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# Serum Progesterone Concentration During the Luteal Phase in Women with Benign Breast Disease*

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**Abstract**—*Earlier studies of the serum concentration of progesterone during the luteal phase in women with benign breast disease have produced conflicting results. Serum progesterone profiles were therefore measured in relation to cyclical breast pain and in biopsied benign disease. No evidence of progesterone deficiency was found.*

## INTRODUCTION

THE AETIOLOGY of benign breast disease is unknown, but since the breast develops and functions under endocrine control, it is possible that some forms of benign disease may be related to an underlying hormonal abnormality. Recent reports [1–9] have suggested a luteal phase deficiency of progesterone in women with various forms of benign disease, including cyclical breast pain (mastodynia) and 'fibro-cystic disease'. This theory is attractive for several reasons. The premenstrual occurrence of cyclical mastodynia is very suggestive of a luteal phase abnormality and, indeed, some women with these symptoms benefit from progestogen supplements [10]. More important, however, is the link that progesterone deficiency would provide between benign disease and breast cancer. The 'inadequate luteal phase hypothesis' [11] has been proposed as an explanation for the increased breast cancer risk in nulliparous women [12]. The demonstration of an inadequate luteal phase and 'unopposed oestrogens' in women with benign disease, proliferative forms of which are also associated with breast cancer [13, 14], would support this hypothesis. Moreover, the identification of a treatable hormone deficiency in women with benign disease raises the possibility of breast cancer prophylaxis [15]. The implications of such a development are profound, and the subject clearly merits further scrutiny. The aim of this study therefore was to investigate the serum concentration of progesterone during the luteal phase in women with benign breast disease.

## MATERIALS AND METHODS

*Patients*

Four groups of women were studied (Table 1).

*Group A.* This group comprised 288 self-referred women who attended a breast screening clinic. Each of these women answered a detailed questionnaire, including a full history of breast symptoms, and provided one blood sample between 8.45 and 11.30 a.m. on a day calculated to be in the luteal phase of her cycle. These women (group A) were separated into those who gave a history of moderate or severe cyclical mastodynia ($n = 82$) and those with trivial discomfort or no symptoms at all ($n = 206$). From the single sample assays, progesterone profiles were derived from the women with and those without significant symptoms.

The three smaller groups, B, C and D, provided at least three luteal phase blood samples in the evening, between 4.15 and 6.30 p.m.

*Group B.* This control group comprised 14 women who gave no personal history of breast disease, no family history of breast cancer and no

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*Table* 1. *Subjects studied*

| | *n* | Age Mean | Median (yr) | Range (yr) | Blood samples | |
|---|---|---|---|---|---|---|
| Group A (screened women) | 288 | 41.9 | 44 | 22–52 | 288 | (morning) |
| Group B (control group) | 14 | 42.5 | 43 | 37–47 | 51 | (evening) |
| Group C (mastodynia patients) | 33 | 33.4 | 34 | 21–50 | 102 | (evening) |
| Group D (biopsied women) | 63* | 38.8 | 40 | 23–50 | 200 | (evening) |

*Group D includes nine women from group C, aged 31–50, mean age 35 yr, who provided 26 blood samples.

history of involuntary infertility. In addition, their breasts were normal clinically and radiologically.

*Group C.* This group comprised 33 patients with persistent severe cyclical mastodynia which was defined as pain lasting for at least 7 days before menses.

*Group D.* This group comprised 63 women—nine of them also belonging to group C—who had undergone breast biopsy for a benign condition between 3 months and 2 yr previously. These women were identified from the records of the Department of Pathology. The histology slides of each patient were reviewed by one of the authors (I.W.M.) and the microscopic findings were graded according to the presence or absence of epithelial proliferatin [16]. Each woman in group D was asked to provide one luteal phase blood sample in each of three consecutive cycles.

All subjects studied satisfied the protocol, which stipulated that all were premenopausal and that none was taking any medication likely to affect hormone concentrations, either at the time of the study or during the preceding 3 months.

Every blood sample was 'dated' according to the number of days that elapsed between venepuncture and the confirmed date of the next onset of menstruation.

Blood samples were drawn from an antecubital vein using a Sarstedt Monovette syringe, centrifuged at 4°C and the serum separated within 2 hr of venepuncture. All samples were stored at −20°C until the completion of the study when they were assayed, in duplicate, in 19 batches composed of a random selection of samples.

*Methods*

*Analytical.* Progesterone radioimmunoassay was performed according to the method described by McGinley and Casey [17], using unextracted serum. In this method, progesterone is displaced from its binding sites by the ethisterone derivative, danazol. A quantity of unknown serum (10 μl) was incubated for 20 min at 37°C in phosphate-buffered saline with tritiated progesterone (The Radiochemical Centre, Amersham, Bucks, U.K.), danazol and antibody raised in rabbit to BSA-linked progesterone. After cooling at 4°C for 40 min, unbound progesterone was removed using dextran-coated charcoal and the supernatant pipetted for counting. Non-specific binding was insignificant. The antibody was highly specific and showed no cross-reaction with danazol, which was used in a final concentration in the assay tube of 400 ng/ml. Standard curves and both low- and high-concentration quality control samples were included in every batch. All very low titre specimens were repeated using an assay volume of 25 μl, for greater sensitivity, in three batches.

Interassay variation for all 22 batches was 13.3%; overall variation was 14.6%. Titres are quoted in ng/ml.

Validation of the assay method: Since the method used here (the 'danazol method') is relatively untried, steps were taken to establish its validity.

At a preliminary stage the danazol method was compared with a method using extraction of the serum [18]. Parallel assays of 59 samples by one of the authors (D.Y.W.) showed good agreement between the two methods (10 μl assay volume: $y = 0.99x + 0.52$, $r = 0.88$; 25 μl assay volume: $y = 0.98x + 1.38$, $r = 0.88$, where $y$ = titre by conventional method, $x$ = titre by danazol method).

Further validation of the danazol method was sought at the completion of the study. Duplicate samples of 20 sera, covering high, intermediate and low titres, assayed by the danazol method and selected from among the different assay batches, were submitted to an independent reference laboratory for enzyme immunoassay, in triplicate, in one batch. The titres obtained by the two methods showed good agreement ($r = 0.96$). The results obtained in the present study are also in good agreement with those for normal women already published in the literature.

The method was therefore considered valid.

*Statistical.* The data were normally distributed. They are presented as the mean, plus or minus one standard error of the mean, of values obtained during each two-day interval before menstruation. The values within each progesterone profile showed significant quadratic regression and

profiles were therefore compared by quadratic regression on the least squares method.

## RESULTS

The progesterone assay results obtained from the women attending the breast screening clinic (group A) were initially analysed in four separate age subgroups and according to differing degrees of severity of symptoms. No differences were found between symptomatic women in any age subgroup and results have therefore been presented as two profiles, each composed of women, with or without cyclical mastodynia, drawn from all subgroups combined (Fig. 1). There is no suggestion of progesterone deficiency in the women with cyclical mastodynia; there is no significant difference between the two profiles.

The progesterone profiles of the control group (B) and the patients with persistent severe cyclical mastodynia (group C) are shown in Fig. 2. Although there is a trend towards a higher progesterone concentration during the early part of the luteal phase among the patients (group C), this is not statistically significant [$F(1, 149) = 0.44$, N.S.].

The progesterone profiles of the control group (B) and the patients who had undergone breast biopsy (group D) are shown in Fig. 3. The data from the nine patients in group D who are also represented in group C have been omitted from this profile. There is no significant difference between the progesterone concentration in these two groups.

Finally, the total of 63 women in the biopsy group were separated according to the grading of the histological appearance found in their breast biopsy specimen. Nine women were found to have fibroadenoma only and these were excluded from the further comparison. The progesterone profile

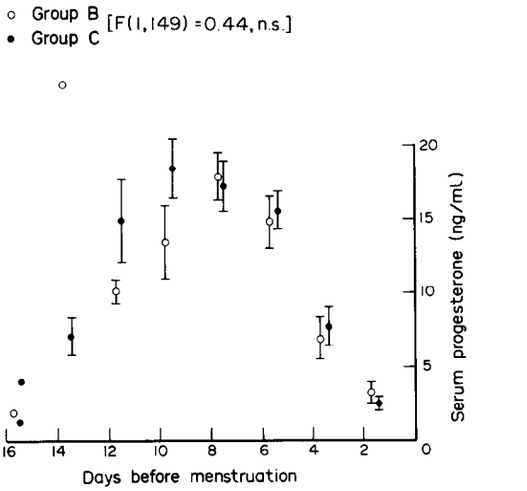


*Fig. 2. Control group and patients with severe cyclical mastodynia.*

derived from these nine patients was incomplete but otherwise unremarkable. The remaining 54 patients were separated initially according to age and varying degrees and types of epithelial proliferation. No subgroup was found to display an abnormal progesterone profile. The profiles presented here were derived from the progesterone assays of patients whose biopsies demonstrated epitheliosis ($n = 30$) or no epitheliosis ($n = 24$) (Fig. 4). There is no significant difference between these two profiles and no suggestion of progesterone deficiency among the patients whose biopsy demonstrated epitheliosis, either in comparison with the patients whose biopsy showed no epitheliosis or in comparison with the progesterone profile of the contrast group, shown in Fig. 3.

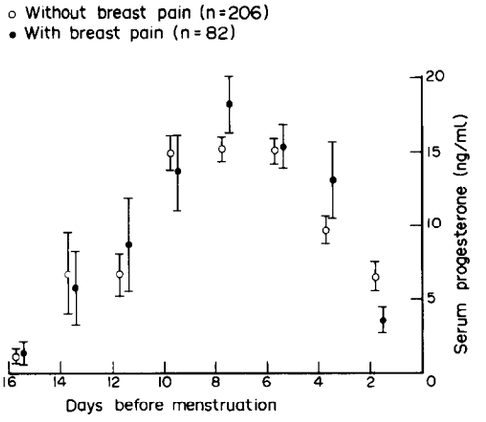


*Fig. 1. Group A: screened women with and without cyclical breast pain.*

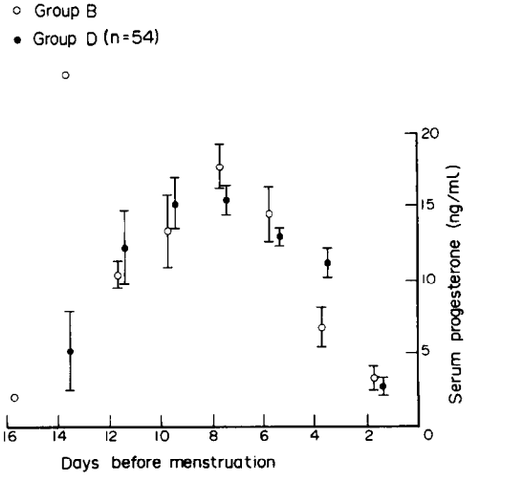


*Fig. 3. Control group and patients who have undergone breast biopsy.*

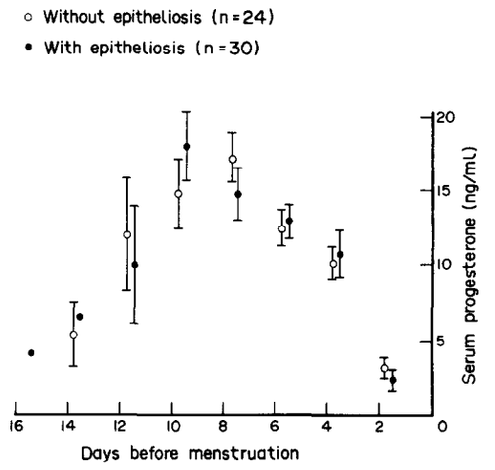


*Fig. 4. Group D: biopsied women with and without epitheliosis in biopsy specimen.*

## DISCUSSION

This study could be criticised for using only single or multiple rather than daily blood samples, since it can only be inferred that the progesterone titre from one blood sample is representative of the circulating concentration during the rest of that luteal phase. Nevertheless, it could be reasonably expected that if one group or subgroup of women persistently or frequently exhibited a subnormal concentration of progesterone during the luteal phase, it would be made apparent by the method used in this study, yet no such tendency was detected. The progesterone profiles found in all groups studied are in good agreement with the luteal-phase progesterone profiles described for normal women in previous reports [19, 20].

Most of the previously published studies of the concentration of progesterone during the luteal phase in relation to benign breast disease have failed to detect any difference between normal women and women with benign disease [19, 21, 22]. The conflict between these reports—including the present study—and the reports from Mauvais-Jarvis and his colleagues is difficult to explain. It is possible that the difference may arise from the timing ('dating') of blood samples. The present study dated blood samples with reference to the onset of menstruation subsequent to venepuncture. England and his colleagues [19], using daily blood sampling, related each sample to the mid-cycle luteinising hormone peak. These two methods have produced comparable progesterone profiles. Mauvais-Jarvis and his colleagues used the first day of the basal body temperature 'thermal plateau' as the reference point for dating each blood sample. This is an unreliable method for determining the day of ovulation and the beginning of the luteal phase, however [23]. An alternative explanation is that the patients studied by Mauvais-Jarvis and his colleagues did indeed exhibit progesterone deficiency and were therefore different from the patients with benign breast disease who have been studied elsewhere. A third possible explanation is that the patients studied by Mauvais-Jarvis and his colleagues were using progesterone supplements at the time of the study with a resultant depression of endogenous progesterone concentration.

This study found no evidence of progesterone deficiency in women with cyclically painful or recently biopsioed breast disease. While this does not preclude transient episodes of luteal deficiency at other times in the life of the women studied, the findings of this study do not lend support to the theory that benign disease is associated with a subnormal circulating concentration of progesterone.

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