PRENATAL DETERMINATION OF FOETAL SEX

by

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Perinatal medicine (W.H.O. Chronicle June 1971) is slowly and rightly evolving itself into a speciality which requires special training and learning. The resurgence of a battery of knowledge in genetics and genetically transmitable diseases verily emphasises the importance of genetic counselling (Eastman and Hellman 1966; Carter 1970; Fergusson Smith *et al* 1971). The sex linkage of certain diseases warrants the knowledge of the sex of "the patient in utero" desirable to plan the neo-natal management in some and to prevent the birth of such babies in others. Hence the importance of pre-natal determination of foetal sex.

Efforts had been made from the very early times to detect the sex of the foetus which varied from time to time depending upon the fancy and fashion of the days. But, scientific search in this direction is of only about 16 years duration. The discovery by Barr and his associate (1953) of a definite chromatin mass in the intermitotic nuclei of human female skin biopsies, paved the way to cytological diagnosis of sex, the presence of chromatin body denoting a female and its absence a male. The demonstration by Marberger *et al* (1955) that the sex chromatin can be displayed also in desquamated cells prompted Riss and Fuchs (1955) Serr *et al* (1955), Dewhurst and Shettles (1956) to utilize the aminotic fluid cells in studying the sex of the foetus. Various authors used different methods in studying the sex chromatin. Shettles used Feulgen Stain, Keynor *et al* (1957) dispensed with staining problems and utilized Phase contrast—Microscopy while Klinger and Ludwig preferred thionine staining. Guard (1959) introduced the differential staining method using Beibrich Scarlet and fast green. Riis (1960) favoured Cresyl Fast violet which is the method used in the present study.

Works of Price (1957), Price and Paunabecker (1959), Salhanick (1959) and Solomon (1966) suggest that there may be correlation between the presence of parabasal cells in vaginal smear and the foetal sex.

Aim

This study was mainly aimed at prediction of foetal sex from the percentage of sex chromatin in the cells of aminiotic fluid following the technique of Riis, Fuchs, Moore and Barr. A corollary would be the assessment of the value of the study of vaginal smear pattern in the prediction of foetal sex.

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Material and Method

Fifty gravidae were studied from the inpatients of S. A. T. Hospital during the period 1969-1971. Liquor amnii was obtained during various phases of pregnancy either by abdominal amniocentesis, transvaginal route or during caesarean section. After amniocentesis the gravidae are kept in hospital for the rest of their pregnancy. After delivery the babies were studied as to their sex as compared to the predicted sex, and for any incidence of trauma.

A total of 238 cases were also studied using vaginal cytology to assess their validity in predicting foetal sex.

Method of Smear Preparation and Staining

About 10 ml. of amniotic fluid was taken in a clean test tube and centrifuged for 30 minutes at 2000 r.p.m. The sediment of desquamated foetal cells and vernix was used for staining. Smears were made on slides previously coated with egg—albumin which prevents washing off of cells. The fixative was a mixture of equal parts of 95% ethyl alcohol and ether. The staining was effected as follows; in distilled water for 5 minutes, in 1% aqueous cresyl fast violet for 7 minutes, in 95% alcohol for 2½ minutes, in absolute alcohol for 5 minutes and finally in Xylene for a minimum of 15 minutes.

The stained preparations were scanned with ordinary microscope. When a cell was thought to have a sex chromatin body, it was studied in detail under oil immersion. The scoring of a cell as sex chromatin positive required a flat nucleus free of folds with the sex chromatin apposed to its nuclear membrane in a plane convex, diamond or circular configuration (Fig. 1).

Observations

1. *Age*: The maximum number of women studied belonged to the age group 16-29. There were only two cases belonging to above 35 age group. (Table I).

TABLE I
Age Distribution

Age group	No. of cases	Percentage
16-20	17	34
21-25	12	24
26-30	11	22
31-35	8	16
Above 35	2	4

2. *Gestational age*: It was found that the proportion of satisfactory smear preparations was somewhat higher in later pregnancy. Three cases studied in the 16-20 weeks period did not show any sex chromatin even though the foetuses were female. After 24 weeks the accuracy rate was high. (Table II).

TABLE II
Correlation with Gestational Age

	Gestation in week					
	16-20	24-28	32-36	37-38	39-40	Above 40
Total cases	3	2	5	10	28	2
Sex correctly assessed	0	2	3	8	22	2
Percentage of accuracy	0	100	60	80	76	100

3. *Hydramnios*: There were three cases of hydramnios in the 32-38 weeks group. In all the three the amniotic fluid drawn was crystal clear and yielded no deposit on centrifugation. This may be due to the tendency of the particulate matter to sink down hence leaving clear supernatant fluid which presents for aspiration.

4. *Correlation with Parity*: There was no correlation between the parity and the sex of the baby. But an interesting observation was that the percentage of female babies increased as the maternal age was above 30. (Table III).

TABLE III

Age Group and Sex

Age group	Male	Female
16-19	6	3
20-24	6	13
25-29	5	5
Above 30	—	12

5. *Percentage of chromatin positive cells and the sex of the baby*: It was seen that with male babies amniotic fluid cells showed no chromatin positive cells. In the study of Sachs *et al* (loc. cit) the cells showed 0.5% Barr Body in male babies.

In case of female foetuses 18-44% of amniotic fluid cells showed chromatin body, the average being 28%. (Table IV)

One baby with male external genitalia had 26% of chromatin positive cells. But detailed chromosomal study could not be undertaken.

6. *Maternal Blood Group and Sex of the Baby*: There was no correlation between the blood groups of the mothers and the sex of the baby. (Table V)

7. *Parabasal cells in the vaginal smear and sex of the baby*: Sixty gravida who showed parabasal cells (Fig. 2) varying from 8-12% were analysed. The results showed that 39 of them delivered male babies giving a percentage of correlation of 65.

But on examining 238 gravida only 60 showed parabasal cells (29.8%). So only a minority could be predicted this way. (Table VI)

In the series of 238 gravida studied with reference to vaginal cytology, 118 delivered male babies and 120 female babies. Among the 118 with male babies 39 showparabasal cells in the vaginal smear. But

TABLE IV

Correlation with Percentage of Chromatin Positive Cells

Percentage of chromatin positive cells	Male	%	Female	%
0- 5	0	0	4	8
15-20	0	0	5	10
21-30	1	2	17	34
Above 30	0	0	7	14

TABLE V

Maternal Blood Group and Foetal Sex

Blood group	O	A	B	AB
Male	5	3	8	1
Female	9	4	18	2

TABLE VI

Gestational Age and Parabasal Cells

	Upto 24	25-30	31-35	36-40	Above 40
Total cases with parabasal cells	3	12	18	27	4
Male Baby	2	7	10	18	3
Percentage	66.6	57.2	42.2	66.6	75

among the 120 female baby series only 21 showed parabasal cells. Inference is that when 100 male babies are born 33.2 of mothers will have parabasal cells in their vaginal smear, whereas with 100 female babies only 17.2 will be showing them.

Among the 50 gravida studied in 43, the sex of the foetus could be predicted accurately using the Barr-body study of amniotic fluid cells, 3 smears in early pregnancy being false negative, 3 from hydramnios being cell free and one male being chromatin positive.

Discussion

Dewhurst and Shettles in 1955 first applied the study of chromatin body in the amniotic fluid cells in the determination of foetal sex. Independent reports from various groups like Riis and Fuchs, Keynor and Sachs *et al* then followed. The major difference in the methodology was only in the staining techniques used. The results obtained by these observers ranged between 90-99%.

The present study was carried out using cresyl fast violet stain and the results were similar to the original workers (86%).

It is apparent from these studies that by careful examination of nuclei of epithelial cells obtained from the amniotic fluid, the sex of the foetus can be accurately predicted. Cell shrinkage causing artefacts simulating chromatin body, debris obscuring nuclear details and mucoid material may come in the way of proper interpretation. In some samples sufficient nuclei could not be found to allow correct prediction. In the present series there were such examples, all in cases of hydramnios.

Fuchs *et al* stated that they had confusion due to maternal cells in about 5% of cases. But we had no such problem.

The value of prenatal sex determination in sex linked hereditary disorders is now well established. Haemophilia, pseudoglioma with grave mental deficiency, cystic fibrosis, hereditary storage diseases, Duchenne and Beker type of muscular dystrophy, X-linked variant of mucopolysaccharidosis and Lisch-Nyham hyperuricaemia are some of them.

In this era of liberalised/legalised abortions the prenatal sex determination may help the parents to choose to retain or reject a given foetus! Of course, this may tantamount to what Jeffcoate (1967) feared for human reproduction degenerating to selective breeding.

Amniocentesis, the present method of collection of foetal cells has its own hazards. Bloodytap, risk of isoimmunization, foetal damage, premature labour, amnionitis and haemoamnion are some of them. However, meticulous attention to detail and aseptic ritual will minimise

.the dangers as the present study revealed.

The drawback of the present method is that diagnosis of the male is a negative fact. This has recently been rectified by the introduction of "Dyeing of the Y chromasome" with quinacrine mustard (Lancet leading article 6 Feb. 71). Hence simultaneous/consecutive use of both the methodology will help to diagnose foetal sex in a positive manner in male as well.

The other aspect of the study viz. sex determination from vaginal smear pattern needs further study. The present study showed 65% correlation. But in 25% of female babies also there were parabasal cells. None of the cases with parabasal cells developed any sign of foetal distress, 95% being delivered at term of healthy babies and 5% going past term.

It is pertinent to ask whether the foetal androgens have any effect at all in the presence of the high concentrations of maternal oestrogen and progesterone. It is well known that if vaginal epithelium is subjected to the action of combination of hormones its reaction may not parallel with the level of hormone concentrations, but to the sensitiveness of the end organ. (Brown and Dixon 1970).

Prenatal foetal sex determination has come to be accepted as an invaluable ancillary in the genetic counselling and advent of perinatal medicine in its wider sphere will only emphasize its importance.

Summary and Conclusions

1. A series of 50 gravida for prenatal determination of foetal sex was studied using amniotic fluid cytology and Barr body staining.

2. The method of smear preparation and staining is described.

3. Two hundred and thirty-eight gravidae were also studied using vaginal smear for parabasal cells to assess its correlation to foetal sex.

4. The percentage of sex chromatin in amniotic fluid cells ranged between 15-38 with the average of 28 for the females, whereas in the male it was zero.

5. The accuracy of prediction was 86%.

6. Effects on the mother and child due to the procedure in this study was nil.

7. In the study of vaginal smear pattern, among those having parabasal cells there were 65% male babies.

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