

STUDY OF VAGINAL CYTOLOGY IN THIRD TRIMESTER OF PREGNANCY

by

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In the last few decades science has made astounding progress. Medicine also has progressed hand in hand with it. The break through in various fields of obstetrics is no less remarkable, but, in spite of all these achievements, there still remains a hard core of day to day problems yet to be answered.

One of the most difficult decisions an obstetrician has to make is, to decide when to deliver a foetus in cases of pregnancy disorders like pre-eclamptic toxæmia, hypertension postmaturity or placental insufficiency. Moreover, in a country like ours many of the hospital cases do not remember their last date of menstruation correctly; this forgotten date also poses a big problem to determine the true maturity of the foetus, in case they require termination of pregnancy.

The study of vaginal cytology in the third trimester was undertaken to consider whether it is possible to

tell the obstetrician with reasonable certainty of the correct duration of pregnancy or the state of the foetus in utero in one of the pregnancy disorders.

Material and Methods

Hundred pregnant women in the third trimester were selected for the study irrespective of age and parity. Thorough clinical and obstetrical histories were taken and every case was examined clinically.

Smears were taken bi-weekly between 30 to 38th weeks. From 39 to 40 weeks they were obtained weekly and also at the onset of labour. Smears were also taken in the immediate postpartum period, that is, 2nd day after delivery.

Smears were collected from the posterior and lateral wall of the upper third of vagina by swab stick after introducing bivalve speculum without lubricant. They were fixed immediately in equal volume of ether and alcohol and were stained with Papanicolaou technique.

General appearance of the smear and presence of clumps, discrete cells, leucocytes, red blood cells, cytolysis and mucus were noted. Differential

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Received for publication on 27-3-67.

count of superficial, intermediate, navicular and parabasal cells was made. The percentage of superficial karyopyknotic cells was expressed as Karyopyknotic Index.

Observations

When comparative study was made between the smears taken from the posterior fornix and lateral wall of the upper third of vagina, it was noted that the lateral wall smears were generally cleaner in appearance. There was hardly any significant difference in the number of superficial, intermediate, navicular and parabasal cells amongst the smears. Sometimes leucocytes were more in the posterior fornix than in the lateral wall smears.

One hundred cases were studied under the following groups.

Normal pregnancy delivered at term	60
Prematurity	13
Postmaturity	4
Toxaemia of pregnancy	9
Severe anaemia in pregnancy	4
Twins	3
Antepartum haemorrhage	2
Syphilis with pregnancy	2
Intrauterine death	2
Quadruplegia with pregnancy	1
Total	100

Normal pregnancy delivered at term

These 60 cases had similar smear patterns during 30 to 38 weeks of gestation. The smears were characterised by heavy desquamation of cells forming big clumps, very few isolated cells, leucocytes few and slight to marked cytolysis in a number of smears having Döderlein bacilli. Navicular cells were 73.93 per cent between 30-37 weeks and 64.82 per cent at 38 weeks. Intermediate cells between 30-37 weeks were 22.16 per cent and at 38 weeks 29.62 per cent. Thus it was seen that navicular cells were inversely proportionate to intermediate cells. Superficial cells between 30-37 weeks were 3.01 per cent and at 38 weeks 6.55 per cent. No parabasal cell was observed during this period. (Table I).

The picture was of marked progesterone activity with navicular cells in clusters dominating the field. The clusters were mainly composed of navicular and intermediate cells with few superficial cells and hardly any discrete cells. There was absence of parabasal and red blood cells. Rarely leucocytes and mucus were found. The vaginal flora was mostly made up of Döderlein bacilli

TABLE I

Showing Mean Cytology at different weeks of gestation upto second postpartum day in 60 normal pregnant cases who delivered at term

Duration of pregnancy in weeks	Superficial karyopyknotic cells in %	Navicular cells in %	Intermediate cells in %	Parabasal cells in %
30-37	3.01	73.93	22.15	0
38	6.55	64.82	29.62	0
39	8.58	46.98	43.98	0.44
40	10.6	31.13	58.00	0.36
During labour	12.65	23.53	63.20	0.61
Postpartum	21.12	1.03	76.24	1.71

This type of smear was termed 'pre-term' smear pattern. Fig. 1.

Vaginal smears during the last two weeks of gestation, that is 39th and 40th week, showed gradual and progressive changes in the 'pre-term' smear pattern. Clumps diminished in size and number and were composed of lesser number of cells having clear margins. Many cells were found to be lying discrete. Number of navicular cells diminished gradually from 46.98 per cent at 39th week to 31.13 per cent at 40th week. Intermediate cells proportionately increased, that is, 43.98 per cent at 39th week to 58.00 per cent at 40th week. There was slight rise in superficial Karyopyknotic Index, that is, 8.58 per cent at 39th week to 10.6 per cent at 40th week (Table 1). In 9 cases parabasal cells were observed between 1 to 6 per cent. These smears had marked diminution of cell clusters, increased number of isolated and flattened cells and a rise in the Karyopyknotic Index, the smear background being clearer than before with diminished cytolysis. This type of smear was labelled as 'at term' smear pattern. Fig. 2.

With onset of labour the smears were characterised by absence of clumps, increase in number of discrete cells, proportionate decrease of navicular, increase in intermediate with definite increase of superficial cells (Karyopyknotic Index). In 8 cases parabasal cells were observed between 1.8 per cent. Cytolysis was rarely encountered, mucus increased in quantity and there was leucocytic infiltration in the smear. Fig. 3.

The characteristics of vaginal cytology after 2 days of delivery were paucity of navicular cells and appearance of 'postpartum' cells in large numbers. Superficial cells were seen in increased number and true parabasal cells were very few. Smears also contained large number of red blood cells and leucocytes along with histiocytes and mucus. No uterine cell or muscle tissue was encountered. Postpartum cells appeared in sheets having thick prominent cellular margin and partly pyknotic nuclei with vacuolated cytoplasm. The individual cell was smaller than intermediate cell but bigger than parabasal cell.

In the present series, out of 60 cases who delivered at term 44 showed 'at term' and 16 'pre-term' smear patterns at 39th week. Out of 44 showing 'at term' smear 14 delivered and out of 16 'pre-term' type of smear, one delivered at 39th week. At 40th week out of the remaining 45 cases, 43 showed 'at term' and two 'pre-term' pattern; 34 from 'at term' and two from 'pre-term' delivered at 40th week. The remaining 9 of 'at term' group delivered within a day or so of 41st week.

Prematurity

Thirteen cases were delivered prematurely; One of them delivered at 35th week, 6 at 37th week and the remaining 6 at 38th week. The mean cytological picture indicated that, as labour approached, superficial cells gradually increased while navicular cells decreased in inverse proportion to intermediate cells. Similar findings were observed in 60 cases who

delivered at term, only difference being that in this group the changes became apparent in the early part of the third trimester.

At 35th week two women had 'at term' smear pattern of whom only one delivered. At 36th week 3 of them had 'at term' smear pattern, but none delivered. At 37th week, out of 12 having 'at term' pattern 6 delivered and the remaining 6 delivered at 38th week.

Postmaturity

Four cases under study delivered normal healthy babies, one at 42 weeks, two at 43rd and the last at 44th week of gestation. All four patients were absolutely definite regarding their last menstrual period. At 41st week all smears had 'at term' smear pattern. Smears of two patients did not reveal parabasal cells at any stage whereas in the other two, 1-2 per cent were noted during the last week of gestation. Rest of the cytological picture did not reveal any difference from that of the normal pregnancy group. In two cases labour was spontaneous, the third baby was delivered by cesarean section, and the fourth was also delivered by cesarean section following failure of induction of labour. In the fourth case induction had failed though the smear pattern was of 'at term' type for over two weeks.

Twins

All three cases of twin pregnancy under study had 'at term' smear pattern at 39th week of gestation. Their cytological pictures were similar to those of normal pregnancy group.

Severe anaemia with pregnancy

Four cases having severe anaemia with pregnancy did not show any marked deviation from that of the normal group in their cytological picture. Three of them gave birth to normal healthy babies. The fourth, who had a fresh still-birth, revealed 2 per cent of parabasal cells in the smear taken just prior to labour.

Toxaemia of pregnancy

Nine cases under study could be classified as mild to moderate toxæmia. There was no severe case of toxæmia or eclampsia. One of the cases with normal range of superficial cells and 4 per cent of parabasal cells in the smear taken two days prior to labour had meconium-stained liquor amnii during labour. The baby was distressed and had to be revived.

The second case with high Karyopyknotic Index (40 per cent) and 2 per cent parabasal cells at 40th week also had normal delivery of a healthy baby. The third case in the 40th week with 2 per cent of parabasal cells and the rest being normal had also normal deliveries of a healthy baby. In the fourth case with blood pressure of 184/110 mm Hg. Karyopyknotic Index was never more than 3 per cent and there was no evidence of parabasal cells. In the remaining five cases cytological picture did not reveal any deviation from that of the normal group. Smears in all these nine cases had 'at term' pattern at 39th week.

Antepartum haemorrhage

Out of two cases under study one had slight bleeding at 38th week and

another moderate bleeding at 39th week lasting for one full day. Both showed normal cytological picture and had uneventful labour with normal healthy babies. Placenta did not reveal any abnormality.

Syphilis with pregnancy

Two cases were studied in the present series. Both had positive Wasserman reaction and were treated adequately.

One had a normal delivery of a healthy baby but the other delivered a fresh still-born foetus. Cytological picture was similar to that of normal group. No parabasal cell was seen in any of the smears taken from the second case.

Quadruplegia in pregnancy

The patient who had a fall during the third month of pregnancy developed quadruplegia due to cervical spine injury. She delivered a baby of 4 lb. 14 ozs. at 39th week. Forceps was applied to cut short the second stage of labour as the abdominal muscles were weak. Cytological picture, except for 2 per cent parabasal cells at 37th week and 1 per cent at 38th week, did not reveal any abnormality. The smears had 'at term' pattern from 38th week onward.

Intra-uterine death

Among two cases studied the foetus had died 15 days and 10 days respectively prior to delivery. From the cytological analysis of these cases it was evident that both had high Karyopyknotic Index, specially the second case who had successive values of 18.38 and 48 per cent. During 39th and 40th week their

smears were of 'at term' type. No parabasal cells were seen in any of these smears.

Comments

Vaginal smear study was carried out in 100 pregnant women in the third trimester and immediate postpartum period in both normal and abnormal pregnancy. The aim was not only to study the exfoliative vaginal cytological pattern, but also whether it was possible to predict the date of onset of labour and warn the obstetricians regarding postmaturity.

As Pundel and Lichtfus vehemently supported taking of vaginal smears from lateral wall, smears were taken both from lateral wall and posterior fornix, under direct vision, for the sake of comparison. Except that the lateral wall smear was clear, no other difference in hormonal set-up was observed.

From 30-38th weeks there was no change in the pattern of vaginal picture. It was of 'pre-term' type having big clumps or occasional discrete cells, but after 38th week, pattern changed gradually into 'at term' type with small clumps of very few cells and many discrete cells till most of the cells were discrete prior to labour. At 38th week 82 per cent of smears were of 'pre-term' type which decreased to 3 per cent by 40th week. Lemberg-Seigfried and Stamm and Lichtfus had also observed change from 'pre-term' to 'at term' pattern about 15 days prior to labour, but Pundel found this change only 5 days before the onset.

In the present study it was found to occur 15 days earlier. Similarly navicular cells decreased till they be-

came fewer in number before labour, and intermediate cells increased inversely to navicular cells. Superficial cells which were 6 per cent before 38th week became 13 per cent prior to labour. Spira and MacRae observed gradual decrease of superficial cells from 25 per cent to 12 per cent at term.

In the premature group the cytological picture was like that of the normal group except that changes were apparent in early part of the third trimester. In this group, out of 13 cases two, who had 'at term' smears at 35th week, had normal deliveries of healthy babies. The rest had 'at term' smears by 37th week and delivery was complete by 38th week. Thus though the gestational age predicted immaturity, biologically it was "at term" as predicted by cytological pattern and proved by normal delivery of healthy babies by 38th week.

In four postmature cases where delivery was at 42, 43 and 44 weeks respectively no apparent difference from that of normal in cytological picture was noted. Parabasal cells were not noted in two cases and in the rest the range was from 1-2 per cent only. In normal pregnancy group about 1-8 per cent of parabasal cells were observed and all of them had normal healthy babies. This finding could not confirm observation of Lichtfus and Pundel that the parabasal cell in the smear was an indication of postmaturity and hence induction of labour. Sammour observed parabasal cells in the 88 postmature cases. Kamnitzer found these cells in smears with living babies without any sign of foetal dis-

tress. Zidovsky commented that parabasal cells were a totally unreliable sign of foetal damage in prolonged pregnancy. However, we can say that prolonged pregnancy did not necessarily imply postmaturity as in none was a dysmature foetus born.

It has been suggested by Browne that placental margin of safety is greatly reduced in cases of twin pregnancy and term is reduced from 40th to 38th week. In the present study of three cases of twin pregnancy, two delivered in early 39th week and the other at 40th week. The cytological picture was also similar to that of normal pregnancy group. Walker and Turnbull in cord blood oxygen, Shearman in urinary pregnanediol and Banerjee in urinary oestrogen did not observe any difference from those having single foetus.

No marked change in cytological pattern from that of normal in patients with severe anaemia was observed.

Parabasal cells of 4 per cent were observed in one case having toxæmia in pregnancy. Foetal distress was present in this case. High Karyopyknotic Index was seen in one patient only. Spira and MacRae and Wood *et al* had also similar observations in long standing toxæmia. The remaining patients had normal cytological picture and this can be explained by the fact that toxæmia of short duration did not produce placental insufficiency which was reflected in the vaginal cytology.

In none of the cases of antepartum haemorrhage was bleeding severe or prolonged and thus no placental margin of safety was encroached upon, and there was no fall of placental

hormone thereby giving a normal vaginal cytology.

In cases of syphilis in pregnancy, though a fresh still-born foetus was delivered to one, no change in cytological picture was noted. Similarly no abnormality was observed in the vaginal cytology of quadruplegic case.

In cases of intra-uterine death high Karyopyknotic Index was observed. Though foetuses were dead 10 and 15 days before delivery no parabasal cells were seen in smears at any stage. Spira and MacRae observed high Karyopyknotic Index in these cases complicated by toxæmia and hypertension, but in our cases none of these complications were seen.

Conclusion

When 'pre-term' smear pattern was seen it could be predicted with reasonable certainty that labour would not start within 10-15 days.

The 'at term' smear pattern was observed about 15 days before the onset of labour, but it was impossible to predict the exact date of onset of labour.

No specific cytological picture was observed in cases of postmaturity or in any abnormalities of pregnancy.

Parabasal cells had no practical significance in cases of intra-uterine death, postmaturity, toxæmia of

pregnancy or in other pregnancy disorders.

Vaginal cytology could only predict that the foetus had reached term and may be delivered in about two weeks' time, irrespective of the gestational age.

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Figs. on Art Paper III