

THE
Journal of Obstetrics & Gynaecology
of India

VOL. IV. NO. 3.

MARCH, 1954

THE VALUE OF ENDOMETRIAL BIOPSY IN STERILITY
IN THE FEMALE

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The modern study of the endometrium can rightly be said to date from Recamier's introduction of the curette. The changes which occur in the endometrium during a menstrual cycle are most fascinating. There is no other tissue in the body which undergoes such a rapid succession of changes in so short a period as 28 days. The changes in the pre-ovulatory phase are not so marked, but during the post-ovulatory phase the sequence of changes from day to day in the histological appearances are so definite that a histologist can almost predict the date of the following menstruation. Rock and Bortelett correlated the dating of 200 endometrial biopsies in the

secretory phase with the day on which menstruation actually occurred. Allowing a "plus or minus" 1 day error in interpreting the biopsy, 48% were found to menstruate at the time predicted. The usefulness of dating the endometrium lies in the fact that it gives a rough idea of quantitative progesterone effect and also indirectly of the time of ovulation. This progesterone effect depends on the duration of action as well as the amount of steroids acting and possibly on the sensitivity of the endometrial end-organ. The examination of endometrium during the secretory phase gives more information about the time of ovulation, degree of progestational changes,

normality or abnormality of endometrium than any other single test done in cases of sterility.

Not all endometrial variations are useful for dating. For example, the tortuosity of endometrial glands and coiling of spiral arterioles increase so gradually that quantitative differences from day to day cannot be detected. There are few components that change rapidly, constantly and characteristically enough under hormonal influence to indicate how long such action has been effective. The criteria most useful for dating of endometrium are:—

1. *Gland Mitosis:*

Gland mitoses indicate proliferation. They occur during menstruation because repair and breakdown are progressing simultaneously.

2. *Pseudostratification of Nuclei:*

This is characteristic of the proliferative phase but persists until active secretion begins. It is not resumed until the glands have involuted during menstruation.

3. *Basal Vacuolation:*

This is the earliest morphological evidence of ovulation found in the endometrium. It begins approximately 36 to 40 hours following ovulation.

4. *Secretion:*

Secretion appears in the lumen of the glands. In the later stages secretion becomes inspissated.

5. *Stromal Oedema:*

This factor varies with the in-

dividual, particularly oedema during proliferative phase, which may be almost absent. The oedema which accompanies secretion is more constant.

6. *Pseudo-decidual Reaction:*

It is evident first around the arterioles and progresses until a superficial compact layer is formed just before menstruation.

7. *Stromal Mitosis:*

These are most abundant during the proliferative phase, absent during active secretion and reappear during the stage of predecidual formation.

8. *Leucocytic Infiltration:*

A few scattered lymphocytes may be found in proliferative and early secretory phase, but the differentiation of predecidual is accompanied by a sharp increase in lymphocytic infiltration. Polymorpho-nuclear leucocytes appear about 26th day of the cycle.

The development of cellular pathology has shed great light on the organic diseases affecting the endometrium. The rapid advances of last three decades and a more enlightened understanding of various clinical phenomenon have greatly broadened the scope of our knowledge. In general, it may be said that the endometrium reflects the activity of the ovaries and the functional menstrual disorders can result from dysfunction of the ovaries. This may be due primarily to ovarian disease or secondarily to extra-ovarian causes such as diseases of the pituitary, thyroid or other endo-

crine glands and various types of constitutional diseases. Emotional factors communicated through the complex avenues of the sympathetic—parasympathetic system are often etiological factors in gynaecological diseases, particularly in functional disorders. The changes caused by such factors are evaluated by changes in the endometrial histology.

To study the endometrial histology it is desirable to obtain the tissue from the same patient at frequent intervals. This has led to the development of the suction endometrial biopsy curette. With this instrument bits of endometrium may be obtained frequently with little discomfort to the patient. Neither anaesthesia nor dilatation of cervix is required. The instrument therefore is indispensable in the armamentarium of a sterility clinic.

An abundant literature is available on the use of endometrial biopsy curette. The technique is significantly free of complications. However, it is not without danger, as perforation of the uterus and pelvic inflammation can result if it is used indiscriminately. Due attention should be paid to asepsis and the technique of operation. The danger of perforation can be minimised by preliminary determination of the length and direction of the uterine cavity with a small sound. Undue force must be avoided in passing the instrument. Over 2,000 endometrial biopsies have been performed at the Sterility Clinic during the past 18 months without complication. The endometrial biopsy is done as an outdoor procedure. The operation is done in the operation theatre with

proper aseptic precautions. With this technique adequate tissue is obtained and the diagnosis of ovarian function, malignancy or presence of infection of the endometrium is easily made.

There have been objections to the use of endometrial biopsy as compared to curetting, and these are based chiefly on Bartelemesz's observations. He states that the endometrial tissue shows variations throughout the uterus. Meigs has stated "we have rarely found that two pieces of tissue vary microscopically and morphologically and do not believe it necessary to strip the endometrium with the idea that many different types of endometrium may be found". In general, the information obtained by endometrial biopsy, curettings or specimen of uterus obtained by surgical removal is more or less the same. The endometrial biopsy is therefore a valuable technique in the study of the diseases of the endometrium.

The highest level of ovarian activity is reached a few days before commencement of menstruation and this is reflected in the endometrium. To minimise the possibility of taking biopsy from an early unsuspected gravid uterus, the occurrence of bleeding should be awaited and biopsy taken as soon after the onset of menstruation as possible. For all practical purposes the endometrial reaction at that time will indicate whether ovulation during that cycle had occurred or not. The endometrial architecture however does not afford any clue to the duration of subsequent menstrual flow. This is dependent on a number of complex and

yet unanalysed factors affecting the vascular system of the endometrium.

The endometrium is an indicator not of hormone production but of effective endocrine activity. The ovarian hormones may be normal but if the endometrium is diseased its response to the hormones may be slight or absent. There are three relative levels which clearly distinguish the classical phases of endometrial cycles.

- (1) *First degree*: There is a menstrual abnormality associated with an endometrium that is secretory in type but less fully developed than a normal menstruating type of endometrium. This suggests that although ovulation has occurred, a deficiency of progesterone exists. Such an endometrium is unfavourable for nidation of the fertilised ovum.
- (2) *Second degree*: A proliferative or hypertrophic endometrium is present, suggesting an absence of progesterone or failure of ovulation.
- (3) *Third degree*: An atrophic or senile type of endometrium suggesting not only absence of ovulation but diminution of oestrogen stimulation also.

The endometrial specimen removed at the onset of bleeding, therefore, indicates the relative severity of ovarian dysfunction. It does not however reveal whether deficiency is due to intrinsic ovarian deficiency endocrine disturbance, or constitutional disease affecting ovarian function.

In comparing endometrial biopsy with other methods of study of

ovarian function, such as hormone assays, it is certain that the information available by histological study of endometrial tissue is far greater.

In the study of sterility in the female, the endometrial biopsy technique is used extensively for determination of:—

1. *Evidence of Ovulation*:

The biopsy technique provides practical and reliable data on evidence of ovulation during the cycle studied. It may be necessary to study several cycles before concluding that the patient does not ovulate. Many women may ovulate only once or twice a year but have anovulatory menstruation oftener. The well known dictum of Robert Mayer "Without ovulation no corpus luteum, without corpus luteum no menstruation", no longer holds true. Apparently normal and healthy women may bleed cyclically from oestrogenic endometrium. The obvious inference would be that ovulation has not occurred, otherwise progesterone would have been found by biopsy.

The diagnosis permitted by biopsy data is only inferential. All the three criteria of Bartelemez (1937) in the definite diagnosis of anovulatory bleeding cannot always be fulfilled, viz:—

- a) Both ovaries should have been studied and definite proof established that no recent or active corpora lutea are present.
- b) The endometrium should have been available for study on the

first or second day of bleeding, i.e. before regression has occurred.

- c) There must have existed a reliable menstrual history which established the fact that bleeding was experienced at the expected time.

Because it is not practical to study the ovaries microscopically the other two criteria must be depended upon. When these are fulfilled the data obtained by study of endometrial biopsy are conclusive. It is true that women who bleed from oestrogenic endometrium are sterile. When they bleed from progestational endometrium, if no other significant fertility reducing factors exist, conception may occur.

The practice at this clinic is to do the endometrial biopsy in the premenstrual week usually 3 to 4 days before the commencement of menstruation. This is a more convenient technique than waiting until the patient starts menstruation, for this may delay her arrival at the hospital. The picture of endometrial histology of a biopsy taken premenstrually is clearer and has less debris than that taken during menstruation. However, the practice of obtaining endometrial tissue in advance of expected menstruation has several objections:-

- 1) It is not applicable to the investigations of irregular cycles.
- 2) The timing of onset of menstruation may be obscured by traumatic bleeding incidental to biopsy.
- 3) If the menstruation does not occur within a day or so following the biopsy, considerably

more differentiation of endometrium may occur before the ovarian cycle ends and therefore the specimen obtained does not permit conclusive interpretation of ovarian function.

- 4) An early pregnancy may be disturbed.

Histological study of the endometrium was made in 1,411 sterile women attending the clinic. 106 were diagnosed as suffering from endometrial tuberculosis on histological evidence. The evidence of ovulation was seen in 62% of the whole group of sterile patients investigated as compared to 8.5% in the group suffering from endometrial tuberculosis.

II. *Study of level of ovarian function:*

A simple system of grading ovarian function from endometrial biopsy data is as follows:—

- 1) Hypo-oestrogenic endometrium.
- 2) Normal oestrogenic endometrium.
- 3) Hyper-oestrogenic endometrium.
- 4) Mature progestational endometrium.

III. *Histological evidence of endometrial tuberculosis:*

It may be pointed out that endometrial tuberculosis is more prevalent than is generally assumed. Considering the high incidence of tuberculosis in general in this country it is not surprising that the incidence of endometrial tuberculosis is found to be high. Endometrial tuberculosis exhibits an unusual feature of being

present in the latent form in a majority of cases. This has been found to be the case in the women studied at the sterility clinic. The diagnosis is made during the routine study of endometrial biopsy primarily done with the object of detecting evidence of ovulation. The incidence of endometrial tuberculosis as reported by various workers is 2 to 15 times higher in sterile women than in fertile women. In a large number of symptom free women, the diagnosis of endometrial tuberculosis is made. The detection of this disease is more important in some cases than the diagnosis of occurrence of ovulation. The incidence of endometrial tuberculosis as reported by various workers ranges, from 1 in 600 endometrial biopsies by Grant and Mackey in Australia to 5.5% by Halbrecht in Tel Aviv.

1,411 cases of sterility studied at the Sterility Clinic showed an incidence of 7.5%. The incidence of endometrial tuberculosis in cases of primary sterility was 6%, while in cases of secondary sterility the incidence was 13%. This is contrary to the figures of other workers who have found higher incidence in the primary sterility group.

The recognition of this disease at a time when new methods of treatment are available has resulted in considerable attention being focussed on the disease. Just as mass radiography of the chest is an aid in detecting silent and unsuspected pulmonary lesions, so the increasing use of the endometrial biopsy curette in

gynaecology is accumulating a mass of data on one aspect of pelvic tuberculosis, namely endometrial tuberculosis. The presence of tubercles in the endometrium clinches the diagnosis of tuberculous lesion higher up in the genital tract whereas absence of tubercles in the endometrium does not always exclude such a lesion.

Further use of endometrial biopsy material is made by staining the tissue for tubercle bacilli with Ziehl-Neilson stain. Part of the endometrial tissue is cultured for tubercle bacilli on Lowenstein medium. Growth of tubercle bacilli is obtained in a number of cases, particularly those cases which show caseation on histological examination.

IV. *Evaluation of results of therapy:*

A. *Hormone Therapy:* Study of endometrial histology is a valuable aid in assessing the results of hormone therapy in sterile women. The endometrial biopsy is done before the commencement and at the cessation of therapy to note whether the hormone therapy has been successful or not.

B: *Therapy with antituberculous drugs:* The advent of streptomycin and PAS has stimulated their use in endometrial tuberculosis. The recent discovery of a powerful antituberculous drug, namely, iso-nicotinic acid hydrazide and its derivatives, has created interest amongst workers in the tuberculosis sanatoria. This drug seems to exert a powerful effect on tuberculous toxæmia with rapid deferves-

cence of fever and gain in weight.

The results of therapy in tuberculosis elsewhere in the body are based on systemic response and radiological change, for it is not possible to examine the diseased tissue histologically. Since the endometrial tissue can be removed easily with little discomfort to the patient the progress of healing can be watched by repeated study of endometrial biopsies.

A series of 30 cases of endometrial tuberculosis has been treated with iso-nicotinic acid hydrazide. As the disease in most cases is in the latent form, the improvement cannot be estimated by changes in general symptoms. Endometrial biopsy was repeated every week. The changing histology during therapy was a guide to the healing process. After cessation of therapy, biopsy was done every month, and the recurrence or quiescence of the disease was noted. In some cases regeneration of healed endometrium occurred to the extent of commencement of menstruation, after an amenorrhoea of $1\frac{1}{2}$ -4 years' duration.

The study of endometrial biopsy therefore is a valuable method in determination of the response to antituberculous drugs.

Our thanks are due to the Scientific Advisory Board of Indian Council of Medical Research for grant-in-aid for studies of endometrial biopsies. We are grateful to the Principal, Lady

Hardinge Medical College, New Delhi, for permission to publish this paper.

Summary.

The examination of the endometrium during the secretory phase gives more information about the time of ovulation, degree of progestational change, normality or abnormality of endometrium than any other single test done in cases of sterility. There are certain components in the histology of the endometrium that change rapidly, constantly, and characteristically enough under hormonal influence and some of these criteria are most useful in dating the endometrium.

The development of cellular pathology has shed great light on the organic diseases affecting the endometrium. The rapid advances of last three decades and a more enlightening understanding of various clinical phenomenon have greatly broadened the scope of our knowledge. In general it may be said that the endometrium reflects the activity of the ovaries.

The endometrial biopsy is done either in the premenstrual week or on the first day of menstruation. This technique is used extensively for:—

- 1) Evidence of occurrence or non-occurrence of ovulation.
- 2) Study of level of ovarian function.
- 3) Histological evidence of endometrial tuberculosis.
- 4) Evaluation of results of hormone therapy and therapy with antituberculous drugs.

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