VAGINAL CYTOLOGY AND ABORTION

by

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In recent years cytological method in diagnosis and evaluation of sex hormonal status has become firmly established.

The present analysis was done to evaluate the aid that vaginal smear method provides in guiding treatment and in judging the efficacy of progesterone in the treatment of abortion.

Papanicolaou (1925) described the characteristic navicular cells of pregnancy in vaginal smears and since then several studies of exfoliative cytology have been made by Pundel and Van Meensel (1951), Spira and Mac Rae (1960), Wood, Osmond Clarke and Murray (1961). Despite suggestions from these writers and in spite of the fact that the vaginal cytologic characteristics of gynaecological patients have been studied throughout the world, similar study in obstetric patients has received little attention. Traut (1936) emphasized that during pregnancy superficial layers of cornified and precornified cells are practically non-existant, whereas the midzone of vaginal epithelium undergoes tremendous hypertrophy and most of the cells seen in smears taken from pregnant patients are derived from these intermediate zones.

Flecther (1940) suggested the vaginal smear as a means of recognizing incomplete abortion in women whose vaginal bleeding was of undetermined significance. He believed the appearance of a layer of basal cells, with many phagocytes, to be diagnostic of incomplete abortion. Hall (1942) reported the use of vaginal smear in correlating the phases of vaginal cornification with progesterone deficiency and formulated a quantitative progesterone estrogen ratio. Schuman (1940) also considered the possible significance of vaginal smear in disturbed pregnancy as well as in diagnosis of pregnancy. However, a complete knowledge of hormonal play in ovulatory menstrual cycle is essential for interpreting the smears. At ovulation, estrogen usually reaches its peak level in the circulation. As Rakoff (1946) pointed out, its effects upon the smear may appear more marked at this time since it is unopposed by any appreciable amount of progesterone. A smear taken at this point shows the highest proportion of highly staining eosinophil cells of polygonal shape having pyknotic nuclei with sharp distinct borders.

Once the corpus luteum begins to

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function, progesterone starts to exert its effect upon the cells that previously had been under the sole influence of estrogen. As a result some regressive effect appears in smears since progesterone opposes the cornified effect of estrogen. This regressive phase promotes changes in both morphologic characteristics and staining reaction of the squamous cells which tend to appear clumped together with curling and folding of their edges.

(2) 404 cases of threatened and recurrent abortion:
   (a) threatened abortion — smear examination was done once a week from the time of onset of vaginal bleeding till 22nd week, followed up to term by clinical examination,
   (b) the pattern of investigation in recurrent abortion was the same.

TABLE I

Type of smear	Intermediate cells %	Navicular cells %	Cornified cells %
Normal	65	20	15
Abnormal (slight)	45-65	20	15-35
Abnormal (moderate)	45-55	10	35-45
Abnormal (severe)	30-55	0	45-70

These changes become predominant with supervention of pregnancy. Navicular forms may become predominant and nuclei tend to become vesicular. The cytoplasm of most cells now becomes basophilic. These navicular cells represent the so-called normal cells of pregnancy. The present investigation is an attempt to assess the prognostic value of the change in pattern of vaginal smears during disturbed gestation.

Material and Method

The material consisted of

(1) 75 cases of normal pregnancy who had smear examination up to the 22nd week of pregnancy and thence followed by clinical examination till the pregnancy terminated in full-term delivery. Vaginal smears were taken once a week,

Table 1 gives the proportion of cornified and uncornified cells in normal pregnancy and in various grades of abnormal pregnancy.

As we believe that increasing cornification connotes threatened or impending abortion for the purpose of evaluation when smear showed abnormal pattern, we arbitrarily graded the increase in cornification into three classes, slight, moderate and severe. In the first group scattered cornified cells were present between 15-35%. In the second group 35-45% of cells were cornified and in the last, most of cells were cornified, 70%.

It seems paradoxical that during pregnancy when it is proceeding normally the cornification index falls. Cornification index is generally accepted as a sensitive indicator of concentration of estrogen. There is a large quantity of oestrogen during

pregnancy and yet in normal pregnancy the cornification index is low rather than high. However, the cornification index is known to be reduced by anti-estrogens such as progesterone and androgen; since progesterone is known to be present in large amounts in normal pregnancy, this could explain the low cornification index. Therefore, we believe that increase in cornification connotes threatened or impending abortion. When the cornification index was between 15-35% the abortion rate was only 12%; 80% of them had normal term delivery. The rest who continued beyond 22nd week of gestation and had palpable foetal parts in ante-natal examination at 22nd week, did not turn up for further ante-natal examination. But it is presumed that they had normal uneventful pregnancy and delivery as they would have reported for check up if any abnormality had occurred. Those who had moderate type of abnormal smears, 43%, had abortion before the 22nd week of gestation; some of them showed tendency to increasing rise of cornification index. Of those who had shown high cornification index, above 70%, 83% aborted.

Threatened Abortion

There were 340 cases of threatened abortion between 6-22nd week of pregnancy. Every case had one bout of bleeding before admission to the hospital. The following criteria were expected to be fulfilled before the case was taken up for investigation: —(i) normal anatomy of the uterus and cervix according to the period of gestation; (ii) exclusion of general aetiological factor, (iii) absence of incompetent os, (iv) negative serological test. Though threatened abortion is defined as uterine bleeding before 28 weeks of pregnancy without any evidence of expulsion of the products of conception, Clark and Murray (1963) took up cases only between 6-22 weeks; we agree that hormone level plays very little part in abortion after 20 weeks, therefore, it is futile to search for abnormal hormone pattern in threatened abortion after 22nd week of pregnancy.

The cases of threatened abortion under investigation did not receive any hormone treatment. They had sedation, bed rest and avoidance of sexual intercourse. The results are tabulated in Table II.

TABLE II

Type of smear	No. of cases	Abortion	Pregnancy continued to 22nd weeks	Full-term delivery
Slight	210	25 (12%)	185	180 (85.7%)
Moderate ..	60	26 (43%)	34	32 (55.1%)
Severe	70	58 (83%)	12	8 (11.4%)

Recurrent Abortion—64 Cases

Majority of these were taken from private clinics. Total number of cases were collected from 1962, 1963 and 1964 between ages of 20-30 years and gave reliable history of two or more consecutive spontaneous abortions before 20th week of pregnancy; none

were included in whom complicating factors such as positive serological tests, extensive cervical tear, uterine malformation, and associated medical diseases of obvious potential significance had been found. All the routine investigations for cervical incompetence and syphilitic infection were carried out in the selected cases. Preliminary investigation in 15 of these cases had been done. They were subjected to extensive investigation for uterine abnormalities before conception, and had endometrial biopsy to see the secretory changes in the endometrium. Vaginal smears were taken as soon as patient attended the clinic, which usually happened to be between 6-10 weeks of pregnancy. All of them were seen and investigated from the 6th week of conception.

Thirty-two cases were chosen at random to study the effect of progesterone in preventing abortion. They were given in addition to sedation etc. 250 mg. of intramuscular Proluton Depot once a week till the 22nd week.

Thirty-two others had treatment only with bed rest, sedation, tranquilizers and avoidance of sexual intercourse.

TABLE III

Outcome of Pregnancy in 32 Cases of Recurrent Abortion without Hormone Therapy

Type of smear	No. of cases	Abortion	Pregnancy proceeded beyond	Full-term delivery	Foetal abnormality
Abnormal:—					
Slight—15-35%	10	2 (20%)	8 (80%)	8	Nil
Moderate—35-45%	8	4 (50%)	4 (50%)	3	Nil
Severe—4570%	14	10 (71%)	4 (28.5%)	4	Nil

TABLE IV

Results in 32 Cases of Recurrent Abortion with Hormone Therapy

Type of smear	No. of Cases	abortion	Pregnancy proceeded	Full-term delivery	Foetal abnormality
Abnormal:—					
Slight	8	2 (25%)	6	6 (75%)	Nil
Moderate	16	4 (25%)	12	12 (75%)	Nil
Severe	8	3 (37.5%)	5	4 (62.5%)	Nil

Table III shows the outcome of pregnancy in 32 cases of recurrent abortion.

Comparison of tables III and IV shows that there was definite improvement in the total salvage rate with progesterone therapy in moderate or severe degrees of hormonal abnormality. In cases where there was slight abnormality the abortion rate remained the same whether there was added therapy or not. This

suggests that the abortion was perhaps due to some other cause rather than deficiency of progesterone.

Case Report

S. S., age 28 years, was admitted with the complaints of previous two recurrent abortions before 16th week of gestation. Examination both of the patient and her husband revealed no abnormality. She was serologically negative. She had been examined in the present pregnancy at the 6th week of gestation. Her last menstrual period was 4th September, 1964. She had a weekly vaginal smear and administration of proluton depot (250 mg.) once a week when cornification index showed signs of rising. She had an uneventful pregnancy, ending in an elective caesarian section on 23rd June 1964, for post-maturity. No foetal abnormality was detected.

Discussion

The importance of hormonal control over pregnancy has been the subject of much study, as demonstrated by the classic work of Nelson and Evans (1954) and changes in proggresterone and oestrogen secretion during pregnancy have been described by various writers. The key role of progesterone in the maintenance of pregnancy has long been appreciated both experimentally and clinically. In normal pregnancy the plasma pregnanediol rises from 5 mg. per cent in the early months to 50 mg. per cent at term, a very steep increase occurring at the 28th week (Despande and Sommerville, 1958); similar results have been shown by Shearman (1959). Davies and Plotz (1957), using known quantities of progesterone, found that the urinary estimations of pregnanediol were very erratic. Therefore, it is an imperfect indicator of progesterone metabolism. As regards oestrogen, a great increase in urinary oestrogen occurs during pregnancy. This has been shown by several workers including Clayton and Marrian (1950), Aitken *et al.*, (1957), and reliable and less laborious methods of oestrogen assay have been described by Brown *et al.*, (1957). Estimations of both hormones, however, can be performed only by expert pathologists and are costly, in time and money, and we feel that examination of exfoliated vaginal cells is an adequate and accurate measure of the final hormonal balance of oestrogen progesterone ratio.

Clark and Murray (1961) have taken into account various other aspects of vaginal smear along with cornification index to predict good or poor prognosis in cases of abortion. According to them the assessment of vaginal smear was made from amount of cell desquamation, size of cells, pattern of cells and cornification index.

As a result of the work of Pundel and Van Munsell (1951), Spira and Mac Rae (1960), Wood, Esmond Clarke and Murray (1961), it became evident that taking of vaginal smears offers a simpler means than biochemical essay.

Exfoliative vaginal cytology can reveal hormonal imbalance and thus serve as a guide to treatment and prognosis. Cornification index is taken as the major criterion for distinguishing abnormal and normal patterns of vaginal smears during pregnancy by the majority of authors, while Clark and Murray (1961) have taken into account various other aspects of vaginal smear

along with cornification index to predict the prognosis in cases of abortion. In this study cornification index has been taken as the most important criterion for prognostic significance in various abnormal pregnancies.

In early pregnancy the vaginal smear is progestational carrying on the normal luteal phase of normal menstrual cycle but with navicular cells of Papanicalaou which are prominent. According to Hochstaedt Lange and Spira (1960) the cornification index of vaginal smear should not exceed 25% in the first half of pregnancy. In our work the average cornification index was 15%. We have taken cases of abortion between 6-22 weeks of pregnancy, because during that period hormones play a dominant role in bringing about abortions and after that period other incidental factors play a more important role. Therefore, we have limited our investigation between 6-22 weeks of pregnancy.

Threatened Abortion

Our work is in complete agreement with that of Menon et al., (1959). In this series when the cornification index was between 30%-40% the incidence of abortion was 63% and when the cornification index was 50% the abortion rate was 100%. The results of this series of threatened abortion also compares favourably with that of Rami, Daftary & Masani (1963). In their series, 60% of cases who aborted had a cornification index of above 60%. Therefore it is logical to presume that cornification index has a prognostic significance in the outcome of threatened abortion.

Three hundred and forty cases of threatened abortion were selected for investigation. The incidence of endocrine factor as a cause of abortion was high in our series but high frequency was not surprising when one considers that our cases were selected after exclusion of all other organic pathology which might have acted as an etiological factor for abortion. Abortion occurred in only 12% of cases where the cornification index was between 15-35%, in 43% where the cornification index was between 35-45%, and in 83% of cases where cornification index was above 45%. This supports the view of Hochataedt Lange and Spira (1960) and Wood Osmond, Clarke and Murras (1961). In our cases maximum abortion occurred when vaginal cytology gave cornification index above 45% (Refer Table II).

Recurrent Abortion

Rami, Daftary Masani (1963) had noted record of cases of recurrent abortion where progesterone deficiency was indicated by raised cornification index and responded favourably to progesterone therapy. Therefore they suggested that the patients showing cornification index between 10—16% might benefit by different doses of progesterone.

Tables III and IV give results of analysis of 64 cases of recurrent abortion with or without hormone therapy.

The use of hormones, to prevent abortion, is a common practice. Spontaneous and recurrent abortion occurred at rates between 5-10%. When general and local causes are eliminated there is still a large num-

ber to be accounted for. It is here that hormone deficiency is postulated as an important cause, and progesterone is commonly used to preserve pregnancy. In their first series Bishop, Richards and Doll (1950) reported 45 cases of habitual abortion treated by progesterone implantation, their success rate being 86% for those patients who had two previous abortions, 89% for those who had three and 75% for those with four or more. They compared these results with the calculation of Eastman on the expected outcome of pregnancy after repeated abortions and concluded that, provided true incidence of recurring abortions is not less than 0.1% their treatment had been of real value. In their second series, Bishop and Richards (1952) added a further 40 cases and for the combined group of 85 cases the success rate was 85%, 76% and 67% for patients with histories of two, three and four or more previous abortions respectively. Their conclusions remained the same, though they acknowledge the report by Bevis (1951) on 32 patients with three or more successive abortions who had 81% of live births without any specific therapy but who had been subjected to extensive investigation. The efficacy of this treatment, however, has been questioned by Swayer and Daley (1953), who compared the results of progesterone implants in 60 cases with the results of no treatment in 53 cases. In patients who had two or more previous abortions 80% of the treated and 75% of the untreated had live babies, and in patients who had three or more abortions 74% of the treated and 55% of the untreated had live babies. This may, however, also indicate that true hormonal deficiency has spread unequally in both groups before treatment.

Jones and Delfs (1951) who studied extensively the endocrine patterns in women with histories of abortion, based the therapy (with thyroid, progesterone, vitamin E) on the assumption of deficiencies of these factors. There were 74 pregnancies in their series in women who had three or more consecutive abortions, 41 gave indications of hormone deficiency and were treated appropriately, 35 going to term (85% success); 23, also apparently deficient, were left untreated, and only 5 went to term (22%).

Malpas (1938) gave success rate, with pregnancy going to term, of 62% patients who had two abortions and were not given treatment, but he states that chance of a living child was only 27% after 3 abortions. It is unfortunately impossible to rely on previous obstetrical history as an indication of hormone deficiency and it is common experience that a patient, who had several past abortions, may have a normal pregnancy without treatment. It is also suggested that indiscriminate use of hormones may cause abortion (Bender, 1947). The controversial issue is whether treatment with aspirin, bed rest, psychotherapy has got the same value as hormone treatment. But, in spite of this scepticism, evidence has recently been produced which would suggest that some cases of recurrent abortions may occur as a result of endocrine imbalance. This has been shown by evidence of vaginal cytology by

Haechstaedt Langer and Spira (1960), and by Shearman and Gurett (1963). The former authors found abnormal cytology in 108 out of 140 cases of recurrent abortions. In patients with abnormal smears they found that 75% of present pregnancy survived following progesterone therapy, while 25% of pregnancy survived without progesterone therapy. In our series of cases, the abortion rate was 50% when cornification index was 35-45% and 71% when the cornification index was 45-70%. But when there is added progesterone therapy in those cases where cytological pattern was moderately or severely abnormal, the abortion rate fell to 25% and 37% respectively. Table IV shows the effect of progesterone in cases of recurrent abortions. According to Clark and Murray (1961) abortion occurred only in 8% of cases when vaginal smear was leveled as good. Birtch (1960) emphasized that from 123 spontaneous abortions there were 76 normal pregnancy smears but his work does not compare with other authors.

Osmond Clark and Murray (1963) postulated that patient with persistant normal smears has 97% chance of successful pregnancy, whereas with persistant abnormal smears this chance is reduced to approximately 9%.

The spontaneous cure rate in initial abnormal groups not treated with hormones is approximately 33%, while in those treated with hormones it is one-third higher.

It is surely important before treating a patient to decide whether or not any hormone treatment is necessary. "Blind" treatment solely on history of recurrent abortions is both expensive and inconvenient; vaginal smear should therefore be taken to detect hormone deficiency before treatment is given and biweekly smears taken to control therapy. This is clearly shown for cases of early pregnancy by Hochstaedt Lange and Spira (1960) who report a success rate of 73% in 89 cases where there was progesterone deficiency as shown by vaginal smear. These cases were treated by intra-muscular progesterone therapy in doses varying from 25-100 mg. daily controlled by weekly vaginal smears, improvement occurring is 10-14 days. The average length of treatment was one month.

On the other hand in 29 cases, some with progesterone deficiency and having no treatment, these workers could report only 24% success. In our series of cases we gave 250 mg. progesterone intramuscularly once a week in 32 cases; the success rate was 75% in severe progesterone deficiency. In those without hormone treatment, where abnormal smear was seen, the abortion rate was between 50-70%. Therefore, it would seem conclusive that where progesterone deficiency exists in early pregnancy, treatment by hormones, controlled by vaginal smears is advisable.

Masculinization of Foetus from Progesterons

Attention has recently been focussed on non-adrenal female pseudohermaphroditism occurring in babies whose mothers were given various hormonal preparations during pregnancy.

The first series was reported by Lawson Wiekins and his colleagues, in 1958; twelve of the 17 patients had been born to mothers who, because of habitual or threatened abortion, had received 17-a ethinyl-testosterone (ethisterone) during pregnancy. Several other writers have since reported series of similar cases most of which have been summarised by Wilkins in his paper which covers 101 patients. Thirty-four of these were associated with use of ethisterone, 35 with 17-a ethinyl-19-nortestoterone (norethisterone) and one with norethignodreal and ethimyl estradiol-3-methyl ether. Foetal masculinization occurred with lower doses of norethisterone than of ethisterone. Many of these patients' mothers received other hormones (especially estrogen) as well as progestogens.

Wilkins and his colleagues have reported cases of 10 masculinized foetuses whose mothers received no hormone treatment at all and two others whose mothers received intramuscular progesterone. Bongiovanris and his colleagues described four similar babies whose mothers received only stilboestrol during pregnancy. Finally, G. H. Valentine reported the case of a baby whose mother had received norethisterone; the baby, in addition to having an enlarged clitoris, also showed manifestations of estrogen stimulation, including vaginal bleeding, when 10 weeks old. It seems possible that these drugs as well as maternal hormones themselves may in some individuals undergo unusual metabolic transformation to produce androgenic or estrogenic metabolities. Yet a further possibility suggested by Bongiovanris and colleagues is that foetal adrenals may be stimulated to secrete androgens.

Our patients received Prolution Depot therapy. All the babies were examined for evidence of musculinization; no foetal abnormalities were found in any case.

Summary

Seventy-five cases of normal pregnancy, 340 of threatened abortion, and 64 cases of recurrent abortions were analysed with weekly vaginal smear examination.

The vaginal smears were arbitrarily graded into slightly abnormal (15-35%), moderately abnormal (35-45%) and severely abnormal (45-70%).

In threatened abortion, abortion rate was 12% when cornification index was slightly abnormal, 43 and 83% when cornification index was moderately and severely abnormal.

In 32 cases of recurrent abortions without hormone therapy abortion rate was 20%. 50% and 71% when cornification index was slightly, moderately and severely abnormal respectively.

In 32 cases of recurrent abortions with hormone therapy abortion rate was 25%, 25% and 37% with various grades of abnormal smear.

There were no foetal abnormalities in any of these cases when progesterone hormone was given.

References

1. Aitken, E. H., Preedy, J. R., Eton, B. & Short, R. V.: Lancet, **2**: 1096, 1958.

2. Bender, M. S.: J. Obst. & Gynec. Brit. Emp. **56**: 783, 1947.
3. Bevis, D. C. A.: Lancet, **2**: 207, 1951.
4. Birtch, P. K.: Clin. Obst. Gynec. **4**: 1062, 1961.
5. Birtch, P. K.: B.M.J. **2**: 123, 1960.
6. Bishop, P. M. F., Richards, N. A. & Doll, R.: B.M.J., **2**: 130, 1950.
7. Bishop, P. M. F. & Richards, N. A.: B.M.J., **1**: 244, 1952.
8. Bongiovanris, A. M. D., George, A. M. & Grumbach, M. M.: J. Clin. Endocrinol, **19**: 1004, 1959.
9. Brown, J. B., Bulbrook, R. D. & Greenwood, F. C.: Endocr. **16**: 41, 1957.
10. Clayton, B. E. & Marrian, G. F.: J. Endocr. **6**: 332, 1950.
11. Davies, E. M. & Plotz, E. J.: Am. J. Obst. & Gynec. **76**: 939, 1958.
12. Deshpande, G. N. & Sommerville, I. F.: Lancet, **2**: 1046, 1958.
13. Fletcher, P. F. A.: Am. J. Obst. & Gynec. **39**: 562, 1940.
14. Hall, S. J.: J. Clin. Endocrinal, **5**: 34, 1945.
15. Hochstaedt, B., Lange, W. & Spira, H.: J. Obst. & Gynec., Brit. Comm. 67: 102, 1960.
16. Jones, G. E. S. & Delfs, E.: J. Am. Med. Ass. **146**: 1212, 1951.
17. Malpas, P.: J. Obst. & Gynec. Brit. Emp. **45**: 932, 1938.
17a. Menon, M. K. K. et al: J. Obst. & Gynec. India, **10**: 84, 1959.
18. Nelson, M. M. &. Evans, H. M.: Endocrinology, **55**: 543, 1954.
19. Osmond-Clarke, F. & Murray, M.: B.M.J., **1**: 307, 1958.
20. Osmond-Clarke, F. & Murray, M.: B.M.J., 1172, 1963.
21. Papanicolaou, G. N.: Proc. Soc. Exp. Biol. (N.Y.), **22**: 436, 1925.
22. Pundel, J. P., Van Meensel, F. & Jaworski, Z.: Gestation et Cytologic Vaginale, Masson Paris, 1951.
23. Pundel, J. P. A.: Actacytol, **3**: 253, 1959.
24. Rakoff, A. E.: Progress in Gynaecology, ed. by J. V. Meigs, & S. H. Strugis, (N.Y.), Guine, 1946, p. 81.
24a. Rami, J. D., Daftary, V. G., Masani, K. M.: J. Obst. & Gynec. India, **13**: 185, 1963.
25. Schuman, W.: Am. J. Obst. & Gynec., **47**: 808, 1944.
26. Shearman, R. T. & Garrett, W. J.: B.M.J., **1**: 292, 1963.
27. Somerville, I. F., Marrian, G. F. & Kellar, R. J.: Lancet, **2**: 89, 1940.
28. Spira, H. & Mac Rae, D. J.: J. Obst. & Gynec. Brit. Emp. **67**: 597, 1960.
29. Swayer, G. I. M.: B.M.J., **2**: 161. 1955.
30. Swyer, G. I. M. & Daley, Doreen: B.M.J., **1**: 1073, 1953.
31. Traut, H. F., Bloch, P. W. & Kuder, Surg. Gynec. & Obst., **63**: 7, 1936.
32. Wood, C., Osmond-Clarke, F. & Murray, M.: J. Obst. & Gynec. Brit. Comm. **68**: 778, 1961.