SIGNIFICANCE OF RETICULIN STAINING IN DATING OF ENDOMETRIAL BIOPSIES

by

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Introduction

Reticulin staining of endometrial biopsies was done in 107 cases. A study of the reticulin pattern was also made in decidua of early pregnency (18 cases). The distinct and changing morphological pattern of reticulin fibres in different phases of the menstrual cycle gives a better diagnostic aid in dating of endometrial biopsies, as compared to routine haematoxylin and eosin stained sections (Craig and Danziger, 1963; Saito, 1978).

Oestrogen causes increased synthesis of reticulin fibres of the endometrial stroma (Fainstat, 1965; Kao *et al*, 1965; McLennan and Rydell, 1965; Beggish *et al*, 1967; Weinke *et al*, 1968). Their physical and chemical properties and staining reactions have also been investigated (Wilder, 1935; Tomlin, 1953).

Distinct histological features, like the relative thickness of reticulin fibres in the stroma, arrangement of fibres around single stromal cells, periglandular and perivascular network, provide a better, simpler and accurate method for dating of endometrial biopsies, as compared to routine H & E stained sections.

Material and Methods

Endometrial biopsies were obtained from Out Patient's Sterility Clinic of J.N. Medical College and Hospital, Aligarh. Serial sections were stained with H. & E. and silver impregnation method (Wilder, 1935).

One hundred and twenty-five cases were studied, and classified as follows:

(i) Proliferative phase, 42; (ii) Secretory phase, 50; (iii) Menstrual phase, 15; (iv) Decidua of early pregnancy, 18. (Specimens obtained from cases of inevitable abortion—I trimester).

Observations

Reticulin fibres revealed interesting features on different days of the menstrual cycle. This change was more distinct and specific as compared to H & E stained sectiins.

5th-8th day: Reticulin fibres were thin, discrete but increased in number in the zona functionalis. Linear arrangement was seen close to stromal cells. The basement membrane of superficial epithelium and the glands was not visible.

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9th-11th day: Reticulin fibres were coarser and thicker, and increased in number in all the layers of the endometrium. Basement membrane of glands could be seen distinctly (Fig. 1).

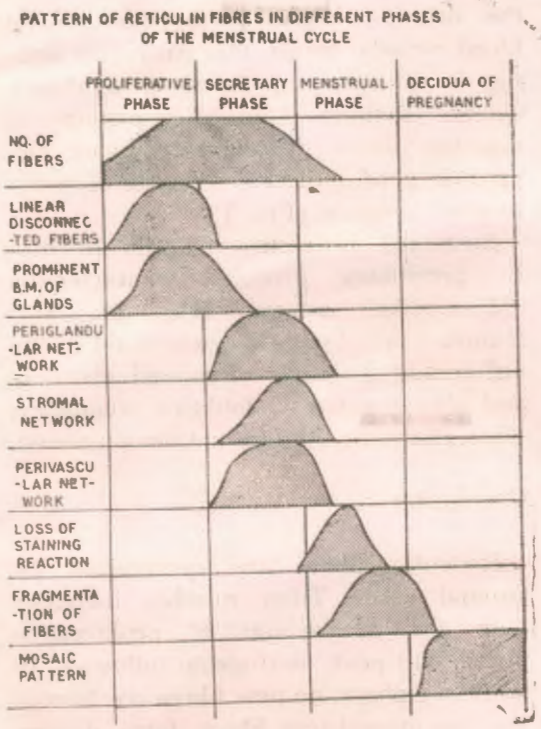

Fig. 4

Graphical representation of the pattern of reticulin fibres in different phases of the menstrual cycle is shown. Diagnostic features are represented along with vertical axis and the results obtained along the horizontal axis.

11th-14th day: Basement membrane of superficial surface epithelium was prominent now. Basement membrane of glands, however, was more deeply staining. Beginning of periglandular nets indicated that ovulation was imminent. H & E stained sections, however, revealed no change except pseudostratification of nuclei in glandular epithelium and few mitosis.

14th-15th day: An abrupt change occurred with the onset of ovulation, periglandular nets were prominent now. H & E sections, however, revealed subnuclear vacuoles.

16th day: The basement membrane of glands disappeared completely and was now replaced by prominent periglandular nets. No change was seen in H & E sections.

18th day: The basement membrane of the superficial surface epithelium disappeared, and prominent interconnected network of reticulin fibres was seen.

19th day: The reticulin fibres encircled stromal cells partially and groups of stromal nets began to appear.

20th day: With the onset of stromal oedema, the reticulin network was disrupted and fibres encircled individual stromal cells completely. Periglandular nets remained unchanged.

22nd day: Periglandular nets and clusters of stromal nets were enmeshed in sheets of continuously encircling reticulin fibres.

23rd day: Perivascular network was seen around spiral arterioles. H & E sections at this stage revealed pre-decidua around blood vessels.

25th day: Perivascular nets were fully formed and deeply staining around all the blood vessels, and offered a sharp contrast with less conspicuous periglandular nets (Fig. 2).

26th day: Periglandular, perivascular and interconnected stromal network of reticulin fibres completely enmeshed the entire endometrial tissue. H & E sections revealed sheets of pre-decidua.

27th day: Reticulin fibres revealed loss of staining reaction. Fragmentation of fibres was seen, with resultant collapse of reticulin network.

28th day: Complete lysis of fibres was a prominent feature. H & E sections reveal-

ed sheets of decidua, leucocytic infiltration and areas of necrobiosis.

Between 1st-4th day of menstruation, fragmentation and lysis of fibres was seen.

Decidua of early pregnancy: Reticulin fibres increased almost twice in diameter, became coarser, darkly staining and completely encircled individual decidual cells in a "mosaic-like" pattern (Fig. 3). This change was characteristic and diagnostic of early pregnancy. Specific arrangement of reticulin fibres in different phases of menstrual cycle is summarized in Fig. 4.

Discussion

Endometrial stromal cells mature, differentiate into cells resembling fibroblasts produce reticulin fibres under the effect of oestrogen in proliferative phase (Baggish *et al*, 1967; Weinke *et al*, 1968). Thickening of fibres, prominent basefent membrane of glands are associated with mid-proliferative phase, when the regenerative ability of the endometrial stromal cells is markdly accelerated.

With the onset of ovulation, however, these fibres encircle stromal cells, lose their linear alignment and periglandular nets begin to appear (Craig and Danziger, 1963). It remains to be seen whether the changes observed from day to day in the secretory phase resulted from oedema, convolution of glands, or whether progesterone produces a physical change in tensile strength of the fibres which results in a circular encircling pattern. Pre-menstrual withdrawal of ovarian hormones causes lysis and fragmentation of reticulin fibres (Momoi, 1975).

In anovulatory cycles, however, these findings will persist, and the onset of periglandular, stromal and perivascular network will be absent. These features are more distinct and prominent as compared to subjective changes like stromal oedema and convolution of glands (Sedlis and Nam, 1971). In cases of primary and secondary sterility these findings will definitely influence the clinical assessment.

Perivascular networks form only when the decidua begins to appear around blood vessels, hence, this particular finding is of value in assessing ovulatory cycles. Definite diagnostic pattern of reticulin fibres in secretory phase can provide good indices for dating of endometrial biopsies (Fig. 7).

Sustained progestrone levels in early pregnancy produces characteristic "Mosaic-like" pattern (Fig. 6). This feature is a valuable diagnostic aid in the differential diagnosis of missed abortion, and also in cases of doubtful pregnancy, when chorionic villi are not demonstrated.

Conclusion

Reticulin fibres are synthesized by stromal cells. Their number increases with the progression of proliferative phase, and peak oestrogenic influence. In secretory phase, no new fibres are formed but the pre-existent fibres form distinct histological pattern which serves as a useful diagnostic index in dating of endometrial biopsies. "Mosaic-like" pattern of the decidua conforms the diagnosis of missed abortion.

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See Figs. on Art Paper III