

A HISTOPATHOLOGICAL STUDY OF ENDOMETRIUM IN PRIMARY STERILITY

V. E. KRISHNAMOHAN • BASANTI NAIR • SHEELA SHENOY

SUMMARY

50 cases of Primary infertility were studied with detailed clinical examination. Accurate dating of endometrium was done and the role of Reticulin, Vangieson, Phloxine tartrazine and PAS staining was assessed. In 80% of cases the uterus was retroverted. In many cases in this study this position of the uterus could have been responsible for the infertility. Majority of our cases underwent endometrial biopsy premenstrually. Dating was done in Hematoxylin and Eosin stained sections. H X E staining was of use in diagnosing corpus luteal defect and anovulatory cycles. PAS was useful to detect scanty secretions which were not visible in H X E. Reticulin stain showed variability in the the amount and distribution of reticulin in the secretory phase. Endometrial biopsy with routine H X E staining is an important investigation because endometrial Biopsy can be practised in socio-economically backward countries like India, where complex expensive immunological and hormone assay procedures are not easily available or affordable.

INTRODUCTION

Infertility is a global problem facing approximately every seventh married couple. Males are responsible in 50% of cases. The endometrium is the most sensitive indicator of ovarian function. Accurate diagnosis is possible only if there is a detailed clinico pathological evaluation. For that the clinician should provide all relevant details. Ideally the biopsy is taken at the end of the menstrual cycle, that

is premenstrually.

MATERIALS AND METHODS

A prospective study was undertaken on endometrial biopsies in 50 consecutive cases of sterility, received in the Department of Pathology, Medical College, Thiruvananthapuram from the Gynaecological Department of Sree Avittom Thirunal Hospital, Thiruvananthapuram, during the period of three months from January 1990 to March 1990. In all the 50 cases, the male counterpart had been

investigated and showed normal sperm count. A control group of 20 patients were included in the study. These represented D & C specimens with normal endometrium, histopathology corresponding to each day of the postovulatory period. H & E and the special stains were utilized in all these cases.

METHODS

(a) **Clinical evaluation:** A detailed clinical evaluation of each patient was undertaken. Age of the patient, marital status, menstrual history, hormonal therapy and any other endocrine disorders were noted. Other diseases of the cardiovascular system, haematological disorders and infections were looked for.

(b) **Pathological evaluation:** The material was fixed in 10% formalin and processed in the automatic tissue processor. Sections of 3-5 μ thickness were cut and stained with the following:

(1) Haematoxylin and Eosin (H & E). Sections were stained with H & E and studied so as to date the endometrium accurately based on the criteria described by Dallenbach - Hell Weg (1981).

Special stains such as Reticulin, Vangieson Periodic Acid schiff and Phloxine tartrazine were employed in these cases and the findings were interpreted in the light of clinical details such as menstrual history, hormonal therapy and other Gynaecological disorders.

(2) Vangieson's staining (Van gieson - 1989) was done to detect polyps, to pick out smooth muscle fibres and fibrous tissue.

(3) Periodic Acid Schiff staining was done to detect small amounts of glycogen or mucous. PAS positivity was graded as Nil, scanty, moderate and intense.

(4) Reticulin staining (Gomori - 1937) was to done study the pattern during different days of the cycle. It was graded as scanty or abundant with a comment as to whether it was intact or fragmented.

(5) Phloxine tartrazine (Lendrum 1947)

was also done on all samples to identify endometrial granulocytes.

All the sections including the special stains were interpreted with reference to the control group.

RESULTS

50 endometrial biopsies and curettage specimen were received in the Department of Pathology, Medical College, Thiruvananthapuram for investigation of primary infertility during a period from January 1990 to March 1990.

The youngest patient was 20 years old and the oldest was 35 years. 32% of the patients were married for more than 2 years. 20% of the patients had more than 4 years of married life.

Pelvic examination done in all these fifty cases showed that 80% of the patients had a retroverted uterus.

Of the fifty cases, 31 were endometrial biopsies and 19 were curettings.

Out of the fifty cases, 14% underwent endometrial biopsy study on more than one occasion.

The time of biopsies in relation to menstrual cycle showed that 72% of the cases

Table I

Histological diagnosis	No. of cases	%
Normal Secretory endometrium	32	64
Menstrual endometrium	8	16
Proliferative endometrium	4	8
Corpus luteum deficiency	3	6
Tissue insufficient	1	2
Early pregnancy	1	2
Acute endometritis (cannot be dated)	1	2

underwent premenstrual endometrial biopsies. In 22% of the cases endometrial evaluation was done during the 1st day of menstruation.

Based on the microscopic picture and following the criteria of Dallenbach-Hellweg, (1981) the endometria were grouped into different categories as per Table I. One case, whose peripheral smear was normal showed features of endometritis where gland destruction was noted with inflammatory cells. Nuclear debris as on 14th P. O. D. (Post Ovulatory Day) was noticed excessive in two cases.

Table II

Secretory endometrium

Secretory endometrium	No. of cases	%
Histopathology Coinciding with that expected	29	90.6
Deficient Secretory glands and stroma, lag of more than 2 days	1	3.1
Glands deficient, lag behind markedly, stroma coincides with the expected period	2	6.3

Table III

Proliferative endometrium

Proliferative endometrium	No. of cases	%
Deficient proliferation (anovulatory)	2	50
Irregular proliferation (anovulatory)	1	25
Normal	1	25

SPECIAL STAINS

(a) Periodic Acid Schiff (PAS) was done and positivity was graded into nil (0), Scanty (+), moderate (++) and intense (+++) PAS positivity in the Control group and Test group were graded.

(b) Reticulin : In proliferative phase, cases had adequate reticulin and menstrual endometrium had scanty reticulin. In early Secretory and mild secretory endometrium scanty reticulin was noted. Late secretory endometrium showed varying amount of reticulin.

(c) On Vangieson staining, 82% of cases showed no significant findings. A nonreactive nodules was delineated in one case. Useful in demonstrating spiral arterioles.

(d) Phloxine tartrazine staining showed phloxino philic cells in 30% cases.

DISCUSSION

A total of 3080 (Three thousand and eighty) biopsies were received in the Department of Pathology, Medical College, Thiruvananthapuram from January 1990 to March 1990. Of these the number of biopsies for infertility was 50 accounting for 1.62% of the total it is an important problem. In India alone there are an estimated 10.2 million couples of infertility (Dawn 1976).

The type of Secretory endometrium according to the maturation of glands, stromal changes, and relating to last menstrual period, based on the histological features and criteria of Dallenbach - Hellweg were grouped as per Table II.

The Subgrouping of proliferative endometrium according to the morphology of glands, stromal changes and maturation as per Table III.

Routine pelvic examination conducted by the Gynaecologist showed that 80% of the cases had a retroverted uterus. The gross abnormality of the uterus, its position, and size are important factors which mechanically obstruct the sperm movement (Fox. H - 1981, Pentti K. Heinonen - 1983). In this study this

position of the uterus could have been responsible for the infertility.

In our study 36 cases underwent premenstrual biopsy, 32 cases showed Secretory endometrium using H X E staining (Table I).

Unusual findings like increased eosinophils in the stroma (Dallenbach - 1981) lymphoid aggregates (Sen & Fox 1967) and increased nuclear debris in the glandular cells were noted. This is an example of apoptosis and it indicates excessive cells destruction which is unusual.

3 cases showed features of anovulatory cycles ie. two cases showed deficient glands; one case showed irregular proliferation (Table III). The most important criterion for making the diagnosis from endometrial biopsy is the absence of secretory changes in the 2nd half of the cycle. The glands and stroma in deficient proliferation were clearly retarded indicating a follicular insufficiency which was the cause of anovulation (Dallenbach - 1981). One case showed features of early pregnancy. The glands were dilated and enlarged and the stroma showed sheets of predecidual cells and plenty of vascular channels. (Rosenfield 1975). In our study 3 cases showed features of corpus luteal defect. A lagging behind of the endometrium by more than two days constitutes luteal insufficiency or luteal lag (Jones G. S. 1976, 1977) (Rosenberg, 1980). In the present series the changes in the glands and the stroma were 4-5 days behind that expected. This fact with a history of regular menses in the past suggested a diagnosis of luteal insufficiency. This may be due to the presence of a corpus luteum that failed to develop normally or had regressed quickly.

In two of our cases the endometrium showed a deficient secretory pattern. (Table II) with a glandular and stromal maturation dissociation. Glands with subnuclear vacuolation were seen side by side with prominent stromal arterioles cuffed by predecidual stroma (Noyes - 1959).

4 special stains were used to assess the

chemical changes that take place in the endometrium. In all these fifty cases Periodic Acid Schiff (PAS), Vangieson, Reticulin and phloxine tartrazine staining was done.

PAS reaction was particularly useful in making functional diagnosis by staining small amounts of glycogen and mucous that are normally inapparent with other stains. In our study we could demonstrate intraepithelial glycogen droplets positive for PAS as early as the mid proliferative phase when the secretions are not visible with H X E staining. Marked variation in positivity in test cases and control cases and from case to case was noted indicating the variations in the intensity of secretory activity from patient to patient.

Reticulin staining showed that in proliferative endometrium reticulin is increased and compactly arranged. Compact reticulin is noted in the early secretory and mid secretory phase. Reticulin is adequate in proliferative phase but in menstrual phase, the reticulin pattern shows fragmentation and becomes scanty. Vangieson, staining did not reveal any obvious change. In one case Vangieson stain brought out a nodule with 2 small resting glands. Vangieson staining was useful to delineate the spiral arterioles and was useful to identify reddish collagen bands corresponding to compressed area in H X E was seen. Reticulin staining showed focal increase in collagen fibres suggesting the possibility of a fibroid.

To demonstrate endometrial granulocytes phloxine tartrazine stain was used. No leucocytic infiltration of normal endometrium takes place before menstruation begins. (Vanbogett - 1978), (Dallenbach - 1981). With phloxine staining endometrial K-cells take bright red colour in a background of yellow. Some of the Neutrophils also stained orange to faintly red with diffuse positivity. Hence the phloxine tartrazine stain did not prove very useful in differentiating between Neutrophils and K cells.

The major draw back of our study was the insufficiency of tissue for all the special stains.

REFERENCES

1. Dallenbach, Hellweg : *Histopathology of the endometrium*, 3rd Edn, Newyork : 1981.
2. Dawn C. S. : *Text book of Gynecology*, 15th Edn. : 224, 1976.
3. Fox H., Buckely C. H. : *Recent advances in Histopathology* : 1981.
4. Gomori G. : *Am. J. of Clin. Pathology* : 13, 993, 1937.
5. Jone G. S. : *Fertil. and Steril.* : 27, 351, 1976.
6. Jone G. S. : *J. Repro. Medicine* : 18, 139, 1977.
7. Lendrum A. C. : *J. of Path. and Bact.* : 59, 399, 1947.
8. Noyes R. W. : *Am. J. Obstet. & Gynec.* : 77, 929, 1959.
9. Pentti K. Heinonen : *Fertil. and Steril.* : 40, 311, 1983.
10. Rosenberg : *Fertil. and Steril.* : 34, 17, 1980.
11. Rosenfield D. L. : *Fertil. and Steril.* : 26, 1088, 1975.
12. Sen. D. K., Fox H. : *Gynaecologia* : 163, 371, 1967.
13. Vagieson : *Laboratory methods of technical method for the hervous system.* Newyork Medical Journal : 50, 57, 1989.
14. Vanbogett L. J. : *Obstet. and Gynec.* : 51, 25, 1978.