# BIOCHEMICAL STUDIES ON THE ENDOMETRIUM IN INFERTILITY

by

BASANT CHADHA,* M.D.

I. N. BHATT,** M.Sc., M.S. (Wisconsin)

and

S. Y. EDULJEE,*** M.D., D.G.O.

*Introduction*

Reproduction is a complex biological process. Quite often, baseline investigations in an infertile couple may fail to reveal the cause of reproductive failure. "Luteal phase inadequacy" implies deficient nutritional bed for implantation and development of the blastocyst. Except for the studies of Hughes *et al* (1963), there has been scant reference in the literature to human endometrial biochemistry and the role of uterine milieu in reproduction. Hence this study was undertaken to determine the biochemical and enzymatic profiles during different phases of the menstrual cycle in infertile women, in order to assess whether this parameter could serve as a guideline for hormonal therapy in cases of disturbed endometrial function.

*Material and Methods*

Women in the age range 20 to 35 years, attending the Gynaecology out-patient department and Infertility Clinic of the Lok Nayak Jai Prakash Narayan Hospital, New Delhi, were studied in the following groups:

Primary Sterility (P.S.).

Secondary Sterility (S.S.).

Repeated Abortions (R.Ab.): Two or more consecutive spontaneous first trimester abortions.

Controls (C): Normal fertile women.

On the basis of a thorough clinical examination and investigation, the primary and secondary sterility cases were each further divided into two groups.

Group I (Gr. I)—where detailed clinical and investigative work-up showed no causative factor for infertility.

Group II (Gr. II)—presence of a definite factor to which infertility could be attributed.

Endometrial biopsies were taken from each patient at 5 or 6 different phases of the menstrual cycle. Immediately after the tissue was homogenised and assayed for glycogen, total proteins and as also its activity for alkaline and acid phosphatase. In all 250 endometrial biopsy specimens were subjected to biochemical analysis.

Timing of endometrical biopsy for estimation of proteins and phosphatases was as follows:

Phase I—(Mid proliferative—6th to 8th day.

Phase II—(Late proliferative)—9th to 13th day.

Phase III—(Early secretory)—14th to 20th day.

Phase IV—(Mid Secretory) 21st to 24th day.

Phase V—(Late Secretory)—25th to 28th day.

*Postgraduate Student.

**Reader in Biochemistry.

***Assoc. Prof.

Deptt. of Obstetrics and Gynaecology, M.A. Medical College, New Delhi.

Endometrial Protein was estimated by the method of Lowry *et al* (1951). The method of King and Armstrong (1937) was followed for estimation of phosphatases. Estimation of endometrial glycogen was done by the method of Morris (1948) using the anthrone reagent as described by Payne and Latour (1955) and Brixova and Dzurikova (1972).

*Observations*

Endometrial protein shows cyclic variations with maximal values being obtained in Phase III in all groups as seen from Table I. A second though lesser peak is observed premenstrually in Phase V in control group, and primary and secondary sterility Group II cases. In contrast, primary and secondary sterility Group I cases, and 75% of repeat abortion cases show a flat protein curve (Fig. I) due to

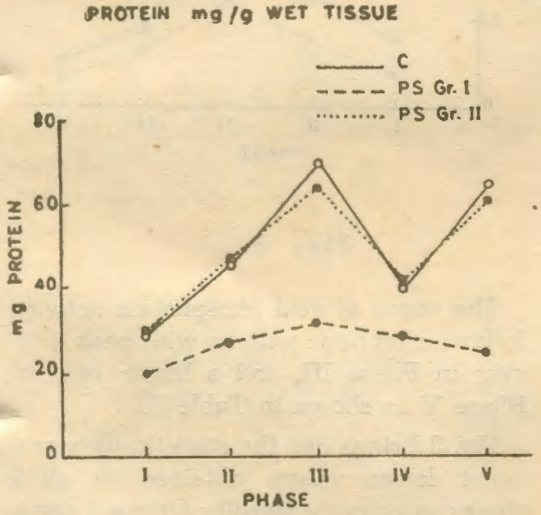


**Fig. 1**

a low peak in Phase III, and no premenstrual rise. Primary Sterility Group I cases show the lowest values, and a comparison of "p" values reveals a highly

TABLE I

*Endometrial Protein in mg/g Wet Tissue*

| Phase | I | | II | | III | | IV | | V | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| No. of cases | 10 | | 10 | | 10 | | 10 | | 10 | |
| C. | 28.30 | ± 4.72 | 44.95 | ± 2.29 | 69.06 | ± 8.37 | 39.49 | ± 6.25 | 64.06 | ± 4.87 |
| No. of cases | 8 | | 8 | | 15 | | 8 | | 11 | |
| P.S. (Gr. I) | 19.90 | ± 2.40 | 27.20 | ± 6.08 | 31.72 | ± 9.46 | 28.44 | ± 6.61 | 27.74 | ± 7.28 |
| No. of cases | 8 | | 8 | | 10 | | 8 | | 8 | |
| P.S. (Gr. II) | 28.62 | ± 5.33 | 45.95 | ± 3.99 | 63.70 | ± 5.66 | 39.81 | ± 6.19 | 61.08 | ± 6.69 |
| No. of cases | 8 | | 8 | | 10 | | 8 | | 8 | |
| S.S. (Gr. I) | 20.02 | ± 2.62 | 29.69 | ± 4.26 | 37.88 | ± 4.82 | 25.59 | ± 2.92 | 26.16 | ± 4.26 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | |
| S.S. (Gr. II) | 29.35 | ± 4.71 | 45.27 | ± 3.19 | 61.99 | ± 5.01 | 38.33 | ± 5.60 | 60.17 | ± 6.20 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | |
| R. Ab. | 22.29 | ± 3.16 | 34.12 | ± 4.66 | 43.45 | ± 4.58 | 30.56 | ± 5.12 | 34.35 | ± 5.09 |

TABLE II

*Endometrial Alkaline Phosphatase Expressed as Micromole Phenol/min/g Wet Tissue*

| Phase | I | | II | | III | | IV | | V | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| No. of cases | 8 | | 8 | | 10 | | 8 | | 8 | |
| C. | 0.408 | ± 0.117 | 0.728 | ± 0.114 | 1.233 | ± 0.316 | 0.496 | ± 0.124 | 0.278 | ± 0.069 |
| No. of cases | 8 | | 8 | | 13 | | 8 | | 8 | |
| P.S. (Gr. I) | 0.212 | ± 0.130 | 0.413 | ± 0.116 | 0.629 | ± 0.186 | 0.354 | ± 0.80 | 0.183 | ± 0.104 |
| No. of cases | 8 | | 8 | | 10 | | 8 | | 8 | |
| P.S. (Gr. II) | 0.409 | ± 0.117 | 0.697 | ± 0.101 | 1.148 | ± 0.141 | 0.461 | ± 0.02 | 0.277 | ± 0.068 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | |
| S.S. (Gr. I) | 0.242 | ± 0.069 | 0.437 | ± 0.085 | 0.698 | ± 0.086 | 0.337 | ± 0.102 | 0.230 | ± 0.089 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | |
| S.S. (Gr. II) | 0.412 | ± 0.081 | 0.667 | ± 0.071 | 1.151 | ± 0.103 | 0.532 | ± 0.083 | 0.313 | ± 0.097 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | |
| R. Ab. | 0.281 | ± 0.080 | 0.396 | ± 0.121 | 0.680 | ± 0.159 | 0.538 | ± 0.80 | 0.236 | ± 0.133 |

significant decrease in protein content in all 5 phases as compared with controls. Table II indicates that endometrial alkaline phosphatase activity follows a constant pattern in all groups with peak values in Phase III. A flat curve of activity is obtained in primary and secondary sterility Group I cases and in repeat abortion cases. The lowest values are obtained with primary sterility Group I as shown in Fig. 2.

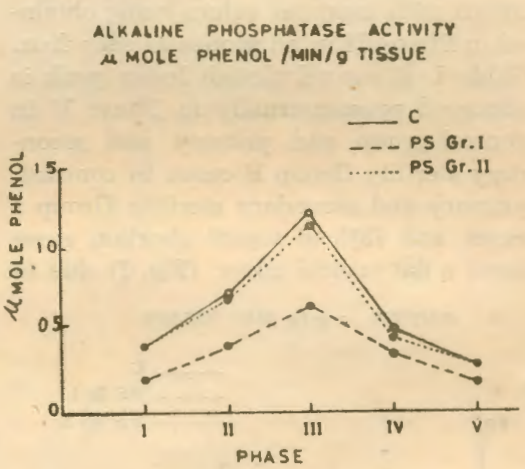


Fig. 2.

The curve of acid phosphatase activity follows a biphasic pattern with peak activity in Phase III, and a lesser one in Phase V as shown in Table III.

Fig. 3 brings out the statistically significant lower values obtained in all 5 phases in primary sterility Group I cases. Table IV: Shows glycogen content of endometrium during 6 different phases of the menstrual cycle. In all groups, peak levels occur between 17th to 20th day of the cycle. However, the values in the infertile Group I cases and repeat abortion cases are very significantly decreased from control values as depicted in Fig. 4.

TABLE III

*Endometrial Acid Phosphatase Expressed as Phenol/Micromole/min/g Wet Tissue*

| Phase | I | | II | | III | | IV | | V | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | |
| C. | 0.399 | ± 0.06 | 0.622 | ± 0.11 | 1.280 | ± 0.18 | 0.718 | ± 0.21 | 1.270 | ± 0.23 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | |
| P.S. (Gr. I) | 0.247 | ± 0.030 | 0.347 | ± 0.051 | 0.602 | ± 0.141 | 0.230 | ± 0.050 | 0.419 | ± 0.101 |
| C/PS (Gr. I) "t" | 0.607 | | 6.250 | | 8.420 | | 14.498 | | 9.615 | |
| "p" | <.001 | | <.001 | | <.001 | | <.001 | | <.001 | |

TABLE IV

*Glycogen Content of the Endometrium mg/g Wet Tissue*

| PHASE | I | | II | | III | | IV | | V | | VI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| days | 6-10 | | 11-13 | | 14-16 | | 17.20 | | 21-25 | | 26-28 | |
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | | 8 | |
| C. | 0.67 | ± 0.119 | 4.02 | ± 0.625 | 6.95 | ± 0.57 | 10.86 | ± 1.20 | 8.21 | ± 0.55 | 5.64 | ± 0.68 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | | 8 | |
| P.S. (Gr. I) | 0.34 | ± 0.101 | 1.92 | ± 0.417 | 3.15 | ± 0.54 | 5.72 | ± 0.87 | 3.76 | ± 0.70 | 2.26 | ± 0.59 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | | 8 | |
| S.S. (Gr. II) | 0.401 | ± 0.085 | 2.67 | ± 0.403 | 4.075 | ± 0.577 | 6.187 | ± 0.86 | 4.099 | ± 0.59 | 2.43 | ± 0.62 |
| No. of cases | 8 | | 8 | | 8 | | 8 | | 8 | | 8 | |
| P.Ab. | 0.43 | ± 0.085 | 2.60 | ± 0.43 | 3.44 | ± 0.509 | 5.75 | ± 0.947 | 4.06 | ± 0.48 | 3.13 | ± 0.50 |

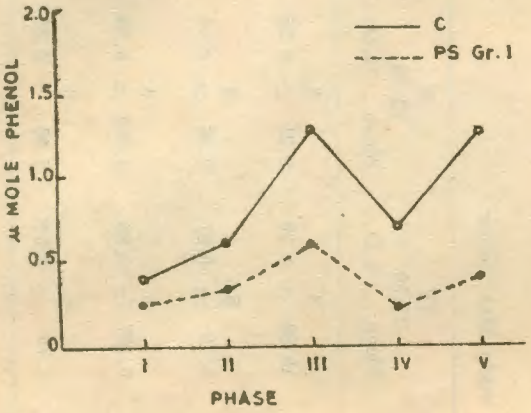


Fig. 3.

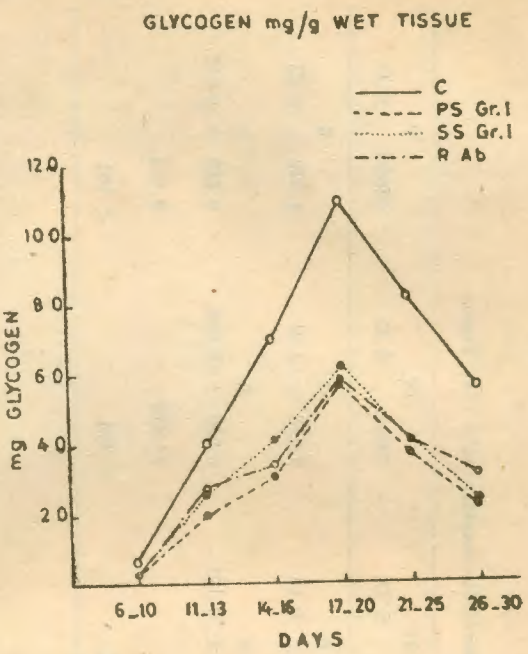


Fig. 4.

*Discussion*

Our knowledge of nutritional physiology of the endometrium and its role in successful reproduction is still incomplete. Hughes *et al* (1963) in their endometrial studies on carbohydrate metabolism in infertile and repeated abortion cases have revealed impaired nutritional physiology in approximately 80% of cases where routine investigative work-up showed no other cause for the reproductive failure. Proper metabolic activity in any tissue is largely dependent on the amount of enzymes, proteins and glycogen present in that tissue and enhanced blood flow. Oestrogens and later progesterone stimulate blood flow and set into action enzyme-coenzyme systems catalysing metabolic reactions and resulting in energy production for tissue growth and differentiation. Hence, there is little biochemical activity during the proliferative phase of the cycle. It builds up at ovulation time and thereafter when fertilization, proper implantation and development of the blastocyst follow closely. In the present study, peak values of proteins and enzymes, alkaline and acid phosphatases were recorded in Phase III i.e. 14th to 20th day. Endometrial glycogen also reached peak levels between 17th to 20th day. This, therefore, is the most vital period for fertilization, implantation and development of the blastocyst. The protein curve of primary and secondary sterility Group II cases (Table I) was similar to that of controls with a second lesser peak also in Phase V, absent in the group I infertile cases and repeat abortion group. This shows that endometrial biochemistry in group II cases was essentially normal as infertility was due to

other causes. Endometrial alkaline phosphatase activity was remarkably lowered in Phase IV, particularly in infertile group I and repeat abortion cases. This may imply faulty nidation and explain early abortions. Hughes *et al* (1963) have suggested that the decreased glycogen storage and energy production at the endometrial level could be due to defects in enzyme, co-enzyme and nucleic acid synthesis in infertile patients. In the present study glycogen levels were significantly decreased in the group I infertile and repeat abortion cases. The decreased glycogen is significant as it is associated with lowered glucose content of the uterine fluid and cervical mucus, an important factor in sperm migration. As this may be corrected by hormonal therapy and thereby improve the fertility potential, it may be worthwhile assessing this parameter in cases of unexplained infertility.

*Acknowledgement*

We are thankful to Dr. S. K. Lal, Dean, Maulana Azad Medical College, New Delhi for allowing us to publish this data.

*References*

1. Brixova, E. and Dzurikova, V.: Clinica. Clinica. Acta. 36: 543, 1972.
2. Hughes, E. C., Jacobs, R. D., Kabulia, A. and Husney, R. M.: Am. J. Obstet. Gynec. 85: 594, 1963.
3. King, E. J, and Armstrong, A. R.: Quoted in Biochem. et Biophys. Acta. 34: 312, 1959 by Ahmad, Z. and King, E. J.
4. Lowry, O. H., Rose Brough, N. T., Farr, A. C. and Randall, R. J.: J. Biol. Chem. 193: 265, 1951.
5. Morris, D. L.: Science. 107: 254, 1948.
6. Payne, H. W. and Latour. J. P. A.: J. Clin. Endocr. 15: 1106, 1955.