ANTENATAL SEX DETERMINATION USING CELLS OF THE AMNIOTIC FLUID

by

B. D. Baruah,* M.B.B.S., (Cal), Ph. D. (Sheffield), F.R.C. Path. (Lond), F.A.M.S.

and

P. K. Gogoi,** M.B.B.S., D.C.P., M.D. (Dib.)

Determination of sex of a baby before it is born is an exciting subject. Since the demonstration of Barr body (X Chromatin) in 1949 this technique was extended for antenatal sex determination from amniotic fluid cells with a variable degree of success. In India, this technique has been applied for the first time by Baruah and Borkotoky (1960) and Rami *et al* (1960) and reported an accurate diagnosis of foetal sex and this was followed by Gayatri and Peters (1972), Hamilton *et al* (1975) and Aggarwal and Devi (1976).

However, with further experience it was revealed that this technique has got its own limitations and incorrect prediction would be made in cases of XO and XXY foetuses, if sex prediction is to be based on X chromatin study alone.

Demonstration of the Y chromatin in interphase nuclei by its fluorescence on staining with dyes such as quinacrine mustard or hydrochloride and its subsequent recognition in amniotic fluid cells from male foetuses further provided a new simple, rapid and reliable technique for antenatal sex determination.

Herein we report the results of a study of determination of antenatal sex in our laboratory using either X or Y chromatin technique alone or by using both these techniques simultaneously to standardise and to assess their comparative accuracy.

*Professor of Pathology.
**Demonstrator of Pathology.
Assam Mtdical College, Dibrugarh, Assam.
Accepted for publication on 13-4-82.

Material and Methods

One hundred and seventy-three antenatal cases were included in the present study where pregnancies were terminated by various obstetrical means like M.T.P., hysterotomy for medical reasons as well as at full term before normal delivery and caesarean section. The sex of the foetus was kept secret by the clinician until the laboratory examinations were completed.

These cases were divided into 3 groups depending on the period of gestation.

Barr body was studied by the following staining technique: (1) Carbolfuchsin, (2) Aceto-orcein, (3) Harris Haematoxylin and Eosin, (4) Feulgen reaction, and (5) Cresyl Echt Violet. Out of these, Carbolfuchsin technique was found to give a very distinct appearance of the Barr body and the results were reproducible. Hence only this technique was followed in subsequent cases.

Staining for X Chromatin

Half of the amniotic fluid was spun at 1,000 r.p.m. for 10 minutes. The cell pellet was then suspended in 3:1 methanol-acetic acid fixative solution; respun,

and resuspended in a small amount of freshly made fixative. Preparations were made from the cell suspension on precleaned, chilled slide and dried at 37°C. The slides were stained with Carbolfuchsin solution for 10 minutes (Eskelund, 1956) and dehydrated in alcohol, cleared in Xylol and mounted in D.P.X., and screened with oil immersion objective. Nuclei which were folded, irregular in outline, pyknotic or deppled with excess deeply staining chromatin were not included in the examination. The X chromatin was identified as a plano-convex body lying adjacent to the nuclear membrane and taking out deep pink colour. (Fig. 1).

Staining for Y Chromatin

The other half of the amniotic fluid was centrifuged at 1,500 r.p.m. for 15 minutes and the cell deposit was placed into albuminised slides. They were partially air-dried and then fixed in 95 per cent ethyl alcohol for minimum 30 minutes. The slides were passed through methyl alcohol, descending series of ethyl alcohol and distilled water for 3 minutes each. They were stained with 0.5 per cent aqueous quinacrine hydrochloride for 15 minutes, treated with distilled water and 0.01M Citric acid-phosphate buffer (pH 5.5). The slides were then dipped in 0.01M phosphate buffer (pH 7.4) and mounted in the same buffer (Hollander and Borgaonker, 1971). Observation with a Carl Zeiss Fluorescent microscope was made with incident light illumination, using 4 mm. B224g ultraviolet excitation filter, 2 mm G241g blue excitation filter and a G247 barrier filter. The Y chromatin body appears as a small, rounded, fluorescent body anywhere within the nucleus which could be identified easily by its greenish yellow fluorescence. (Fig. 2). The stained slides were examined within two hours.

Scoring of both X and Y body was done by examining 100 consecutive cells. Presence of Barr body in less than 10 per cent was labeled as male and above 10 per cent as female. However, in the fluorescent technique, when no nuclear fluorescent body was seen, it was considered as female. In 70 cases both these two techniques were used. In combining both the methods, the prediction of foetal sex was done only in those cases where there was no discrepancy between X and Y chromatin determination.

The results were verified by examining the somatic sex of the foetus after abortion or birth and in equivocal cases by gonadal biopsy of aborted foetus, after the laboratory examinations were completed.

Observations

Using the Barr body (X chromatin) technique alone, the sex of the foetus was correctly predicted in 161 cases out of total 169 (95.27%). Number of incorrectly predicted cases were 8; 6 of which were wrongly predicted as females, and 2 as males, the sexes were actually males and females respectively as confirmed by the apparent sex and subsequent gonadal biopsies. Four samples were found to be unsatisfactory because of contamination with blood.

By using fluorescent technique for Y body with the same material, sex of the foetus was correctly predicted in 80 cases out of 81 (98.76%) (Table I).

By demonstrating both X chromatin and Y fluorescent body in the same case in a total number of 79 cases, correct prediction was done in all 76 predicted cases. In 3 cases no prediction was done due to unsatisfactory smears.

Examination of amniotic fluid cells at

different periods of pregnancy has shown that the percentage of X and Y chromatin positive cells increases with the maturity of the individuals in female and male respectively and highest in late pregnancy (Tables II and III).

TABLE I

Prenatal Sex Determinating Y Fluorescent Body

Predicted sex of amniotic fluid by quinacrine stain	Apparent sex at birth/abortion	No. of incorrect prediction	Percentage of correct prediction
Male 50	Male 50 Female 0	0	100.0
Female 31	Female 30 Male 1	1	96.77
Total: 81	Overall accuracy	..	98.76

TABLE II

X Chromatin Positive Cells (in percentage) in Each Correctly Predicted Sex in Relation to Period of Gestation

No. of weeks of gestation	Total No. of cases	X-chromatin positive cells (%)			
		Females		Males	
		Average	Range	Average	Range
Group—I (10-16 weeks)	66	37.16	15-49	0.63	0-2
Group—II (17-28 weeks)	67	39.05	13-53	0.9	0-3
Group—III (29-42 weeks)	28	40.29	18-58	1.09	0-3

TABLE III

Y-body Positive Cells (in percentage) in Each Correctly Predicted Sex in Relation to Period of Gestation

No. of weeks of gestation	Total No. of cases	Y-Body positive cells (%)			
		Males		Females	
		Average	Range	Average	Range
Group—I (10-16 weeks)	29	46.57	35-58	0	0
Group—II (17-28 weeks)	30	47.39	30-63	0	0
Group—III (29-42 weeks)	21	49.11	45-65	0	0

TABLE IV

Prediction of Sex by Demonstrating both X Chromatin and Y Fluorescent Body in the same Case

No. of weeks of gestation	No. of cases	Predicted sex by combining both techniques		No. of predicted cases	Phenotypic sex at birth/abortion				Overall accuracy (Percentage)
					Predicted cases		Unpredicted cases		
		Male	Female		Male	Female	Male	Female	
Group—I (10-16 weeks)	30	20	8	2	20	8	1	1	100
Group—II (17-29 weeks)	28	16	11	1	16	11	1	—	100
Group—III (29-42 weeks)	21	9	12	0	9	12	—	—	100

Comments

In the present study, a correct diagnosis was obtained by demonstration of X chromatin alone in 95.27 per cent of cases. Baruah and Borkotoky (1960), Amarose *et al* (1966), Papp *et al* (1970) reported 100 per cent of correct prediction of sex by this technique. In 4 cases of our study, prediction could not be done as the smears were not satisfactory due to contamination of blood. There were 6 false X chromatin positive cases, in the range of 11 to 20 per cent, which may be due to contamination with maternally derived cells and 2 false negative cases which contained many pyknotic and degenerated cells with many clumped chromatin particules.

Using Y fluorescent body technique, a correct prediction was made in 98.76 per cent of cases which is quite in agreement with similar observations by Cervenka *et al* (1971) and Rook *et al* (1971). In one case, where a wrong prediction was made, no Y body could be seen under the fluorescent microscope. This may be due to a technical failure or small Y chromosome. Rook *et al* (1971) who in their series of 20 cases could not detect Y body in a few male cases, suggested that many of the cells were damaged or not viable or a small Y chromosome may fail to fluoresce as the possible causes of their failure. Borgaonkar and Hollander (1971) found 6 males with small Y chromosome which did not fluoresce in their study. Casperssol (1972) has also reported cases in which the intense fluorescence of the Y chromatin has been lost due to translocation. Adams *et al* (1973) and Polani (1979) recommended that when considering the foetal sex in diagnostic amniocentesis, a blood smear should also be obtained from the father to confirm the normality of Y chromatin and if possible

chromosome study should be undertaken.

In the present study, by using both X and Y chromatin techniques in 79 cases, there was no discrepancy in the result of prediction by these two methods in 76 cases and the accuracy was found to be cent per cent in all the three groups. In 3 cases, where there were no correlation between the results of both techniques no prediction was made. The percentage of X and Y chromatin positive cells increases with the maturity of the foetus in female and male respectively and reaches the highest in late pregnancy.

For most accurate results where a decision on the termination of pregnancy is necessary, both these techniques should be used together to avoid any fallacious results.

Antenatal sex determination is no longer a test to satisfy the curiosity of the parents or obstetricians. It may have some application in our society for social reasons and in general for family planning purposes. But most important clinical application is in genetic counselling and to eliminate certain sex-linked hereditary diseases like haemophilia, Duchenne type of muscular dystrophy and Hunter's syndrome, where a therapeutic abortion is indicated.

Summary

By study of X chromatin, foetal sex was correctly predicted in 161 out of 169 cases (95.27%); by using fluorescent staining technique for Y chromatin in 80 cases out of 81 (98.76%) and by combining these twin techniques, sex was correctly predicted in all 76 cases (100%). It is concluded that although Barr body technique is quite useful for routine diagnostic procedure, the Y body technique is superior to Barr body technique and for most accurate diagnosis both these techniques should be used to avoid any fallacious results. This can be utilized for detection of certain selected sex-linked disorders and as an important aid for the further management of pregnancy.

Acknowledgement

We are grateful to the Principal and Superintendent, Assam Medical College, Dibrugarh for permitting to publish this paper. Our thanks are also extended to Dr. R. K. Borkotoky, F.R.C.O.G., Professor and Head, Department of Obstetrics and Gynaecology and his staff for their help in arranging the supply of amniotic fluid samples.

References

1. Adams, C., Kilpatrick, B., Kabacy, G., Fralko, G. and Dunors, K. W.: Acta. Cytol. **17**: 233, 1973.
2. Aggarwal, S. and Devi, P. K.: J. Obstet. Gynaec. India. **26**: 245, 1976.
3. Amarose, A. D., Wallingford, A. J. and Plotz, E. J.: New Engl. J. Med. **275**: 715, 1966.
4. Barr, M. L. and Bertram, E. G.: Nature. **163**: 676, 1949.
5. Baruah, B. D. and Borkotoky, R. K.: J. Obstet. Gynaec. India. **11**: 104, 1960.
6. Borgaonkar, D. S. and Hollander, D. H.: Nature. **230**: 52, 1971.
7. Casperssol, T. (1972): Quoted by Reference 14.
8. Cervenka, J., Gorlin, R. J. and Bendel, R. P.: Obstet. Gynec. **37**: 912, 1971.
9. Eskelund, V.: Endocrinol. **23**: 246, 1956.
10. Gayatri, D. and Peters, E.: J. Obstet. Gynec. India. **22**: 19, 1972.
11. Hamilton, S. M., Agarwal, N. and Hingorani, V.: J. Obstet. Gynec. India. **26**: 848, 1976.
12. Hollander, D. H. and Borgaonkar, D. A.: Acta. Cytol. **15**: 452, 1971.
13. Papp, Z., Gardo, S., Herpay, G. and Arvay, A.: Obstet. Gynec. **36**: 429, 1970.
14. Polani, P. (1979): Personal communication.
15. Rami, J., Daftary, V. G., Parikh, M. N. and Masani, K. M.: J. Obstet. Gynec. India. **11**: 99, 1960.
16. Rook, A., Hsu, L. Y. and Hirschhorm, K. M.: Nature. **230**: 53, 1971.

See Figs. on Art Paper II