PRENATAL SEX DETERMINATION BY DIFFERENTIAL CYTOPLASMIC STUDIES OF AMNIOTIC FLUID

by

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Introduction

A relatively recent method based on the colour differentiation of the cytoplasm of various amniotic fluid cells has been made available for prenatal sex deterfnination. This method is based on the earlier observations by Rosa and Fanard (1949) that the cells of the amniotic fluid when stained by Harris-Shorr method take mainly two stains. Those staining pink are called the Eosinophilic cells and those staining blue or bluish green are called the cyanophilic cells. The former arise mainly from the buccal mucosa of the fetus, whereas the cyanophilic cells arise from the vaginal vestibule of female fetus and presumably the vagina. A higher proportion of cyanophilic cells in the amniotic fluid therefore suggests a female fetus and vice versa. We have employed this fact of cytoplasmic colour differentiation in our study of prenatal sex determination.

Material and Methods

One hundred patients in different stages of pregnancy and labour were taken up for study. The amniotic fluid was collected by various means, namely during abdominal hysterotomy, abdominal amniocentesis, artificial rupture of membranes during labour and during caesarean section (Table I). In each case about 10 ml. of the fluid was withdrawn which was centrifuged. The sediment was drawn into even smears on slides which were stained by Modified Harris Shorr Method (De-Neef, 1967). The slides were then examined under the microscope and a minimum of 500 cells were counted. The percentage of cyanophilic cells (blue staining cells) was determined in each case. This was called cyanophilic Index (C1). The sex of the fetus was predicted without prior knowledge of infant sex.

Eighty-five cases studied were between 28-40 weeks of gestation and the other 15 were between 10-28 weeks of gestation.

Observations

Between 10-20 weeks of pregnancy the amniotic fluid contained very few cells on which Cyanophilic Index could not be possible. Therefore, the determination of sex at this stage of pregnancy was not possible. From the 5 cases studied between 22-28 weeks of pregnancy we made a correct prediction of sex in 2 cases only, taking a Cyanophilic Index of 26% or above for the diagnosis of female sex

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and below 26% for the diagnosis of male sex as will be seen later for pregnancies of more than 28 weeks duration.

ents with male infants, whereas it fell in the range of 20-84% in the 40 amniotic fluid samples taken from patients with

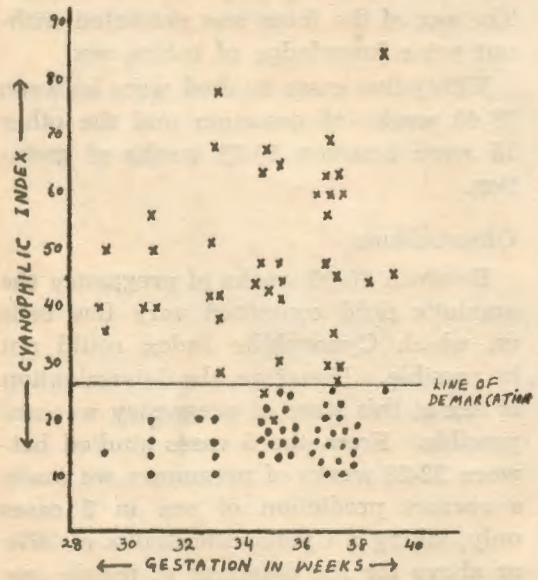
TABLE I
Duration of Pregnancy at the Time of Study of Antenatal Sex

Duration of pregnancy	No. of cases	Method of Amniotic Fluid collection			
		Hyst.	Amn.	ARM	CS
10–16 weeks	5	5	—	—	—
16–22 weeks	5	5	—	—	—
22–28 weeks	5	2	3	—	—
28–34 weeks	15	—	5	10	—
34–40 weeks	70	—	—	50	20

Legend: Hyst. (Hysterotomy); Amn. (Amniocentesis); ARM (Artificial rupture of membranes); CS (Caesarean Section).

Amongst the 85 patients between 28-40 weeks of gestation, 45 delivered male and 40 delivered female infants. The cyanophilic Index fell in the range of 10-25% in the 45 amniotic fluid samples taken from patients with male infants (Fig. 1). Taking 26% of Cyanophilic Index as the line of demarcation and predicting male outcome in those who had an index below it we predicted the sex correctly in 83 out of 85 patients, (97.6% accuracy). The accuracy in predicting male outcome was 100% while in predicting female outcome was 95% only. The error by this method is the incorrect prediction of male sex in both instances. The wrong male prediction was seen in one patient with 23% CI giving birth to a full term female baby with no apparent abnormalities and the other patient with 20% CI who had hydramnios and delivered an anencephelic female infant.

FIGURE 1
SCATTERGRAM SHOWING THE CYANOPHILIC INDEX IN 85 PATIENTS BEYOND 28 WEEKS
(DOTS → MALE INFANTS
CROSSES → FEMALE INFANTS)



Discussion

The basis of the differential cytoplasmic studies for sex determination is the presence of the vagina in the female fetus. The origin of various cells in the amniotic fluid has now been confirmed and traced to various fetal surfaces (mouth, skin and genetalia) and the amnion and umbilical cord (Huisjes, 1968a, 1968b; Wachtel *et al*, 1969; Huisjes, 1970; Mehrotra *et al*, 1974). The Cyano-

philic cells arise mainly from the vaginal vestibule and presumably from the vagina of the female fetus. The buccal mucosa gives origin to some of the Cyanophilic cells as well as the Eosinophilic cells and the fetal skin is the exclusive source of the faintly staining polygonal cells. Therefore, the amniotic fluid from the patients with female fetus will show a much higher Cyanophilic Index than those with male fetus. The explanation for this cytological pattern appears to be a hormonal one. The maximum and minimum limits of Cyanophilic Index for antenatal diagnosis of male and female sex show variation from different centres where the work has been conducted. In the period of gestation over 28 weeks taking a maximum CI of 25% in the amniotic fluid for male diagnosis and a minimum CI of 27% for female diagnosis, sex was correctly predicted in 83 of the 85 samples of amniotic fluid giving 97.6% correct prediction. The prediction of female fetus was cent per cent correct while the two errors involved the incorrect prediction of male fetus. One such incorrect male prediction was made on the amniotic fluid sample from an anencephelic female fetus, the CI being 20%. The fetus however had no external malformation of the mouth or vagina which could explain a low CI, though the baby was stillborn. In the other instance, the fetus was phenotypically normal female. No explanation is forthcoming for the lower CI in these two cases.

The maximum and minimum limits of CI for the antenatal diagnosis of male or female sex show variation from different centres where the work has been conducted. In the earliest work of this type Huisjes (1968) mentioned only the average CI of 22.5% in male and 51.9% in female without setting the limits for the diagnosis of either sex. Arendzen and Huisjes (1971) found 20% CI as the maximum for the diagnosis of male sex and 22% CI as the maximum for the diagnosis of female sex. Bennett *et al* (1970) set the limits at 20 and 25% respectively. Both the works are comparable to our observation in patients of over 28 weeks pregnancy (Table II). However, Nelson (1973) using the simple Shorr stain reported a much higher percentage of Cyanophilic cells in male and accordingly put the maximum limit of CI for male at 42% and minimum limit for female at 50%. Using these criteria she made only one error in 30 samples. She explains that the high percentage of Cyanophilic cells in males may be due to the use of a different method of fixation and simple Shorr stain rather than the Harris modification and due to the length

TABLE II

Comparision With Other Reported Works on Cytoplasmic Staining Technique, Antenatal Sex Determination Employing

Authors	Staining technique	No. of cases	Maximum CI (male)	Minimum CI (female)	% correct prediction
Arendzen & Huisjes (1971)	Harris–Shorr	36	20%	22%	98.2%
Bennett *et al* (1972)	Harris–Shorr	31	20%	25%	99%
Nelson (1973)	Simple-Shorr	29	42%	50%	97%
Present study	Modified Harris–Shorr	85	25%	27%	97.6%

of time that the amniotic fluid cells were stored before examination, though how these factors could affect the CI is not explained. These different diagnostic percentage in different published series (Table II) only point out that each laboratory should set its own limits of maximum and minimum CI for antenatal sex determination.

Antenetal sex determination was not possible by this method in early weeks (10-20 weeks) of pregnancy because of the paucity of cells and only 40% correct prediction was possible in the 5 patients whose gestation was 22-28 weeks. It will be wrong, however, to draw inference from 5 cases only. The only other work on these lines in early pregnancy is by Nelson (1971) who cultured and stored the cells for 1-21 months and reported only 33% correct prediction in pregnancies of less than 20 weeks duration.

Summary

Prenatal sex determination was carried out by the relatively new technique of differential cytoplasmic staining of amniotic fluid cells on 100 patients in different stages of pregnancy. Modified Harris-Shorr stain was used. The method was found easy, quick and accurate after 28 weeks of gestation but unreliable in pregnancies of less than 28 weeks duration.

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See Figs. on Art paper II