FOETAL MATURITY ESTIMATION BY AMNIOCENTESIS

by

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Foetal maturity by staining amniotic fluid with Nile Blue Sulphate was determined in a series of 600 cases. More than 20 per cent orange staining cells in the amniotic fluid indicated foetal maturity of 38 weeks or more in 87.9 per cent cases, and counts below 20 per cent indicated foetal maturity below 37 weeks in 72.7 per cent cases.

There are many methods of determining foetal maturity, but no test is completely accurate. The need for assessment of foetal maturity becomes imperative where a woman cannot recall her dates of last menstrual period (about 16 per cent in advanced countries and more than 50 per cent in India), or where a woman has conceived during lactational amenorrhoea or has irregular menstrual cycles or complications like toxaemia, hypertension, antepartum haemorrhage, postmaturity or threat to life of the foetus. The presence of exfoliated cells in the liquor amnii has long been recognised (1942 Bourgeois) in vaginal smears of patients with ruptured membranes. Recent reporters have suggested that presence of these cells is of assistance in estimation of foetal maturity. Hopman (1952, 1957) corroborated the findings. The use of cell count in amniotic fluid after staining with Nile Blue Sulphate as an index of foetal maturity has been investigated.

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Received for publication on 24-9-1973.

Material and Method

Amniotic fluid in 600 patients attending S. M. H. S. Hospital, Department of Obstetrics and Gynaecology, was studied, irrespective of maternal age, parity of socio-economic status. Patient's period of gestation varied from 14 weeks' to 44 weeks'. They were divided into two groups:

Group I: This group consisted of 320 patients who did not remember the exact date of last menstrual period. Period of gestation varied from 32-42 weeks.

(a) 185 patients did not remember the last menstrual period exactly.

(b) 55 patients had only an approximate idea of last menstrual period.

(c) 35 patients had conceived during lactational amenorrhoea.

(d) 20 patients had irregular cycles.

(e) 15 patients had bleeding per vaginam in the first trimester of pregnancy.

(f) 5 patients had some menstrual irregularity due to oral contraceptives.

(g) 5 patients had conceived with the loop intact.

Group II: Comprised of 280, whose estimated date of delivery, as calculated from the menstrual history, using Naegeles rule was not in doubt. The period of gestation varied from 14-44 weeks.

(a) In 190 patients the period of gestation corresponded with the period of

amenorrhoea.

(b) In 80 patients there was postmaturity.

(c) In the rest 10, clinical findings did not correspond with the period of amenorrhoea.

Method

Five ml of uncontaminated liquor amnii was taken by two routes:

(1) *Abdominal*

(i) abdominal percutaneous paracentesis—180 cases

(ii) at the time of caesarean section—90 cases

(iii) at the time of hysterotomy—240 cases

(2) *Vaginal route*

(i) low rupture of membranes—240 cases

(ii) high rupture membranes—10 cases

(iii) leaking—30 cases

One drop of fresh, uncentrifuged amniotic fluid was put on a clean glass slide by means of a sterile pipette and mixed with one drop of 0.1 per cent aqueous solution of Nile Blue Sulphate. No fixative was required. The stain and the amniotic fluid were thoroughly mixed. Cover slip was put on the preparation and an immediate microscopic examination was performed under low power (X 10). Total 500 cells were counted, including both blue staining cells and orange staining cells. The percentage of orange stained anucleated foetal cells occurring singly or in clusters was calculated, and related to the period of gestation. Neurological examination of the new born baby was done 48 hours after birth, as the tone changes in the days that follow the birth, by the method of Koenigsberger, 1966.

Results

TABLE I

Percentage of Orange Cells at Completed Weeks of Gestation

Period of gestation in completed weeks	Number of patients	Percentage of oragnce cells	
		Mean %	Range %
14	18	0	0
16	15	0	0
18	10	0	0
20	3	0	0
22	2	0	0
24	2	0	0
28	20	0	0
30	13	0	0
32	26	0.5	0-1%
33	20	1.9	1.8-2.0
34	13	0.8	0-1.6
35	31	9.7	2.2-17.2
36	23	23.7	2.3-57.0
37	41	18.5	0-54.5
38	51	38.8	13.4-69.2
39	71	38.4	13.8-69.4
40	161	44.2	8.4-80.0
41	47	54.6	43.4-65.8
42	21	37.0	8.4-58.4
43	8	59.3	32.4-71.0
44	4	–	–

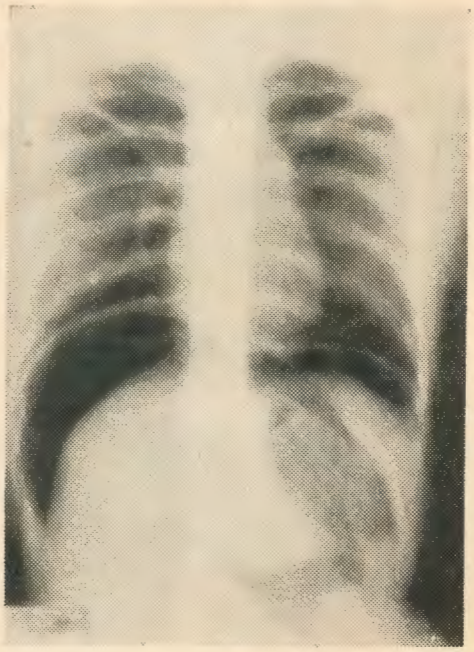
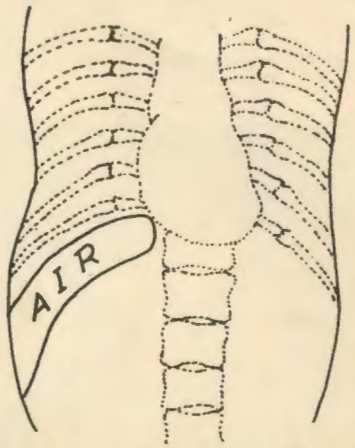
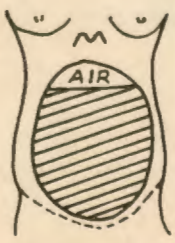


Fig. 1
X-ray shows air under the diaphragm.



AIR UNDER THE DIAPHRAGM

Fig. 2



UNILOCULAR CYST

MULTILOCULAR CYST

Fig. 3

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Fig. 1
Anencephaly with umbilical hernia.

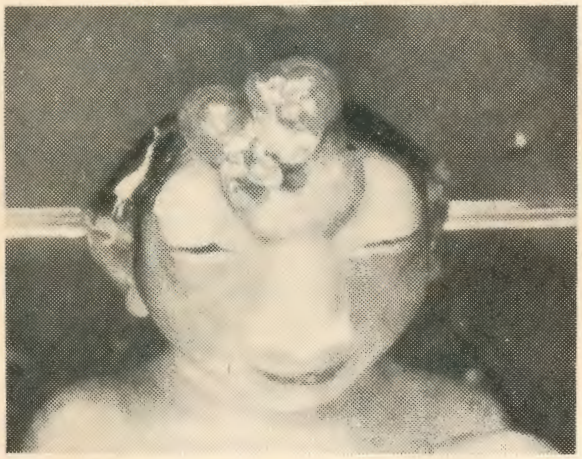

Fig. 2
Anencephaly with herniation of rudimentary brain.

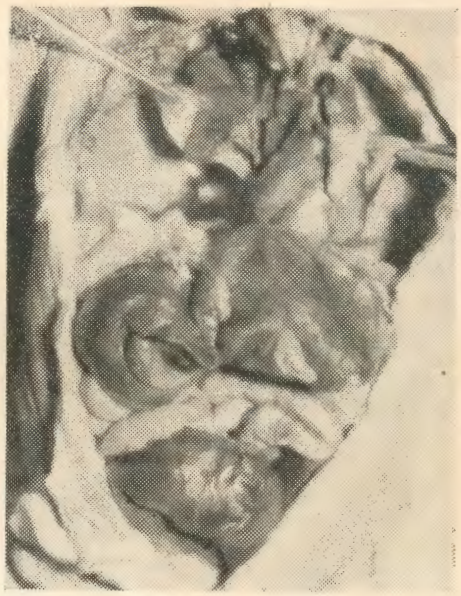

Fig. 3
Case of diaphragmatic hernia showing small sized (hypoplastic lungs after reduction of herniated viscera.

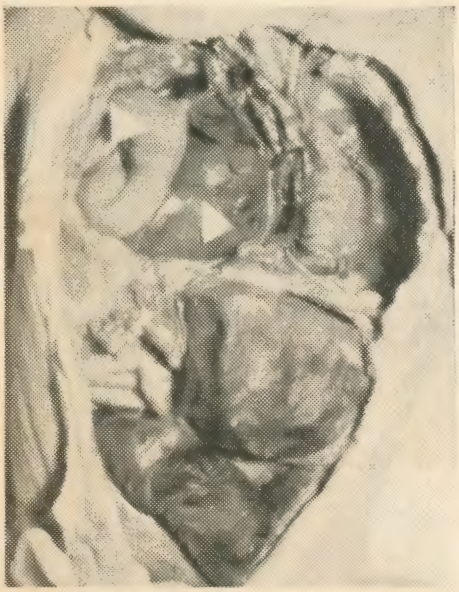

Fig. 4
Diaphragmatic hernia showing herniation of intestines and liver in thoracic cavity, associated with defect of right leaf of diaphragm.

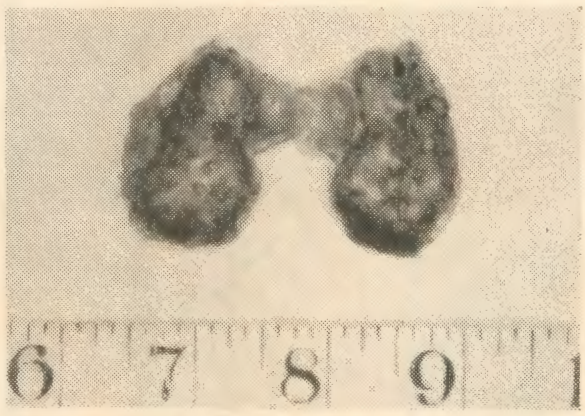

Fig. 5
Polycystic kidneys on cut section showing multiple cysts of variable sizes.

Fig. 6
Microphotograph of polycystic kidney showing cysts lined by two cuboidal epithelium with increased interstitial tissue and island of cartilage (H & E x 100).

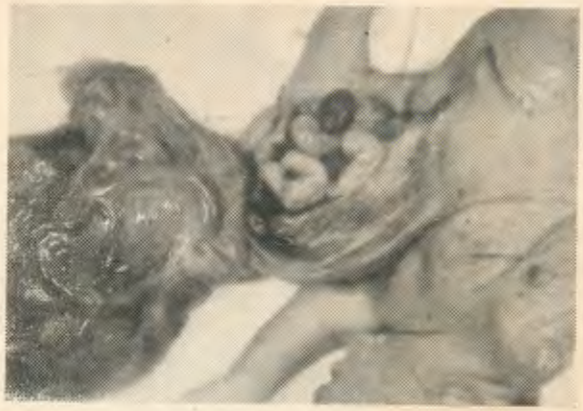

Fig. 7
Eventeration of abdominal viscera associated with large defect in the anterior abdominal wall.

Fig. 8
A case of stillbirth showing sacrococcigeal dermoid.

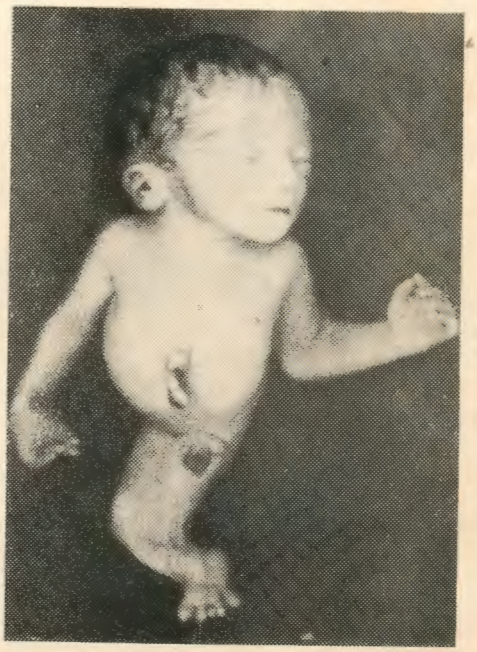

Fig. 9
A case of sympodia.

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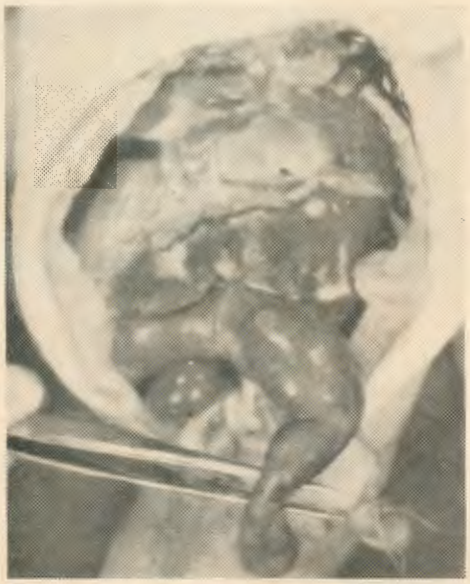

Fig. 10
A case of sympodia showing colon ending in a blind loop which is filled with meconium.

Fig. 11
A case of still birth showing multiple congenital malformations—anophthalmitis, agenesis of nose, cleft palate, ascitis etc.

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Fig. 1
(Magnified View) Blunt horn of uterus pseudodelphys with haematometra with haematosalpinx.

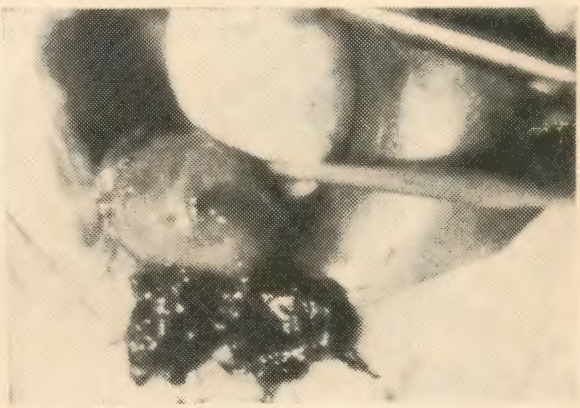

Fig. 2
Uterus pseudodidelphys, the two horns attached by thick membrane old clotted menstrual blood from the blunt horn.

Fig. 1
Ovarian tumour showing inner ragged wall covered with whitish flakes.

Fig. 2
Metastatic nodules in the mesentry and wall of the bowel. Some are solid while others cystic.

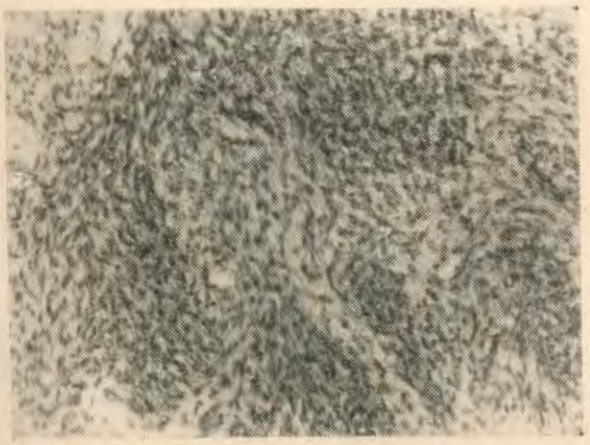

Fig. 3
Tumour cells arranged in bundles and whorls with myxomatous degeneration. H. & E. x 60.

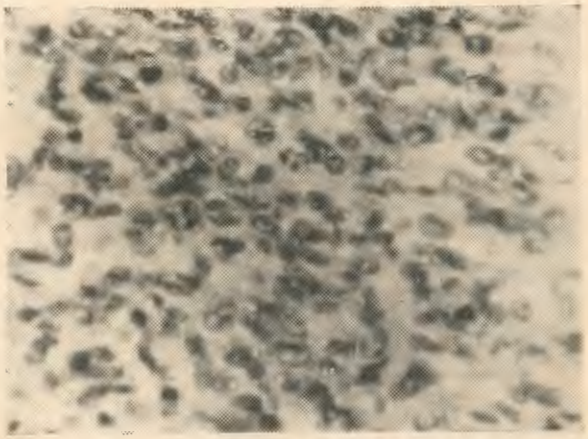

Fig. 4
Plumpy tumour cells showing hyperchromatism and fair number of division figures. H. & E. x 270.

Discussion

Liquor amnii from a total of 600 patients was studied. They were divided into two major groups. Knowledge of the last menstrual period was the only criterion of division into two main groups. No selection was made as regards age, parity or complications like hypertension, diabetes, toxaemia and anaemia.

There was increase in the turbidity of amniotic fluid with increasing period of gestation. Before 36 weeks it was clear, sparkling and straw coloured, whereas after about 38 weeks it became whitish in colour. In 71 cases there was slight meconium staining of liquor amnii after 40 weeks of gestation suggestive of placental insufficiency and postmaturity (Devi *et al*, 1970).

The amount of vernix caseosa was found to increase after 38 weeks of gestation. Bigger flakes of vernix were found to float in the liquor amnii at or after 40 weeks of pregnancy. It may be related to the shedding of primary lanugo hairs. Prior to 38 weeks of pregnancy liquor amnii was freely obtainable.

The cells seen microscopically in amniotic fluid after staining with Nile Blue Sulphate using the method of Brosens and Gordon could be divided into blue, orange and unstained. The cells of all the three groups may be nucleated or anucleated. It is on the basis of orange staining, anuclear foetal cells that diagnosis of ruptured membranes is made and their percentage helps us in foetal maturity assessment (Saraf and Purandare, 1966).

Foetal cells are polygonal or ovoid in shape. They are found singly or in clusters. The cellular diameter varied from 40 to 505 microns. The cytoplasm is non-vacuolated, translucent and orange stained. These cells are anucleated or are nuclear ghosts and nucleoli are absent.

A total of 500 cells was counted and the percentage of orange staining cells was calculated. It was noted that orange cells were very few in number before 34 weeks. No foetal cell was found between 14-29 weeks of gestation.

After 34 weeks foetal cells gradually increased in number till near term the percentage of orange cells came to 50 per cent. After term it was noted that the percentage of orange cells exceeded 50 per cent and cells had a great tendency to clump together and form clusters. Free fat globules were also noted after 38 weeks. The orange cells increased significantly with increase in the period of gestation (P/0.01). This is in agreement with previous workers—Brosens and Gordon (1967); Anderson and Griffiths (1968); Sharp (1968); Bishop and Corson (1968). Blue staining cells predominate the field before 36 weeks and after this their number decreased till after 40 weeks very few blue cells were found. The percentage of orange cells from 32-34 weeks is in agreement with that reported by other workers (Brosens and Gordon, 1966, 1967); Sharp (1968); Bishop and Croson (1968). From 34-38 weeks the range is from 0 per cent to 69 per cent. The percentage of zero per cent in this group was found only in a few patients. The result is, however, in agreement with Anderson and Griffiths (1968), who reported that there are no orange cells before 36 weeks. Chan *et al*, (1969); Barnett and Nevin (1970) also recorded that orange cells may be absent and zero counts may occur with immature, mature and postmature babies and so zero counts are not of much value. Whether twin pregnancy had a bearing on percentage of orange cells cannot be said as twin pregnancies were few in the

whole series; however, low counts can be explained on the basis of associated hydramnios. The percentage of orange cells beyond 40 weeks ranged from 13.8 per cent to 79.8 per cent. The lower figures obtained in this period of gestation are, however, in agreement with previous workers.

It was found that age, parity and complications of pregnancy did not have any effect on the percentage of orange cells as was reported by Brosens and Gordon, (1965, 1968); Anderson and Griffiths (1968) and Sharp (1968). Low readings were demonstrated in hydramnios complicating pregnancy as reported by Sharp (1968) and Huijes (1968). Whether dilution factor plays any part, requires further study.

It appears that low counts are encountered very rarely even when the baby is mature. If counts were more than 20 per cent it was almost certain that in 87.7 per cent the period of gestation was beyond 38 weeks and if counts were less than 20 per cent the period of gestation was less than 37 weeks in 72.7 per cent of cases. No importance was given to zero counts.

In a few cases of confirmed IUD, cellular differentiation was very difficult due to presence of thick meconium as the fluid was taken very late after the spontaneous rupture of membranes.

In about 50 patients, in whom liquor amnii was obtained at the time of hysterotomy and duration of pregnancy varied from 14 to 24 weeks, percentage of foetal cells was zero which can be explained only on the basis of foetal cell origin.

Beyond 40 weeks of pregnancy percentage of orange cells exceeded 50 per cent and usually the foetal cells were found in clusters.

Vernix caseosa and free fat globules must also be considered in giving an opinion as to the maturity because in a few patients with proved foetal maturity the percentage of orange cells was lower than expected from the general trend after 38 weeks.

False negative results were reported in 13 cases, where the flow of amniotic fluid was too low and contaminated.

The study suggests that Nile Blue Sulphate test is an accurate, simple and inocuous method of predicting foetal maturity, and could find a place in routine clinical practice where dates of last menstrual period are not known or periods are irregular.

Acknowledgement

We thank Dr. S. N. Ahmad Shah, M.R.C.P., T.D.D., Principal, Government Medical College and Dean, S. M. H. S. Hospital, Srinagar, for permiting us to investigate the patients and also staff of Unit I, Department of Obstetrics and Gynaecology, for their full co-operation extended to us in carrying out the investigation.

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