiring surgical interruption of pregnancy. Since the publication of these preliminary reports, scientists all over have evinced great interest and are engaging in the study of the determination of sex before birth, (Dewhurst, 1956; James, 1956; Sachs *et al*, 1956; Keymer *et al*, 1957; Baruah *et al*, 1960; Rami *et al* 1960; Riis and Fuchs, 1960; Agarwal, 1963; Abbo and Zellweger, 1970; Rook *et al*, 1971). Today, the methods for sex determination before birth have developed and proved to a point of precision, so that the sex of an unborn child can be predicated with high degree of accuracy. With the introduction of Medical Termination of pregnancy Act in April 1972 in India, increasing requests were received for prenatal sex determination and it became necessary to establish a simple and accurate test for this purpose.

Methods and Material

Amniotic fluid for this study was obtained from 67 cases, admitted to the All India Institute of Medical Sciences Hospital, New Delhi, for termination of second trimester pregnancies.

Patient was first asked to empty the bladder. Then she was made to lie on the table and head end was lowered. Suitable point on the lower abdomen, midway between the pubic symphysis and uterine fundus was selected for amniocentesis. This was infiltrated with 1% xylocaine. Amniocentesis was then performed with No. 18 needle and 10 ml of liquor was collected in sterile container for sex determination studies. If the amniocentesis gave a bloody tap, that site

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was abandoned and another site was selected.

The amniotic fluid was centrifuged at 800 rpm for 15 minutes. The supernatant fluid was discarded and 3:1 aceto-alcohol added to the cell pellet. The mixture was shaken and then centrifuged for another five minutes. The fixed cell button was then left as such in the centrifuge tube for about 15 minutes. At the end of this period a drop of the cell pellet of the amniotic fluid was dropped on a slide, 2-3 drops of 1% aceto-orcein added over the cells and the slide covered with a cover slip. The slide was then passed through a low flame 2-3 times (taking care that the slide was not over heated). Excess of the stain was removed from the slide by pressing it between a blotting paper. The slides were then sealed with nail polish and studied under the oil immersion. If necessary the slides were preserved as such, for a day or two in the deep freeze.

Observations

A total number of 67 cases were studied. The studies were carried out before expulsion of the foetuses. Eleven smears were unsatisfactory because of too many pyknotic cells and very few cells suitable for analysis. Non-nucleated and disintegrated cells constituted the majority in the amniotic fluid sediment. These are probably the squamous cells from the skin. Many other cells were markedly pyknotic and still others which were poorly stained. Thus a very small percentage of cells remained for study which had well preserved and satisfactorily stained nuclei. A hundred such cells were counted in each case.

The interphase nuclei from the amniotic fluid cells showed the sex chromatin mass lying typically adjacent to the nuclear membrane in the form of a dark, dense, plano-convex or rounded mass (Fig. 1 & 2). When this mass was observed in 15-60% of the amniotic fluid cells nuclei, this was recorded as sex chromatin positive and a female foetus was predicted. A male foetus was predicted when the sex chromatin limits ranged from 5-8% of the cells. There were 26 sex chromatin positive smears and 30 sex chromatin negative. Out of these fifty six samples there was error in diagnosis in two. Both these were in the twenty weeks of gestation group. One was diagnosed as sex chromatin negative but was later on found to be a female. Another case diagnosed as sex chromatin positive was actually a male. External physical examination of the abortuses revealed 30 males and 26 females. Table I shows the results in detail.

Discussion

Of the many authors who have worked on sex determination in amniotic fluid cells, very few have mentioned the percentage of incidence of the sex chromatin.

Shettles (1956) found 28-65% typical sex chromatin body in cells of amniotic fluid when the foetus was a female Makowski *et al* (1956) have reported variable counts as counts were made by different observers. In males their count was 6-17% or 6-22% in females 49-71% or 42-70%. However, they state that the mean incidence of the sex chromatin in nuclei found in amniotic fluid cells of female foetuses, is many times of that found in male foetuses. They observed that the minimum count in females is approximately twice the maximum count in male foetuses.

The sex chromatin limits ranged from 14-70% in females and 1-10% in male according to Keymer *et al* (1957) who

TABLE I

Distribution of Patients in Relation to Period of Gestation, Sex Chromatin Determination and Phenotype of Aborted Foetus

No. of wks. of Gestation	Total No. of cases	Sex Chromatin		Phenotypic Sex		No. of unsatisfactory smears	No. of cases with correct diagnosis	No. of cases with wrong diagnosis
		Positive	Negative	Female	Male			
14	2	–	1	–	1	1	1	–
15	1	–	–	–	–	–	–	–
16	17	9	7	9	7	1	16	–
18	16	5	6	5	6	5	11	–
19	1	–	1	–	1	1	1	–
20	30	11	14	12	15	3	25	2

used fresh, unfixed and unstained material and studied under phase contrast. According to James' study (1956) the presence of nuclear chromatin with male foetus varied from 6-22% of nuclei and for female foetuses from 14-71% thus showing an overlap in the reported percentages. Abbo and Zellweger (1970) reported the presence of Barr bodies in 5% or more than 1000 cells as indicative of female foetus.

Inspite of the variation appearing in counts in the incidence of the sex chromatin in amniotic fluid cells as reported by workers using different staining techniques, which might introduce an element of confusion, we can safely conclude that with careful study of properly prepared and stained slides of cells from the amniotic fluid, the sex of the infant can be determined with a very high degree of precision.

In the two instances where erroneous determinations were made the amniotic fluid was insufficient, contaminated slightly with blood and very few well preserved nuclei were present for analysis. Thus it must be emphasized once again that in order to make a correct diagnosis the smears must be obtained and examined under ideal conditions.

It must be mentioned here that we are also carrying out fluorescence microscopic studies in the amniotic fluid for the identification of Y-chromosome using quinacrine dihydrochloride. In the present study we confirmed our results in four instances with the fluorescence method.

Summary

Antenatal sex determination studies were carried out in 67 cases admitted in the All India Institute of Medical Sciences Hospital, New Delhi for termination of second trimester pregnancies with the Intra-amniotic administration of prostaglandin and 20% saline. The sex of the foetus can be determined antenatally by study of the sex chromatin in well preserved and stained cells in amniotic fluid sediment. A rapid method for this study has been described.

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See Figs. on Art Paper I