HISTOLOGICAL STUDIES OF VAGINA AND ENDOMETRIUM IN HABITUAL ABORTION

by

E. PETERS** F.R.C.O.G.

PURNAKALA SHARDA* M.S.

Lataste (1892) established the concept of vaginal rhythm and related it to oestrus and ovulation in several animals. However, it was not until 1917 when Stockard and Papanicolaou (1917), by studying the cytological changes produced in the vaginal smear of the guinea pig and correlating them with ovarian function, could establish the presence of sexual cycle in them. The histological studies of sex organs in primates, as conducted by Corner *et al* (1927), suggested that similar types of ovarian as well as endometrial cycles did exist in women. This concept constituted the basis of our knowledge pertaining to changes occurring in the human vaginal epithelium (De Allende, 1958).

Habitual abortion, though it is one of the commonest conditions met with in clinical practice, from the point of view of its varied aetiology, pathology, histology and endocrinal pattern and multiplicity of the methods of treatment, has always posed a problem to the obstetricians. The period quoted by Bishop (1951) as "Sweeping horses in mid-stream" is critical and it is at this time that many abortions occur. One cannot help feeling that the erratic outpouring of chorionic gonadotrophins represents a determined attempt to ensure that either the placenta or the rapidly waning corpus luteum must maintain the optimum concentration of progesterone.

Study of vaginal smears during pregnancy and endometrial biopsy during the premenstrual phase seems to be useful in evaluating the hormonal imbalance. This work has been undertaken with a view to assess the value of the cytological studies of vagina and the endometrial biopsies in cases of habitual abortion.

Material and Methods

These studies were conducted on 45 subjects who attended the gynaecological department of the State Zenana Hospital, Jaipur. Patients with a history of three or more consecutive abortions were taken as cases of habitual abortion, and were divided into two groups; (i) pregnant, and (ii) non-pregnant, each consisting of 15 subjects. Fifteen non-aborters were divided similarly into group of (i) 10 pregnant and (ii) 5 non-pregnant women, and were considered as control.

A careful record of the past and the present illnesses, as well as the menstrual and obstetrical histories of

*Reader in Obst. & Gynec.

**Prof. of Obst. & Gynec., S.M.S. Medical College, Jaipur.

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each case, was made. General and systemic examinations were carried out. In addition to the local examination of the external genitalia and cervix, a speculum examination was also performed. The following investigations were undertaken:—

(1) *Blood* for total RBC, WBC and Hb, differential leucocyte count, ESR, cholesterol, glucose, Kahn's test and Rh-factor.

(2) *Urine* for routine and microscopic examination.

(3) BMR- was calculated by Ried's formula, i.e., BMR-0.75 (PR + 0.74 PP)- 72, where PR and PP represent the pulse rate per minute and the pulse pressure respectively.

(4) *Vaginal smears* were prepared by Papanicolaou's technique (Papanicolaou's and Traut, 1943).

(5) *A test for cervical incompetence* was performed by introducing a 8 mm. Hegar's dilator through the internal os. Definite resistance associated with discomfort was experienced in cases with competent os, while no resistance was encountered, nor did the patient complain of any discomfort during its passage in cases with incompetent os.

(6) *Endometrial biopsy* was done only in cases of competent os and the tissue was stained by haematoxylin and eosin. The results of the vaginal smears and endometrial biopsies have only been presented here.

Vaginal cytology:—Cytological interpretations were derived by counting 200 cells from portions of the vaginal smear showing a thin and uniform layer of cells.

Cornification Index:—During the preconceptional phase, the standard values for comparison were taken as described by Novak and Novak (1958). The relative percentage count of navicular, precornified and cornified cells has been represented as n/p/c respectively. (In pre- and post-ovulatory phases they were 0/0/100 and 5/90/5, indicating a 'shift to the right' or a 'shift to the left' respectively). During pregnancy standard values have been quoted by Hochstaedt *et al* (1960). In the earlier half of pregnancy smears showing cornified cells upto 25% were considered as normal. As the progesterone level declined, the number of cornified cells indicated a rise.

In smears, cornified cells have been regarded as an indicator, and the index denoting their value is called the cornification index (CI).

In non-pregnant subjects, vaginal smears were examined weekly, and endometrial biopsy was taken during the premenstrual phase. In pregnant women, the vaginal smears were taken at monthly intervals starting from the earliest period of pregnancy, when the subject could be available for examination for the first time.

Results

63% of aborters fell between the age groups of 20-30 years. The average age for abortion was found to be 27 years and no case above 36 years of age was present in our studies. No correlation between the build of the patient and abortion could be established. The maximum number of abortions occurred during the first 8-12 weeks of pregnancy.

I. *Non-Pregnant Group*:—The results have been shown in Table I.

TABLE I.

Shows the Cornification Index (CI) and the results of the endometrial biopsy in non-pregnant group of subjects

Subjects	CI (n/p/c)	Endometrial biopsy	Inference
CONTROL (5)	1/90/9	Secretory phase	Normal progesterone effect
ABORTERS (15)			
A (7)	3/79/18	Secretory phase	Varying
A_1 (3)	6/73/21	Secretory phase	Progesterone effect
A_2 (3)	0/93/7	Late secretory phase	Mild progesterone deficiency
A_3 (1)	0/60/40	Secretory phase	Moderate progesterone deficiency
B (7)	6/42/52	Proliferative phase	Varying
B_1 (3)	0/13/87	Proliferative phase	Anovulatory cycle
B_2 (2)	28/63/10	Proliferative phase	Mild progesterone deficiency
B_3 (2)	0/59/42	Proliferative phase	Mild progesterone deficiency
C (1)	0/70/30	Mixed activity	Varying

n/p/c indicates navicular, pre-cornified and cornified cells respectively under the CI (cornification index), and figures in parentheses refer to the number of subjects studied.

1. Control:- All the five cases had an average C.I. of 1/90/9 in the post-ovulatory vaginal smear, and the endometrial biopsy showed the normal secretory phase (Figs. 1 and 2).

2. *Aborters:*—Study of 15 cases yielded different results as follows:

(A) Seven subjects had an average C.I. of 3/79/18, indicating the secretory phase of endometrium (Figs. 1 and 2). Three of them (A_1) had normal progesterone effect with an average C.I. of 6/73/21, showing a shift to the right and the endometrial biopsy indicating the secretory phase of activity. The other 3 (A_2) had a mild progesterone deficiency effect with an average C.I. of 0/93/7 and the endometrial biopsy showed a late secretory phase. The last subject (A_3) had moderate progesterone deficiency with a C.I. of 0/60/40 and the endometrial biopsy revealed the secretory phase.

(B) The other seven cases had an average C.I. of 6/42/52 showing the proliferative phase of endometrium (Fig. 3). Three of them (B_1) had an average C.I. of 0/13/87 with anovulatory cycles showing the proliferative phase of endometrial biopsy. Two of them (B_2) had an average C.I. of 28/63/10 showing a shift to the left, but the endometrial biopsy remained of the proliferative type having mild progesterone deficiency. The remaining 2(B_3) had an average C.I. 0/59/42 showing mild progesterone deficiency in the endometrial biopsy with proliferative phase.

(C) One subject with a C.I. of 0/70/30, however, showed mixed activity, some glands showing secretory, others the proliferative phase

of the endometrial biopsy (Fig. 4).

II. Pregnant Group:- The results have been shown in Table II.

TABLE II

Showing the vaginal cytogram in pregnant group of subjects, indicating various degree of progesterone deficiency and also the effect of progesterone therapy on the continuation of pregnancy

Subject		Type of smear	Navicular cells	Pre-cornified cells	Cornified cells	Pregnancy continued beyond 24 weeks
Control (10)		Normal progesterone effect	0-30	56-92	0-15	100%
ABORTERS (15)						
	A (6)	Normal progesterone effect	0-30	67-96	0-15	100%
2 @	B (4)	Mild progesterone deficiency	0-36	46-95	5-28	75%
3 @	C (3)	Moderate progesterone deficiency	2-23	38-90	4-40	100%
1 @	D (2)	Severe progesterone deficiency	2-20	30-88	7-50	50%

@ indicates the number of subjects given progesterone treatment, and all these subjects continued pregnancy beyond 24 weeks.

Figures in parentheses indicate the number of subjects in each series. The values of each type of cells have been shown in range percentage.

(1) *Control:* All the 10 cases showed the normal progesterone effect (Fig. 5).

(2) *Aborters:* The following results were obtained in 15 subjects:-

(A) *Normal progesterone effect:-* In six cases pregnancy continued normally beyond 24 weeks without any treatment, the highest average value of cornified cells being 15%, showing normal progesterone effect (Fig. 5).

(B) *Mild progesterone deficiency:-* Three out of four cases continued pregnancy beyond 24 weeks, out of which two were treated with progesterone and one had no progesterone. One patient who aborted had no progesterone therapy. The highest value of cornified cells was 28%, showing a mild progesterone effect (Fig. 6).

(C) *Moderate progesterone deficiency:* In all three cases pregnancy continued normally beyond 24 weeks, and all of them were given hormonal therapy. The highest average value of cornified cells was 40%, showing moderate progesterone effect (Fig. 7).

(D) *Severe progesterone deficiency:* One of these two cases who was treated with progesterone could continue pregnancy beyond 24 weeks,

while the other who did not receive any treatment aborted. The highest average value of cornified cells was 50%, showing a severe progesterone deficiency (Fig. 8).

Discussion

These results (Table II, and Fig. 5-8) indicate that vaginal cytology is a valuable investigation in patients with a history of habitual abortion. When the smear is normal (Fig. 5), having a low cornification index, the pregnancy will almost certainly continue, but when the smear is abnormal showing high cornification index (Fig. 8), there is every likelihood of an abortion occurring. Consequently an abnormal smear may be used as an indicator for the prophylactic treatment of abortions.

The definition of abnormal smear is still controversial. Hochstaedt, Lange and Spira (1960) regarded the presence of 25% cornified cells as abnormal, while Kishore *et al* (1957) considered cornified cells up to 30% as normal. Paul (1955) had quoted values upto 35% as normal. However, Randall *et al* (1955) and Wood *et al* (1961) had graded the smears as poor, moderate or good on the basis of the amount of cell desquamation, the size of the cells, the pattern of the cells and the cornification index. A smear was classified as good if (1) there was heavy cell desquamation, (ii) the cells were small, (iii) the pattern of the cells throughout the smear showed clumps which included navicular and basal cells, and (iv) the cornification index was less than 10. As the largest number of cases had been studied in the series of Hochstaedt *et al* (1960) and their classification being more up-to-date, we had adopted it.

Malpas (1942) and Eastman (1956) suggested that more than two abortions had a definite bearing on subsequent prognosis. The majority of women aborted between 8-12 weeks of pregnancy as it is an established fact that during this period the function of the corpus luteum was taken over by the placenta. Hence, this transitional period was the most critical period for the fertilized ovum.

Hochstaedt *et al* (1960), as well as Bishop (1951) and Riley (1959), noted a shift to the right in the smears towards the end of the second or the first half of the third month of pregnancy, indicating a hormonal imbalance during this phase of change over from the corpus luteum to the placenta.

The vaginal cytology and endometrial biopsy act as mirrors reflecting ovarian activity. The evaluation of data by one method only, i.e. by studying vaginal smears or endometrial biopsy, is not conclusive. Thus, both these methods have been combined in our studies. These results have been correlated, thus enabling us to arrive at a definite conclusion and to detect the exact nature of the hormonal imbalance.

Conclusions

1. An abnormal smear is not necessarily always indicative of an unfavourable prognosis.
2. High cornification index is very suggestive of an impending abortion.
3. Endometrial biopsy and vaginal cytology, though having a tendency to synchronize in different phases of the cycle, yet occasionally show varia-

tions. Such variations, however, could not be observed in normal cases, thereby indicating a marked correlation between the histology of both the tissues under varying hormonal influence in different phases of the cycle.

4. Endometrial biopsy during the premenstrual phase in habitual aborters show a tendency towards the proliferative phase instead of the secretory phase which is invariably met with under normal circumstances.

5. Vaginal cytology and endometrial biopsy yield fairly accurate information pertaining to diagnosis, prognosis and line of treatment in cases of habitual abortion.

Summary

Fifteen normal non-aborters, and 30 habitual aborters, attending the Gynaecological department of the State Zenana Hospital, Jaipur, have been included in this study. Besides normal investigations, vaginal smears and endometrial biopsies were also studied to evaluate the hormonal imbalance.

Out of 15 non-aborters, 3 had anovulatory cycles, 5 showed normal progesterone effect, and the remaining 7 exhibited varying degrees of progesterone deficiency. Another group of 15 pregnant subjects, 6 had normal pregnancy smears, and 9 indicated a moderate type of progesterone deficiency. The intensity of progesterone deficiency was proportional to the degree of cornification. High cornification index was suggestive of abortion.

All the normal 15 subjects in pre- and post-conceptional states had normal smears and their endometrial biopsy showed the secretory phase, indicative of the progesterone effect.

An abnormal smear in itself was not of much significance but if associated with high cornification index was suggestive of impending abortion. In aborters, unlike normals, an incoordination between the vaginal cytology and endometrial biopsy existed and a proliferative phase instead of the normal secretory phase was found. Thus, vaginal cytology and endometrial biopsy together furnish a fairly correct assessment of the diagnosis and the line of treatment in cases of habitual aborters.

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See Figs. on Art Paper II