ORANGE CELL COUNT AS A COMPLEMENT TEST TO BUBBLE STABILITY TEST FOR PRENATAL ESTIMATION OF MATURITY†

Paper II

by

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Quantitative estimation of bilirubin and creatinine in amniotic fluid in addition to lecithin estimation or lecithin-sphingo-myelin ratio are of immense value in assessing fetal maturity and probability of the newborn developing R.D.S., but these tests require sophisticated biochemical laboratories. Nile Blue Sulphate test (Brosens and Gordon, 1966) and Bubble Stability test (Clements et al, 1972) have the advantage of simplicity and quickness and can be performed by the clinician. When the bubble stability test gives 'positive' result, it is of definite value (Edwards and Baillie, 1973 and Roux et al, 1973). With negative result, there are many 'false positives' i.e., these babies may not develop R.D.S. 'Intermediate' result with bubble stability test is found to be equivocal. Roux et al (1973) also found 'Intermediate' result equivocal as it was found in term as well as in premature babies.

Orange cell count shows wide variation in individual cases as reported by Dhar and Bazaz (1975). They reported counts above 20% in 87.7% of pregnancies above 38 weeks i.e., 12.3% had less than 20% count, such low count being found in 72.7% of pregnancies below 38 weeks.

The above two tests together may help in deciding the optimum time of intervention in complicated pregnancies.

Material and Methods

Amniotic fluid was obtained by vaginal, or abdominal route or at the time of caesarean section in 100 normal pregnant women who had their confinement in Lady Hardinge Hospital, New Delhi. Colour of the fluid and its turbidity was noted and bubble stability test was performed according to the technique of Clements et al (1972). The 5 tubes contained, amniotic fluid, 0.9% saline and 95% ethanol as shown in Table I. It was graded as positive, intermediate and negative. The presence of a complete ring of bubbles in first 3 or more tubes was taken as a 'positive' result. The absence of such a ring in the first tube indicated a 'negative' result, while the presence in the first and second tubes indicated 'intermediate' result.

Nile Blue Sulphate Test: One drop of amniotic fluid was directly mixed with one drop of 0.1% aqueous solution of commercial nile blue sulphate dye on a clean glass slide. Heating of the slide

TABLE I
Constitution of Tubes

Tube	1	2	3	4	5
Dilution	1:1	1:1.3	1:2	1:4	1:5
Amniotic fluid (ml)	1.0	0.75	0.5	0.25	0.2
0.9% Sodium chloride (ml)	0.0	0.25	0.5	0.75	0.8
95% Ethanol (ml.)	1.0	1.0	1.0	1.0	1.0

for 1-2 minutes was not constantly observed. Under low power, hundred cells were counted and the percentage of orange cells calculated. Small, moderate and large clusters were considered equivalent to 5, 10 or 20 cells (Brosens and Gordon 1966). Blue cells did not tend to cluster.

At the time of birth, weight of the newborn was recorded and maturity was scored according to ten neurological and eleven external criterion suggested by Dubowitz *et al* (1970). Observations were by the same person to avoid subjective error. The use of combined score, i.e. 35 for external and 35 for neurological is better than when only either is considered.

Results

1. Turbidity of liquor increases after 35 weeks of pregnancy. It was turbid in 91.7% of the cases between 37 to 38 weeks gestation, 96% of the cases between 39 to 40 weeks. Liquor was clear in only 2 out of 52 (3.9%) cases after 37 weeks of pregnancy (Table II).

2. In 61 cases where duration of pregnancy was known, orange cell count was less than 1% before 34 weeks gestation, but such a low count was also obtained between 35 and 36 weeks in 1 out of 5 cases (Table III).

3. Orange cell count above 30% was always found after 40 weeks gestation and in 96% of cases between 39 and 40 weeks gestation.

4. Orange cell count of 1 to 30% was not decisive of maturity. Wide scatter of maturity score (Table IV) and birth weight (Table V) was found with this cell count.

TABLE II
Turbidity of Liquor Amnii

Period of gestation in weeks	Samples	Appearance of Liquor Amnii	
		Clear	Turbid
34 <	4	4 (100%)	—
35, 36	5	4 (80%)	1 (20%)
37, 38	12	1 (8.3%)	11 (91.7%)
39, 40	26	1 (3.85%)	25 (96.15%)
>40	14	—	14 (100%)

TABLE III

Orange Cell Percentage in Relation to the Period of Gestation

Period of gestation in weeks	Samples	Orange Cell Percentage		
		1<	1-30	>30
34 ≤	4	3 (75%)	1 (25%)	—
35, 36	5	1 (20%)	4 (80%)	—
37, 38	12	—	4 (33.3%)	8 (66.7%)
39, 40	26	—	1 (3.8%)	25 (96.2%)
> 40	14	—	—	14 (100%)

Chi-Square 57.72; p <0.001

TABLE IV

Relationship Between Maturity and Orange Cell Count

Orange cell Percentage	Sample	Maturity Score		
		20 <	20-30	≥ 30
Less than 1	4	4 (100%)	—	—
1-30	10	4 (40%)	5 (50%)	1 (10%)
More than 30	47	—	9 (19.1%)	38 (80.9%)

Chi — Square 51.005; p<.001.

TABLE V

Relation of Bubble Stability Test and Orange Cell Percentage to the Birth Weight of the Baby

Bubble Stability Test	Orange Cell Percentage	Samples	Birth weight in grams		
			2000<	2000-2500	>2500
Negative	1<	8	7 (87.5%)	—	1 (12.5%)
	1-30	—	—	—	—
	>30	—	—	—	—
Intermediate	1<	1	—	1 (100%)	—
	1-30	15	7 (46.6%)	6 (40%)	2 (13.4%)
	>30	2	—	—	2 (100%)
Positive	1<	—	—	—	—
	1-30	3	—	—	3 (100%)
	>30	71	1 (1.4%)	10 (14.1%)	60 (84.5%)

5. When bubble stability test was negative, orange cell count was less than 1%, i.e. it predicted maturity of 34 weeks or less. Birth weight was less than 2000 gm (7/8) and maturity score was less than 20, (Tables V and VI).

6. When bubble stability test was positive and orange cell count above 30%, the predicted maturity was more than 38 weeks, birth weight was more than 2500 gm in 84.5% (60/71) and maturity score was always above 20 and above 30 in 80% (58/71).

7. 'Intermediate' bubble stability test had earlier been found inconclusive in predicting maturity. Out of 18 such cases orange cell count was above 30% in 2 cases and in both birth weight was

TABLE VI
Bubble Stability Test and Orange Cell Percentage in Relation to Maturity Score of the Baby

Bubble Stability Test	Orange Cell Percentage	Samples	Maturity Score 20	20-30	30
Negative	1<	8	8 (100%)	—	—
	1-30	-	—	—	—
	>30	-	—	—	—
Intermediate	1<	1	1 (100%)	—	—
	1-30	15	8 (53.3%)	7 (46.7%)	—
	>30	2	—	—	2 (100%)
Positive	1<	-	—	—	—
	1-30	3	—	1 (33.3%)	2 (66.6%)
	>30	71	—	13 (18.3%)	58 (81.7%)

above 2500 gm and maturity score above 30. In one case orange cell count was less than one, birth weight was between 2000 to 2500 gm and maturity score below 20. Thus in these 3 cases 'orange cell count' has been helpful in supplementing bubble stability test.

8. (a) Fifteen cases with 'intermediate' bubble stability and orange cell count between 1-30 were analysed further in Table VII. An orange cell count less than 10 may also be considered decisive

as in 7 out of 8 such cases babies were underweight and maturity score was below 22. However, in case 6, liquor was turbid and free vernix particles were also observed.

(b) With orange cell count 10-20% and intermediate bubble stability in 3 cases, one baby was obviously mature or above 38 weeks gestation as maturity score was 30 and birth weight as 2700 gm (Table VII).

TABLE VII
Birth Weight and Maturity Score in Cases with 'Intermediate' Bubble Stability and Orange Cell Count Between 1 to 30 Percent

Orange Cell Count	Appearance of Liquor	Vernix Particles	Orange Cell Count	Birth Weight in gms	Maturity Score
	C	—ve	2	2100	20.0
	C	—ve	2	1470	11.5
	C	—ve	2	1400	12.0
1-9	C	—ve	4	1490	14.0
	C	—ve	5	1800	20.0
	T	—ve	5	3270	26.5
	C	—ve	6	1500	10.0
	C	—ve	8	1800	22.5
	C	—ve	10	1800	20.0
10-20	C	—ve	10	2190	15.0
	C	—ve	15	2700	30.5
	T	+ve	26	2050	32.5
20	T	+ve	26	2050	26.0
	C	+ve	27	2250	28.5
	C	+ve	28	2400	31.0

T—Turbid, C—Clear

(c) Above 20% orange cell count, free vernix was present and fluid was often turbid. In this group maturity score was always above 25, i.e. corresponding to above 36 weeks gestation (paper 1) though birth weight was lower than 2500 gms.

Comments

The value of 'positive' bubble stability has been established by Schaffer and Avery (1972), Parkinson and Harvey (1973) and Dewhurst et al (1973). It correlates well with high L:S ratio (Wagstaff and Bromham 1973) and with very high risk of hyaline disease.

Orange cell count collaborated well with 'negative' bubble stability result, i.e. cell score was also less than 1%. In these cases birth weight was less than 2000 gm (excepting one) and maturity score less than 20, i.e. corresponding to less than 34 weeks gestation.

In the present study, value of nile blue sulphate test in cases with 'intermediate' bubble stability was assessed. Out of 18 cases orange cell count was less than 1 in 1 and more than 30 in 2 cases and position remained doubtful in 15 cases. These are analysed further in Table VII.

Brosens and Gordon (1966) suggested count of less than 10% as indicative of gestation of less than 36 weeks; such count would have caused 1 error out of 8 cases (Table VII). Here turbidity of liquor should have warned us about possible wrong low orange cell count. Between 10 and 20 orange cell score, there are only 3 cases; 1 with count of 15% had good birth weight. The remaining 4 cases with an 'intermediate' result had orange cell count above 25, in addition free vernix particles were observed in all. Though birth weight was low in these, maturity score was above 25, i.e. maturity corresponding to over 36 weeks gestation.

Thus, in cases with 'intermediate' bubble stability, orange cell count of above 25 would be safe for assuming maturity of above 36 weeks gestation. With counts between 10 and 25, one should take cognisance of turbid liquor and free lipids or vernix particles. Liquor is turbid in 96.30% of cases after 36 weeks of gestation. Sharp (1968) has remarked that unexpected low counts may be found but note should be made of turbidity of liquor and presence of free vernix particles or free lipids on microscopic appearance. Bellati et al (1969) also gave importance to the size of clusters.

Shah and Patil (1975) found that with orange cell count of 10% or more, labor could be safely induced, and no infant would weigh less than 2500 gm; this statement was true in 100% of their cases.

Acknowledgements

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