

THE VAGINIAL SMEAR AS AID TO CLINICIAN IN STERILITY AND MENSTRUAL DISORDERS

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Introduction and Aim

The aim of this study is to explore the potentiality of the vaginal smear from the clinician's point of view to help him in dealing with his patients with special reference to sterility and menstrual disorders.

This study has been prompted by the following observation made at the Gynaecological Department of Civil Hospital, Ahmedabad.

1. There is no way of assessing hormonal activity in patients, as blood hormonal and urine hormonal estimations are not done.

2. The usual report of the pathologist on endometrial biopsies does not differentiate the various degrees of oestrogenic activity but merely gives a report that the endometrium is in the proliferative phase. Thus in long-standing cases of amenorrhoea and regularly menstruating cycles the report is the same though the degree of oestrogenic activity varies considerably.

3. Cases of latent pelvic infection which have been missed may occasionally flare up after an endometrial biopsy.

4. Patients often refuse the use of repeated endometrial biopsies.

5. It is common experience in the

O.P.D. to have patients of sterility say that they have taken hundreds of oestroid tablets over a number of years. This sort of indiscriminate use of hormones is to be condemned. This study aims at finding out the particular patients requiring hormonal therapy, because the patients too have a right to the best of treatment, treatment that is rational and controlled.

Because of the general idea of the clinician that the vaginal smear is a time-consuming and specialised procedure, and because of the impression of the pathologist that the vaginal smear is less reliable than the biopsy, the study of the vaginal smear and its utilisation has suffered considerably.

If the vaginal smear, like any other clinical investigation, is to be of more widespread use, it must fulfil three conditions:—

1. Simple technique.
2. High degree of reliability.
3. Ease of interpretation.

These conditions have been adhered to, as far as possible, in the study.

Lastly, it is hoped that the clinician himself will take the smears, study them, and utilise the information obtained in the best interests of his patients.

Review of Literature

Cytological studies were first introduced by Pouchet in 1847, who attempted, with unstained smears, to analyse the normal sex cycle. In 1917, Stockhard and Papanicolau described the technique of obtaining vaginal smears from rodents to study the activity of the ovaries. In 1923, Allen and Doisy applied the procedure as a quantitative method of assaying follicular hormone, oestrogen. In 1933, Papanicolau introduced the smear technique to women. He described cyclic changes in the vaginal smear corresponding to the follicle changes in the ovaries. Papanicolau and Shorr showed the striking changes in the vaginal smear after menopause and castration which are different from those obtained in smears from women with normal functioning ovaries. They also proved that, by administering oestrogens, smears from menopausal women could be changed to resemble those with normally functioning ovaries.

Geist and Salmon classified vaginal smears into four groups to indicate various degrees of oestrogen deficiency.

*Reaction 1 (Advanced oestrogen deficiency):**Characteristics:*

1. Complete absence of squamous epithelial cells.
2. Presence of 'atrophy cells'.
3. Leucocytes and erythrocytes are present.

Reaction 2 (Moderate degree of oestrogen deficiency):

Characteristics: Variable number

of large epithelial cells. Nuclei relatively large. Interspersed among these varying number of atrophy cells are present.

Reaction 3 (Slight degree of oestrogen deficiency):

Characteristics: Predominance of rather large, irregular epithelial cells. Cells are irregular and the outlines blurred and indistinct. Few atrophy cells may be present.

Reaction 4: Smear consists of large, flat, clearly outlined squamous cells with small deeply stained nuclei. Cells large and clear cut and nuclei smaller than in reaction 2. No 'atrophy' cells or leucocytes are usually seen.

De Allende, Ines and Oscar Orias believe that taking daily vaginal smears permits one to know if ovulation is occurring or not. They put down, from the point of view of smears, the significant events as:—

(1) Menstrual phase characterised by presence of erythrocytes.

(2) Post-menstrual phase characterised by cells chiefly of intermediate type with some cornified cells up to 30% which are more or less separate and generally have non-folded cytoplasm.

(3) The inter-menstrual peak of cornification with clean leucopenic smear and a high proportion of cornified cells (45 to 90%).

(4) Luteal phase with dense clumping of cells, moderate to abundant leucocytosis, cells with numerous folds and wrinkles.

They made, further, counts of different types of cells and plotted curves:—

(a) *Eutropic Curve.* Normal curve

of ovulatory cycles. Percentage of cornified cells beginning relatively low, rises more or less regularly to a maximum, from 45% to 90%; cornified cells being present towards the middle of the cycle, thereafter falling suddenly and remaining low till the next cycle.

(b) *Cyclic Hypotrophic Curve*, Same as above but percentage of cornification especially towards the middle remains below those of latter.

(c) *Non-cyclic Hypotrophic*. Here the level of cornification, below 10%, is constant without oscillation throughout the cycle.

(d) *Curve of Persistently High Cornification*. Percentage shows above 40% of cornified cells.

Lastly there is the 'atrophic' curve and depending upon the deep cells there is mild atrophy 1% to 15% deep cells present in the smear. Moderate atrophy when 15% to 40% deep cells are present and advanced atrophy when deep cells are present between 40% to 100%. They advocate daily smears if day of ovulation is to be detected.

Wahi and Mehta working in this country studied 30 selected sterile women with vaginal smears. They observed that anovulatory cycles differ from ovulatory cycles, in the absence of desquamation and decline in cornification after the oestrogenic peak. They took vaginal smears on alternate days, as far as possible, in a cycle, and endometrial biopsy on 12th, 19th and 26th days. The most important change noted was desquamation accompanied by a decline in the number of cornified cells. This picture, following a peak of oestrogenic activity when fully cornified cells

predominate, may be regarded as diagnostic of ovulation.

Material and Method

In all 103 patients were studied with 290 vaginal smears. All these patients were those attending the O.P.D. of the Civil Hospital, Ahmedabad. Of the 103 patients, 80 belonged to the sterility group and 23 to that of amenorrhoea with sterility, the amenorrhoeas ranging from 4 months to 8 years. The sterility patients belonged to either primary or secondary sterility. Except for four patients who did not give definite history of more or less regular menstruation, the rest were, within small limits (18-35 days), menstruating regularly. The ages ranged from 18 to 38 years. Local inflammatory conditions in the sterility group like cervicitis, pelvic inflammatory masses, etc. were excluded. Trichomonas infection was particularly attended to. Cases of absolute sterility, like absent uterus etc., were also excluded. All smears in these series were taken with a glass pipette from the posterior fornix, fixed immediately and stained with Shorr stain. The method adopted is as described in 'Cytology of the Human Vagina' by Oscar Orias and De Allende.

Though initially smears were taken on alternate days, this was soon abandoned as it was found impracticable with the type of patient attending the O.P.D. of this Hospital and secondly, it did not conform with the condition laid down if it were to be adopted by the clinician.

Each menstrual cycle was thus studied by a smear during each of its phases, viz. proliferative; ovulatory

and secretory phase. Therefore a patient was told to report roughly between 8 to 12, 13 to 18, and 20 to 25 days of her cycle. This meant taking a minimum of three smears for each case of sterility. After the third smear was taken a four quadrant endometrial biopsy, so as to avoid misleading interpretations, was routinely taken. If the endometrial biopsy showed a secretory change, no further endometrial biopsies were taken. When it showed a proliferative change, the endometrial biopsy was repeated in as many patients as possible to make sure that the woman was having anovulatory cycles.

In anovulatory cycles there is no ovulatory or secretory phases but in this study, for convenience, these cycles were divided into three parts corresponding to the proliferative, ovulatory and secretory phases of normal ovulatory cycle.

Further the clinical 'triad' of obesity, scanty periods, and small uterus was applied to the patients as grouped by the corrected vaginal smear to be described later. In the amenorrhoea group, smears were taken when the patients reported and in those who followed the treatment

The criteria are as follows:—

Proliferative Smear

1. 'Clean' type of smear.
2. Cells more or less discrete.
3. Outline well demarcated.
4. Wrinkling and curling of cells not marked.
5. Acidophilic cells prominent.
6. Leucocytes scanty or absent.
7. Presence of granules in cytoplasm.

smears were repeated after treatment, be it medical or surgical.

In this group endometrial biopsy was taken only in 9 patients.

Care was taken to see that the smears were not taken too close to menstruation, on either side.

Important Criteria and Data Utilised in Study of Vaginal Smear With Respect to Sterility

The normal menstrual cycle has been divided roughly into two phases, the proliferative phase and the secretory phase.

It has to be kept in mind that a smear may show predominating characteristics of the proliferative phase, secretory phase or may show combinations of both in which case no opinion can be given.

The degree of cornification is an index of the degree of oestrogenic activity. It must be remembered that even in the same individual oestrogenic activity is so fluctuating that smears may show different degrees of cornification, taken at short intervals, in the same individual.

Cells of the O.B.T. (outer basal cells, deep layer) are not normally found in the vaginal smear. When-

Secretory Smear

1. 'Dirty' type of smear.
2. Cells in clumps.
3. Outline hazy.
4. Wrinkling and curling of cells marked.
5. Basophilic cells prominent.
6. Leucocytes prominent.
7. Presence of granules not marked.

ever oestrogenic activity is at a low ebb, as after menopause or before puberty the O.B.C.'s (outer basal cells) appear in the smear. The occasional appearance of an outer basal cell in a smear should not be given any importance.

Leucocytes appear typically in the secretory phase. They are scanty or absent in the typical proliferative smear, provided infection is ruled out.

Study

Ovulatory Cycles:

26 patients showed according to the endometrial biopsy normal ovulatory cycles, as seen by the secretory changes in the endometrium.

A. 22 patients showed in the vaginal smears the characteristics of the secretory smear in the smear taken between the 20th-30th days of the cycle. Photomicrographs 1a and 1b best show these changes.

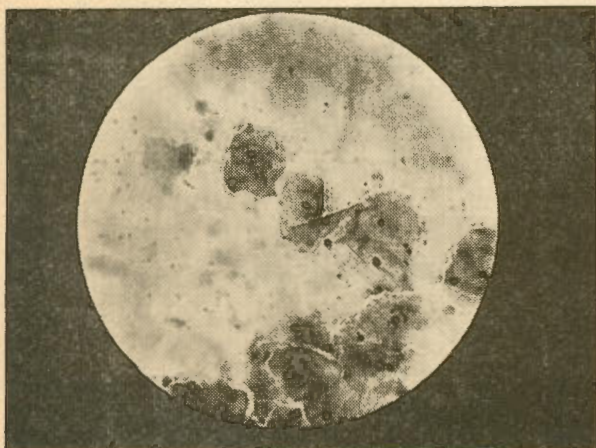


Fig. 1-A

A picture of a smear taken on the 9th day of the menstrual cycle. Note the discrete cells with pyknotic nuclei and a few leucocytes.

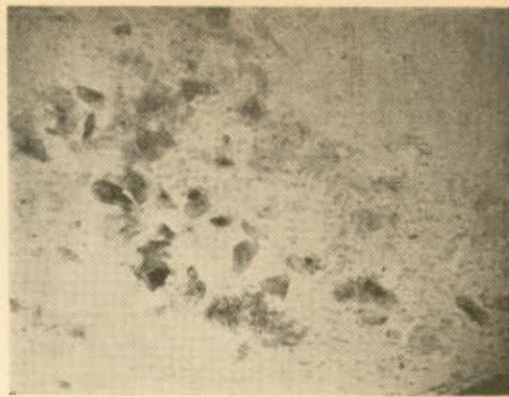


Fig. 1-B

The smear on the 24th day of the same menstrual cycle as 1-A. Note the grouping and clumping of cells and the numerous leucocytes. The endometrial biopsy showed the cycle to be ovulatory.

Photomicrographs 1a and 1b show smears taken on 9th and 24th day of the same menstrual cycle respectively. The smear on 24th day shows numerous leucocytes, clumping and curling of cells and a hazy outline. Contrast this with smear taken on 9th day. Here the smear is 'clean', leucocytes are few and cell outlines clear.

The smears taken in the secretory phase strongly contrasted with those taken in the proliferative and ovulatory phases. The presence of leucocytes in the secretory phase was the most striking change observed. As is sometimes said, the leucocytes appeared in a 'shower'. The presence of these leucocytes in the secretory smear is best shown in photomicrograph 1b. Comparison of this picture with that of the smear taken on 9th day of the same cycle, photomicrograph 1a, shows what a dramatic change has occurred. Grouping and

wrinkling of cells were also noted markedly in the secretory smears as seen in photomicrograph 1b. Throughout the cycle cornified cells were present in varying numbers, especially predominating in the smear taken during the proliferative and ovulatory phases of the cycle.

B. In two cycles the smears were dirty and unsatisfactory throughout and no opinion could be given.

Ovulatory Cycles Showing 'Proliferative' Characteristics in Smears

C. In two cycles the smear remained 'clean' throughout, no leucocytes or curling and clumping of cells was noted in the smears taken during the secretory phase of the cycle.

The endometrial biopsy, however, showed the secretory changes in the endometrium and these two cycles must be typed as 'false' reports according to the vaginal smear. Four of the patients in this group of normal ovulating women have since become pregnant.

The clinical 'triad' as applied to these patients is shown below in tabular form:—

No. of patients	Obesity	Scanty periods	Uterus smaller than normal
26	1	8	3

Anovulatory Cycles

52 patients according to the endometrial biopsy showed anovulatory cycles.

a. *Anovulatory Cycles Showing Outer Basal Cells in Smears*

In 14 cycles, cells from the deep layers, the outer basal cells were seen

in the smears either during a part or throughout the cycle. They varied in numbers from 1-5 per low power field to as many as 15-20. They appeared in two forms:

(I) They may be scattered diffusely throughout the smear, in which case they had to be carefully looked for.

(II) They may be seen in groups, in which case they are easily detected. Photomicrograph 2 shows such a group of four outer basal cells which are rather darkly stained and appear roundish or oval with prominent nuclei.



Fig. 2

A smear in which the outer basal cells are seen in a group of four in the centre, which are stained rather darkly. These cells are round or oval in shape with comparatively large nuclei. In this smear there were other groups of outer basal cells also.

These cells were also seen to take either the basophilic stain when the cytoplasm was seen as green or greenish-blue or the acidophilic stain when it stained, reddish or reddish-orange. One cycle showed the presence of a fair number of outer basal cells in the smear at the 9th day, disappearance of the outer basal cells at the 15th

day, the pictures following the oestrogenic activity quite faithfully.

In these 14 patients, leucocytes appeared frequently in the smears and were at times very prominent. In the 'diffuse' outer basal cell type of smear the presence of leucocytes, often gave, at first glance, an appearance of the secretory smears and only careful examination revealed the outer basal cells concealed among the other epithelial elements of the smear.

Intermediate cells, superficial squamous cells and bacteria complete the picture. In not one of these patients did the endometrial biopsy showed secretory changes.

The clinical 'triad' applied to these patients is seen in tabular form below:—

No. of patients	Obesity	Scanty periods	Uterus smaller than normal
14	2	12	4

b. *Anovulatory Cycles Showing Absence of Cornified Cells*

Seven cycles showed practically complete absence of cornified cells from smears taken throughout their cycles. Smears were made up of intermediate cells and occasional deep cells.

c. *Anovulatory Cycles Showing 'Proliferative' Characteristics*

There were 17 patients in this group. Clumping, curling of cells, presence of leucocytes and other characteristics of the secretory smear were not seen. (Mechanical error, as

where the smear was thick, was not to be regarded as clumping).

In one cycle, leucocytes were seen in fair number throughout the cycle but the cells were otherwise discrete and well outlined.

d. *Anovulatory Cycles Showing Increased Oestrogenic Activity*

Three patients fell in this group. The predominating cell was the cornified cell. The smears were 'clean' with well defined cell margins and pyknotic nuclei. Leucocytes were absent or scanty. The smears throughout the cycles showed an increased oestrogenic activity.

e. *Anovulatory Cycles Showing Dirty and Unsatisfactory Smears*

Six patients belonged to this group. The smears, at times were extremely dirty showing profusion of bacterial flora, leucocytes, cytolysis, distorted cells with ragged margins, so that it was not possible to give opinion.

f. *Anovulatory Cycles Showing 'Secretory' Criteria*

Five cycles showed a change from the proliferative characteristics to the secretory characteristics and were thus typed as ovulatory cycles, while in reality they were anovulatory cycles as shown by the endometrial biopsy. These were thus 'false' ovulatory cycles. The endometrial biopsy was repeated in three of these patients and two of them showed secretory changes in the following cycles.

III. *Koch's Endometritis in Two Patients*

One patient showed fairly good oestrogenic activity as seen by plenty

of cornified cells in the smears. Leucocytes were plentiful. The second patient showed absence of cornified cells in smears taken throughout the cycle. Leucocytes were present. Clinically, the first patient is menstruating regularly but getting scanty periods. The second patient recently had a period of amenorrhoea lasting 2 months followed by a scanty period lasting a few hours.

Excluding the Koch's group and the group showing outer basal cells in the smears, the clinical 'triad' as applied to these patients is seen in tabular form:—

No. of patients	Obesity	Scanty periods	Uterus smaller than normal
38	nil	20	11

Amenorrhoea

In 23 patients belonging to this group, 12 patients showed the presence of intermediate and outer basal cells. In 3 of them as many as 15-20 outer basal cells per low power field were seen.

One patient showed persistently a smear with over 80% cornified cells. Patient was operated on 11th January, 1956, for an ovarian cyst. A month after the operation of ovariectomy the smear showed a sharp fall in the number of cornified cells which fell to 10%. After the last study the patient has not turned up.

The remaining 10 patients showed nothing abnormal in the smears which consisted of varying numbers of cornified and intermediate cells.

Fourteen patients were given two injections, on alternative days, of inj.

oestroprogyn (inj. oestroprogyn contains 2.5 mgm., oestradiol benzoate and 25 mgm. progesterone). Ten patients (which included 6 patients showing intermediate and outer basal cells and 4 showing varying proportions of intermediate and cornified cells) got withdrawal bleeding. The 4 patients who did not respond were (a) a patient showing hyperoestrinism with secondary amenorrhoea of 6 years; (b) two patients showing fairly good oestrogenic activity with secondary amenorrhoea of 2 years and 5 years respectively; and (c) a patient showing plenty of outer basal cells in the smear with amenorrhoea of 5 years.

The endometrial biopsies of both patients showing fairly good oestrogenic activity with secondary amenorrhoea of 2 years and 5 years respectively, and not responding to oestroprogyn, showed Koch's endometritis.

Discussion and Comments

From the study it will be seen that in 15 out of 80 patients of sterility, it was not possible to type the cycles. This includes 8 patients who showed 'dirty' and unsatisfactory smears and 7 whose smears showed the opposite criteria to which they actually belonged. This gives an error of 18.7% for typing of cycles by this method. It is quite possible that daily taking of smears may reduce this error and that local infection, not detected, might have added to the error. It is also quite possible that the apparent error in some of the cases is due to the variability of response of the vagina and endometrium to oestrogens, in any method which utilises

vaginal smears to interpret endometrial changes, this possibility has to be kept in mind, however, as will be seen from this work, in 8.7% of the cases (i.e. in those patients in whom the smears showed the opposite criteria to which they belonged).

It is further seen that 21 patients showed poor or low oestrogenic activity. This group includes patients showing absence of cornified cells or the presence of outer basal cells in the smears. In none of these patients did the endometrial biopsy show a secretory phase. It is in this group of patients that the vaginal smear is invaluable to the clinician. 26.2% of the patients of sterility in this study therefore need not have had an endometrial biopsy to diagnose absence of ovulation.

Proof that the presence of outer basal cells is an indication of poor oestrogenic activity is easily seen in smears taken before puberty or after menopause. Occasional outer basal cells often seen in smears must not be confused with this group.

The present study has shown, in some cases, the disappearance of the outer basal cells at the 15th day, the time when oestrogenic activity is known to be high and their reappearance at about the 25th day of the cycle. A single smear in such a cycle, taken at about the 15th day, will not show outer basal cells and it will not be possible to type the cycle or to know that the patient belongs to the 'outer basal cells present' group. The pathologists report on the endometrium gives the phase in which the endometrium is and throws no light on the degree of oestrogenic activity or ovarian failure. It is essential for

the clinician, if the patient is to be adequately treated, to know the level of oestrogenic activity which indirectly gives the clinician information as regards ovarian activity. Thus, a patient in the anovulatory group showing good oestrogenic activity and another showing anovulatory cycles with outer basal cells present must be treated differently, the latter patient requires oestrogens urgently and quickly and the former does not though both patients are having anovulatory cycles.

Patients having anovulatory cycles may be conveniently classified as below:—

- (i) Anovulatory cycles showing good oestrogenic activity as seen by good number of cornified cells in the smears.
- (ii) Anovulatory cycles showing poor oestrogenic activity as seen by a few cornified cells in the smears or absence of cornified cells in the smears.
- (iii) Anovulatory cycles showing ovarian failure as seen by the number of outer basal cells in the smears.
- (iv) Unclassified:—

This would include smears which are unsatisfactory and cannot give useful information.

Endometrial biopsy would eliminate the 'false' group.

Once the clinician has classified his patients as shown above, the patients can be treated more easily and rationally.

Patients undergoing gradual ovarian failure may perhaps pass through the following stages:—

Normal ovulatory (secretory)
 Non-ovulating (proliferative)
 'Outer basal cells present' stage
 Severe ovarian failure with or
 without amenorrhoea.

It will be seen from the above that the 'outer basal cells present' stage is a very important phase in the path of ovarian failure. The vaginal smear is an excellent method to detect this 'phase' in a patient as is seen by the presence of outer basal cells in the smears.

Once the vaginal smear shows the presence of outer basal cells there should be no delay in treating the patients promptly. Waiting for the endometrial biopsy report is not required as these patients will all show absence of secretory activity. These patients will all show absence of secretory activity. These patients should be treated promptly because they have already delayed enough and have turned up for treatment late. 17.5% of the patients of sterility studied in this group showed outer basal cells in the smears. If these patients were left untreated, they may have gone into more severe ovarian failure.

In cycles that do not show the outer basal cells and are anovulatory, delay is permissible and an endometrial biopsy may be repeated in the following cycle to make sure that the patient is not irregularly ovulating. Treatment, therefore, may be delayed in this group of patients.

The clinical picture can be most deceptive and treatment should not be based solely on it.

The maximum number of patients complaining of scanty periods was in the 'outer basal cells present' group

but 30.5% of the normal ovulating women also complained of scanty periods.

Uterus smaller than normal was seen most frequently in patients with anovulatory cycles. In anovulatory cycles, excluding the 'outer basal cells present' group, it was noticed in 28.9%. In anovulatory cycles with presence of outer basal cells it occurred in 28.9% and in the ovulating cycles 11.5%. Obesity was not found to be common. Two of the patients in the 'outer basal cells present' group were obese and one in the normal ovulating group.

The clinical picture should be an additional aid to hormonal therapy as even a clinically 'normal' patient may show outer basal cells in the smear. A patient complaining of scanty periods and with the uterus smaller than normal should therefore be carefully studied by smears.

Amenorrhoea

This group can be conveniently divided into:—

- (a) Smears showing hyperestrinism.
- (b) Smears showing presence of cornified and intermediate cells.
- (c) Smears showing outer basal cells or absence of cornified cells.

This will make treatment of patients easy. Such a division of patients is again not possible by endometrial biopsy, and in this group also the vaginal smear is invaluable to the clinician.

Hecht has pointed out that hyperestrinism is often accompanied by pelvic pathological lesions. In the one patient who showed hyperestrinism,

an ovarian cyst was present.

Two patients who showed good oestrogenic activity in the smears and did not respond to oestroprogyn were found to be cases of Koch's endometritis. The clinician should keep this possibility carefully in mind and whenever patients of amenorrhoea show good oestrogenic activity in the smears, the suspicion that the uterus may be at fault should be aroused.

Summary and Conclusions

1. A study of 103 patients (80 of sterility and 23 of amenorrhoea and sterility) by the vaginal smear is presented.

2. 17.5% of patients of sterility studied showed moderate ovarian failure.

3. Typing of cycles by this method is not satisfactory as it gave an error of 18.7%. Daily smears might have given better results.

4. This method of studying patients by the vaginal smear is an excellent method of screening sterility patients and picking out patients with moderate ovarian failure, who need more intensive and immediate treatment than others.

5. Cycles showing outer basal cells in the smears may be typed as anovulatory cycles. There was no error in this group.

6. Endometrial biopsy, though it gives the phase in which the endometrium is, fails to throw light on the degree of ovarian failure. Its limitations have been discussed.

7. The incidence of taking endometrial biopsy could have been reduced in these series by 26.2% (i.e. patients showing outer basal cells, absence of cornified cells or an occasional cornified cell in the smears do

not need an endometrial biopsy).

8. Endometrial biopsy must be reduced to a minimum because it is a surgical procedure and overlooked or quiescent pelvic inflammations may be lit up.

9. Hormones should not be administered on the strength of the clinical picture.

10. The 'triad' of scanty periods, uterus smaller than normal and obesity are indications for careful vaginal smears study.

11. In amenorrhoea the vaginal smear is invaluable, and patients should be carefully studied and classified before treatment is begun.

12. In the best interests of sterility patients, therefore, both the endometrial biopsy and the vaginal smear should be studied.

Acknowledgement

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