CYTOLOGICAL STUDY OF AMNIOTIC FLUID FOR PRENATAL SEX DETERMINATION

by

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Antenatal sexing has found a limited, but important clinical application for legal abortion in cases of certain sex linked hereditary diseases like haemophilia, muscular dystrophy and Hunter's syndrome. Ever expanding world population poses a major threat to the future security of the world. Unless a perfect effortless contraceptive is discovered, safe medical abortion on demand will eventually be universal and thus prenatal sex determination may have some application in relation to psychological, social, and religious hindrances to reduction of world population.

Antenatal determination of foetal sex has a very long history of failure. Only recently it has been brought beyond guess work and superstition into scientific reality on the basis of sex chromatin study. (Dewhurst, 1956; Fuchs and Riis, 1956; James, 1956; Makowski *et al*, 1956; Shettles 1956; Keymer *et al*, 1957; and Paqquinucci, 1957).

Many chromatin stains are available, with varying degree of specificity for desoxyribonucleic acid. Cytoplasmic counter stains (Feulgen stain, Guard's stain) can produce a beautiful result, but the procedure is notoriously difficult to reproduce and time consuming. Aceto-orcein squash preparation technique (Sanderson and Stewart, 1961) is used for demonstration of Barr-Body in amniotic fluid smears, which is just as accurate as other methods, but much more rapid. As this method has not been evaluated in our country, a study was undertaken to assess the acceptability and reliability of this method.

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Material and Methods

The present work comprises a study of amniotic fluid in a total of 170 cases for prenatal sex determination by detection of Barr-Body using aceto-orcein stain.

The patients were divided into two main groups.

Group I: 120 patients above 20 weeks of gestation.

Group II: 50 patients in whom the pregnancy was between 14-20 weeks. Liquor amnii for study was obtained by the following methods.

Group I: (20-40 weeks of gestation) Abdominal route—transabdominal amniocentesis—42 cases; at caesarean section—17 cases.

Vaginal route: 1—At artificial rupture of membranes—38 cases.

2. Transvaginal puncture—23 cases.

Group II: (Between 14-20 weeks gestation).

Abdominal Route: 1—Transabdominal amniocentesis—26 cases.

2. At hysterotomy—16 cases.

Vaginal Route: From intact sacs in cases of complete abortion—3 cases.

Buccal smears from adult females and males were used as controls with each batch of slides examined. The sex of the foetus as predicted from the cytological study of the amniotic fluid was correlated with the sex of the foetus as observed after birth or abortion.

Technique

About 5-10 cc of collected amniotic fluid was centrifuged for 5 minutes at 2000-2500 revolutions per minute. Thin smears were prepared from the sediment. Smears were stained with aceto-orcein stain for 5 minutes and examined under oil immersion. One hundred well stained nuclei were counted and the percentage of cells showing positive chromatin body were determined.

The method was found to be preferable to other chromatin stains, because of the rapidity of the procedure and the revelation of the fine details of nuclear structure. These two things are of extreme importance, specially to a clinician who cannot devote several hours for standard chromatin staining like:—Feulgen and Gaurd's staining, each taking 4-6 hours.

Nuclear Morphology: A chromatin negative nucleus has a finely granular nucleoplasm and well defined nuclear membrane with no sex chromatin. A chromatin positive is similar but exhibits sex chromatin adjacent to the nuclear membrane (Figure I).

Observation and Discussion

As shown in Table I the incorrectly predicted cases which were actually male, as confirmed by the apparent sex, were predicted as females. In both the cases amniotic fluid was collected by the transvaginal route and the error in prediction was probably due to admixture of maternal vaginal cells, resulting in the false determination. Lin and Bennett (1960), also attributed the mistake in prediction of the foetal sex in their study was probably due to the same reason.

In the present study, a correct diagnosis was obtained in 98.33% of cases in group I (over 20 weeks). The findings of Sachs, Serr and Danon, Fuchs and Riis, Dewhurst, (1954) and later Kaymer *et al*, (1957) and Amarose *et al*, (1966), who have reported 100% correct prediction of sex, are only at slight variance (less than 2%) with the results obtained in the present study (Table II).

Group II

Results are clear from Table III that sex could be predicted according to the

TABLE I

Correlation Between Predicted Prenatal Sex (aceto-orcein method) and Apparent Sex at Birth

Total 120	Predicted sex of amniotic fluid		Apparent sex at birth		No. of incorrect prediction	%age of correct prediction
Female	Female	56	Female	54	2	96.43
			Male	2		
Male	Male	64	Male	64	0	100
			Female	0		

Overall accuracy 98.33%.

TABLE II

Comparison of Results of Antenatal Sex Prediction From Amniotic Fluid by Various Workers (demonstration of Barr body)

S. No.	Author's name	Year	Total No.	Percentage of accuracy
1.	Dewhurst	1956	40	100
2.	Fuchs and Riss	1956	20	100
3.	Shettles	1956	40	100
4.	Sachs et al.	1956	40	100
5.	Makowski et al.	1956	30	93.3
6.	Keymer et al.	1957	15	100
7.	Lin and Bennett	1960	30	93.0
8.	Daftary et al.	1960	138	93.0
9.	Amarose et al.	1966	27	100
10.	Gayatri et al.	1971	100	92
11.	Rook et al.	1971	20	85
12.	Present series	1972	Over 20 wks. 120 14-20 wks.—46	98.33 100

TABLE III

Correlation Between Predicted Prenatal Sex by Aceto-orcein Method and Apparent Sex at Birth

Sex	Predicted sex of amniotic fluid	Apparent sex at birth		No. of incorrect prediction	Percentage of correct prediction
Female	19	Female	19	—	100
		Male	0		
Male	27	Male	31	In 4 cases no diagnosis was made	100 in predicted cases, (27)
	4?	Female	0		

selection criteria of cells, and number of cells screened, only in 46 cases out of 50 in Group II (below 20 weeks) and the results were found to be cent per cent correct, which are in full agreement with Sachs, Serr, Danon and Fuchs and Riss and other charted in Table II. The chief difficulty with foetal sexing by aceto-orcein stains is the large number of dead or moribund cells in the samples in early weeks of pregnancy. These cells are present in greater poportion as gestation increases, but later the number of exfoliated cells of liquor amnii is so much that screening of suitable cells is a time consuming problem. In Group II—Samples of liquor amnii in 8% cases did not contain sufficient cells according to the selection criteria of cells, so these are not included in the analysis.

Another interesting feature revealed in the present study is that the percentage of sex chromatin cells in female increased with the maturity of the individual being lowest in early intrauterine life, midway in the late pregnancy and highest in early intrauterine life, midway in the late adults as shown in Table IV.

Summary

Aceto-orcein staining technique is quite reliable, specially when the possible source of errors are carefully avoided. In 98.33% in group I, and 100% in Group

TABLE IV

Sex	Percentage of range of sex chromatin body		
	Amniotic fluid smears of foetus below 20 wks.	Above 20 wks.	Buccal smear of adults
Female	5–12	6–18	15–40
Male	0 –1	0– 2	0– 2

II below 20 weeks, sex was diagnosed correctly with simple aceto-orcein stain. Staining of sex chromatin body is so extremely rapid that a smear may be made, stained, scrutinised, all in less than 10 minutes. If, however, decision to terminate pregnancy is involved it would be advisable to confirm the results of aceto-orcein method by another method. Demonstration of 'Y Body' using fluorescent stain, becomes mandatory in early pregnancy where sufficient number of cells fit for examination may not be present.

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See Fig. on Art Paper II