

Study of Corpus Luteum Function in Infertile Women by Timed Endometrial Biopsy and Progesterone Assay in the Diagnosis of Luteal Phase Defect

Col Jasvinder Kaur Bhatia¹, Col Jagdeep Singh Bhatia^{*2}

¹Assoc Prof. Dept of Pathology, Armed Forces Medical College, Pune, India

²Sr Advisor, Command Hospital Southern Command Pune, India

Original Research Article

*Corresponding author
Col Jagdeep Singh Bhatia

Article History

Received: 06.08.2018

Accepted: 17.08.2018

Published: 30.08.2018

DOI:

10.21276/sjams.2018.6.8.32



Abstract: Luteal phase defect is an improper development of endometrium for nidation, either due to decreased secretion of progesterone by the corpus luteum or when the effect of progesterone is limited at the endometrium. Both primary infertility and recurrent abortions have been found associated with this defect, however the diagnosis is difficult as there are no defined criteria for diagnosis. Aim of the study was to evaluate corpus luteum function in infertile women by timed endometrial biopsy and progesterone assay in the diagnosis of luteal phase defect. Cross sectional prospective study. Fifty infertile women with regular periods and a normal menstrual flow were included. The patients were evaluated by midluteal endometrial biopsy and serum progesterone. The tests were done on the same day in each patient, in the mid luteal phase. Delay of more than 2 days was taken as luteal phase defect. Age matched, fertile, normally menstruating women were taken as controls. Result: 44 patients were within two days of the chronological dating. 6 endometria were dated as more than two days short of the chronological date. Four cases showed coordinated delay and 2 cases showed dissociated delay. Six endometria showed proliferative phase endometrium. Mean progesterone level of 6.8+ 1.75 ng/ml was found in patients of luteal phase defect. Conclusion: Endometrial biopsy specimen provides an accurate measure of quantitative progesterone effect on corpus luteum function and also of the response of endometrium to the steroid stimuli and therefore is recommended for the diagnosis of luteal phase defect.

Keywords: Endometrial biopsy, endometrial dating, Luteal Phase defect, Serum progesterone.

INTRODUCTION

The luteal phase of the menstrual cycle of a woman is the period between ovulation and the onset of next menstruation. Luteal phase defect is defined as an improper development of endometrium for nidation, either due to decreased secretion of progesterone by the corpus luteum or when the effect of progesterone is limited at the endometrium. Both primary infertility and recurrent abortions have been found associated with this defect [1]. Assessment of luteal function is an important investigation in the evaluation of infertile women.

The first description of luteal phase defect by Jones preceded the report of Noyes *et al.* Noyes described the criteria for interpreting endometrial biopsies based on basal body temperature charting [2]. Jones advocated two biopsies for diagnosing inadequate luteal phase [3].

At present there is no single diagnostic test available for diagnosis of luteal phase defect[4]. Some of the studies have suggested that both midluteal phase

P level and late luteal endometrial histological examination should be assessed at the same cycle in the diagnosis of LPD [5] Some studies have emphasized the role of endometrial biopsy in diagnosis and management of luteal phase defect [6]

Aim and Objectives

To evaluate corpus luteum function in infertile women by timed endometrial biopsy and progesterone assay in the diagnosis of luteal phase defect.

MATERIALS AND METHODS

Fifty infertile women attending infertility clinic in our hospital were subjects of the study. Infertility was defined as one year of unprotected intercourse without pregnancy [7].

Women belonging to age group 18-35 yrs were included in the study. Women with regular periods and a normal menstrual flow were registered by noting two menstrual cycles before their inclusion in the study. Women taking hormonal treatment were excluded from

the study. After an informed consent, a detailed history was taken from every patient including the following aspects; Age at menarche, menstrual history since menarche, years since marriage/cohabitation, history of medications and any associated disease. Every patient was evaluated by midluteal endometrial biopsy and serum progesterone.

The tests were done on the same day in each patient, in the mid luteal phase. Age matched, fertile, normally menstruating controls were selected, and serum progesterone estimation was done. Endometrial biopsy was not taken from controls as the same was not agreed to by the ethics committee.

Endometrial Biopsy

Endometrial biopsy of all the patients was done either as an outpatient or under general anesthesia in operation theatre. MR (Menstrual Regulation) cannula was used and endometrial tissue was sucked out with a syringe, under aseptic conditions, either as a OPD procedure or under general anaesthesia. Specimen were fixed processed, sections of 6 micron thickness were cut and stained by hematoxylin and eosin. Histological characteristics were assessed in each slide in the form of glands, stroma, ratio of the gland to stroma and pattern uniformity. Dating of endometrium was done by using the criteria of Noyes. We compared this with the chronological date. Luteal Phase Defect was diagnosed in endometria showing a lag of two or more days between the histological dating and chronological dating.

After a date was assigned to each slide, this dating was compared with the chronological date. We used the next menstrual period as the reference point in accordance with the latest recommendations. Luteal Phase Defect endometrium show a lag of two or more days between the histological dating and chronological dating. They were assessed and scored. To quantitate the morphologic changes occurring in the endometrium ten arbitrarily chosen fields were analyzed in each of the slides. In each field we assessed glandular mitosis, tortuosity of glands, pseudostratification of nuclei, basal vacuolation, secretion, stromal oedema, predecidual reaction and leucocyte infiltration. Morphologic changes were quantitated by analyzing ten arbitrarily chosen fields in each of the slides and were graded as none (0), Slight(1), Moderate(2) and Marked(3). The importance of each criterion was assessed in the diagnosis of luteal phase defect.

To determine the impact of intraobserver variability on endometrial dating and the diagnosis of luteal phase defect Intraobserver error was found out. All the slides were assigned a number and randomized. The same slides were seen and reported twice. The criteria for dating of the endometrium were again applied. Variation was defined as the difference of days between the first and second interpretation. The actual intraobserver variation was calculated as the mean of

these individually calculated variances. Intraobserver variation was defined as the difference of days between the first and second interpretation.

Serum Progesterone

Serum progesterone was estimated by radioimmunoassay. Serum progesterone of all the fifty patients was determined using BRIT (Board of Radiation and Isotope Technology) progesterone direct radioimmunoassay kit. (RIAK12). The sensitivity of the assay was 0.24ng/ml. The intra assay variation was less than 10% and intraassay variation was less than 15%. Serum progesterone values of fifty normally menstruating fertile women were also estimated both in the preovulatory and luteal phase as controls.

RESULTS

Clinical Profile

Age Group

Age of the patients varied from 18 to 33 years. Mean age of patients was 24.04 years. Maximum patients were of the age group of 20 to 24 years comprising 52% of the total cases. (Fig1)

Menstrual History

Their age at menarche varied from 13 to 18 years. Mean age at menarche 13.6 years. All these patients had a regular menstrual history, which was checked by noting two periods prior to the study. There was no history of inter menstrual bleeding. Duration of flow varied from 2-5 days and the cycle length was 26-30 days.

Duration of Infertility

Duration of infertility varied from 2-11 years of marriage. Average duration of infertility was 4.84 years. While studying the duration of infertility of these patients it was found that maximum number of patients were between one to five years of marriage.

Serum Progesterone

Serum progesterone of the controls varied from 2.2ng- 34.6ng/ml. Patients were divided into three groups according to serum progesterone levels. A serum progesterone of >3ng/ml was evidence of secretory activity. Serum progesterone of >10ng/ml was taken as found in normal secretory endometria. A serum progesterone value of >3ng/ml and <10ng/ml was taken as Luteal Phase Defect. When classified as per serum progesterone levels six patients (12%) were in the range of luteal phase defect. (Fig2) Serum progesterone of all the patients varied from 0.38-22.6ng/ml. Mean progesterone. Level of 6.8+ 1.75 ng/ml was found in patients of luteal phase defect. Mean progesterone Level in women with a normal cycle was 15.4 + 2.7ng/ml. (Table1)

Evaluation of Luteal Function

A total of 44 endometria were patients were found to be in secretory phase, 38 of which were within

one to two days of the date as per the next menstruation. Six endometria showed proliferative phase endometrium.

Endometrium in six patients was more than two days short of the chronological date. Four cases showed coordinated delay where the endometrium lagged behind more than two days. There was coordinated delay of glandular and stromal maturation and the glands showing subnuclear vacuolations corresponding to days 16 (Fig 3) Two cases showed Luteal phase defect with dissociated delay where the glands were

small and narrow with more maturation of stroma which showed spiral arterioles. (Fig 4)

Clinical Profile of these patients of Luteal Phase Defect was as follows: Age of these patients varied from 21-28 years.(Mean Age 23.5 years).Mean age at menarche 13.5 years and mean duration of infertility 4.2 years.

Intraobserver variation. The magnitude of intraobserver variation ranged from 0-3 days with a variation of day as the most common variation. In none of the cases we found a variation of more than two days between the first and the second reporting (Table 2).

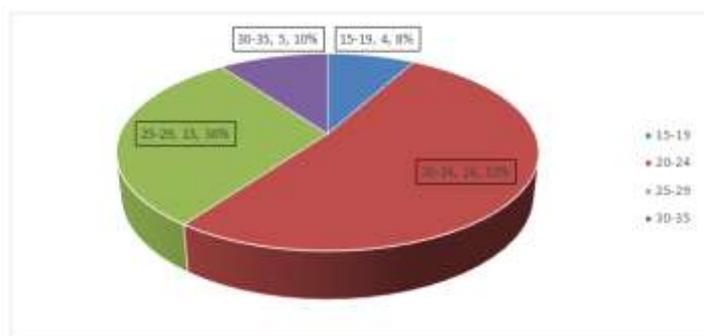


Fig-1: Age group of the patients

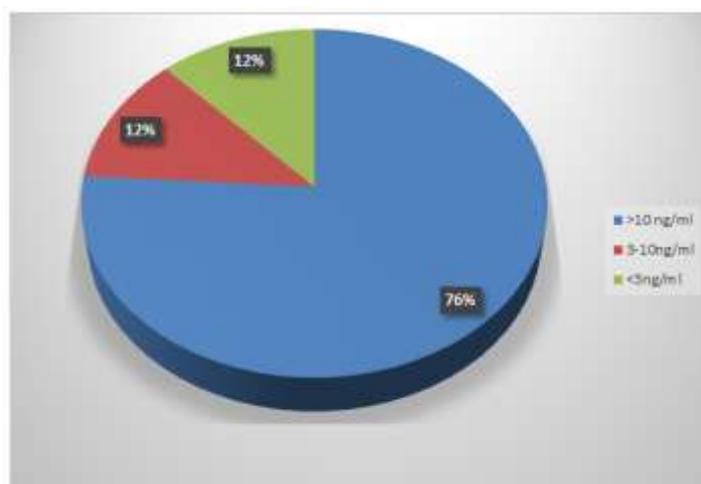


Fig-2: Classification of the patients as per the serum progesterone levels

Table-1: Mean serum progesterone in each group

S.No.	Serum progesterone Levels	Mean Serum Progesterone (mean \pm 2 SD)
1.	>10 ng/ml (11.2 – 22.6 ng/ml)	15.4ng/ml \pm 2.7ng/ml
2.	3-10ng/ml (5.6 – 9.4 ng/ml)	6.8 \pm 1.75 ng/ml
3.	<3ng/ml (0.38 – 1.08 ng/ml)	0.83 \pm 0.4ng/ml

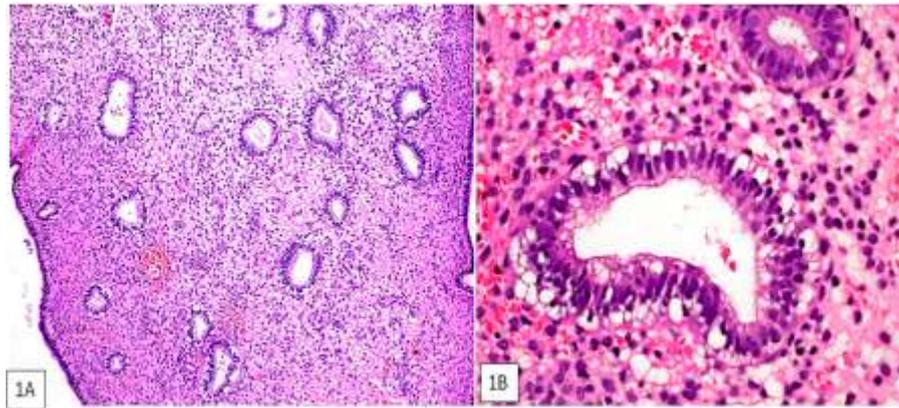


Fig-3(A & B) Luteal Phase Defect with coordinated delay. Glands and stroma lag behind by more than 2 days. The glands show subnuclear vacuolations corresponding to days 16-17

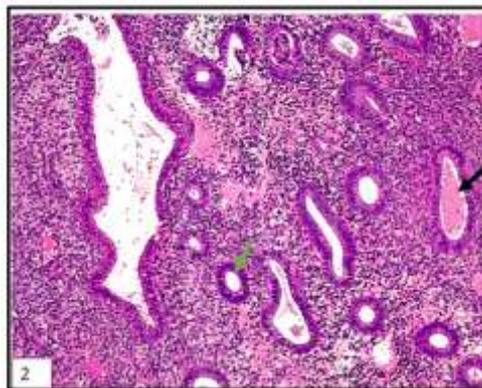


Fig-4: Luteal Phase deficiency with dissociated delay: Small round glands(Green Arrow) with basal nuclei and tall tortoise glands with some showing secretory activity. (Black Arrow)

Table-2: Variation of days and the percentage of intraobserver variation.

Variation of days	No of cases	Percent Total
0	24	48 %
1	26	52%
2	2	0%
3	0	0%

Endometrial Biopsy of the entire patient was done in the midluteal Phase. All the slides were dated by assessing each of the following criteria.

DISCUSSION

Luteal phase defect is a cause of infertility and recurrent abortions. While luteal phase defect can be established in research settings by assaying daily blood samples for progesterone, there is not yet a totally reliable method for the diagnosis of luteal phase defect for use in clinical practice.

Daya S *et al.* in 1988 compared the progesterone profiles of women with luteal phase defect with those of women with normal cycles [8]. In our study we found a mean progesterone level of 6.8+ 1.75 ng/ml in patients of luteal phase defect and a mean progesterone level in women with a normal cycle of 15.4 + 2.7ng/ml. In accordance with their study, we found that the level of serum progesterone was

significantly more in women with a normal secretory phase.

We found that all those patients who had a serum progesterone value of more than 3ng/ml showed a secretory phase in endometrial biopsy. In our study, all patients of luteal phase defect on endometrium biopsy had a serum progesterone value of less than 10ng/ml. Those endometria, which showed a late proliferative phase on endometrial biopsy, had a serum progesterone value of less than 3ng/ml.

It has been seen that the abnormalities of progesterone secretion reflect in the endometrium. Luteal phase defect also may show patterns. Two patterns of luteal phase defect were seen in our study. Luteal Phase defect with coordinated delay: Endometrium shows a coordinated delay of both glandular and stromal maturation. The endometrium lags behind at least by 2 days. A repeat biopsy is needed

to confirm the diagnosis. In these cases, we repeated the biopsy and found the similar histomorphology in the second biopsy. The second pattern which can be seen is Luteal phase insufficiency with dissociated delay which was seen in two patients[9].

We found 100% correlation between endometrial biopsy and serum progesterone levels. Biopsy was performed in the mid luteal phase in our study. Mid luteal endometrial biopsies can pick up retarded endometrial development in cycles in which a second late luteal biopsy, performed in the same cycle, demonstrated "in phase" histologic changes in the endometrium. Castelbaum and colleagues, performed two luteal phase biopsies in the same cycle in 33 infertile women and recommended a mid-luteal biopsy[10].

Li T C *et al.* also found that it is the first half of the luteal phase that endometrium is most sensitive to the progesterone where as in the second half the endometrium is more likely under the control of local factors like the presence of the embryo [11].

Kusuda M *et al.* studied the changes in the endometrium in both the mid and late luteal phase and stressed the importance of endometrial biopsy in the mid luteal phase [15].

The criteria used for defining inadequate luteal phase has varied in different studies. Davis *et al.* performed multiple endometrial biopsies in consecutive menstrual cycle of five women. With a definition of a lag of equal to or more than two days, they found an incidence of 26.7%. When this was changed to a lag of more than three days the incidence was found 6.6%. In our study we found an incidence of 12% with a criteria of 2 days and an incidence of 4% when a more stringent definition of a lag of more than three days. This in part explains the large variation in the figures quoted for the incidence of luteal phase defect in literature.

In our study, we performed only one biopsy as we had the serum progesterone levels in addition to endometrial biopsy to evaluate the luteal phase.

Gautray *et al.* studied the luteal phase by endometrial biopsy and serum progesterone and found a good correlation. Mean serum progesterone in their study in normal cycles was 15.14 ± 1.46 ng/ml on day 21. The mean serum progesterone in patients of luteal phase defect was found to be 10.69 ± 2.21 ng/ml and was found to be statistically significant in accordance with our study [13].

In our study the cases of luteal phase defect were 12 % of total cases. Another study found cases of Luteal phase defect making up to 15.6% [14].

LPD may warrant renewed interest in the unexplained sub fertile population. It is possible that the underlying impaired ovulation and subsequent hormonal derangement may contribute to subfertility[15].

CONCLUSIONS

Our study was aimed at evaluating endometrial biopsy in the diagnosis of luteal phase defect in infertile women. We recommend that, as interpretation of endometrial biopsy specimen provides an accurate measure of quantitative progesterone effect on corpus luteum function and also of the response of endometrium to the steroid stimuli, a single serum progesterone should not be used a substitute to an endometrial biopsy, both the investigations complement each other. Using both serum progesterone and endometrial biopsy can also avoid the second biopsy.

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