

Correlation of serum progestagen-associated endometrial protein levels with endometrial biopsies serum steroid levels and therapy for luteal phase defects

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The progestagen-associated endometrial protein (PEP) level rises from the early to the late luteal phase. A study was performed in infertile women where late luteal phase endometrial biopsies and serum PEP levels were obtained. The objective of the study was to evaluate the correlation between the PEP levels and the endometrial biopsies and to determine if subnormal PEP levels could be improved by the same therapies used to correct endometrial defects. There was a poor correlation between PEP levels and endometrial biopsies ($r = 0.17$). Similarly, there was no significant correlation between PEP levels and levels of the following hormones: mid- and late-luteal phase progesterone (P) ($r = 0.186$ and 0.282 respectively), mid-luteal phase 17-hydroxyprogesterone (17-OHP) ($r = 0.139$) and mid-luteal phase oestradiol ($r = 0.135$). Furthermore, there was no correlation between PEP levels and the dosage of progesterone used in therapy ($r = 0.07$). There were no statistically significant differences in PEP values (U/ml) depending on whether any fertility drug was taken. Thus our data suggest that progesterone may only have a permissive role, with some other factor(s) controlling the actual rise and fall of PEP.

Key words: endometrial biopsies/luteal phase defects/serum PEP/serum steroid levels

Introduction

The progestagen-dependent endometrial protein (PEP) (also known as pregnancy-associated endometrial alpha₂-globulin (alpha₂-PEG), endometrial protein 15, placental protein 14 (PP14), alpha uterine protein, placenta-specific alpha₂-microglobulin) is the major endometrial secretory protein during the second half of the luteal phase of the cycle and the first trimester of pregnancy (Bell, 1988; Julkunen *et al.*, 1986c). The most dramatic increase in endometrial content occurs in the last 2-3 days of the menstrual cycle (Bell *et al.*, 1987) but has also been found to increase from the early to mid-luteal (Joshi *et al.*, 1981) and throughout the luteal phase (Julkunen *et al.*, 1986b). Immunoreactive PEP (Joshi *et al.*, 1982), placental protein 14

(Julkunen *et al.*, 1986a) and alpha₂-PEG (Bell, 1988) have been detected in sera throughout the menstrual cycle and increase from basal levels after day 21 and peak in the late luteal phase (Joshi *et al.*, 1986). Joshi *et al.* (1986) speculated that the discrepancy between the highest level of progesterone occurring during the mid-luteal phase yet the peak PEP levels being delayed to the late luteal phase may be related to an increase of progesterone-receptor in the early luteal phase, which triggers the synthesis of long-lived messenger RNAs. These RNAs would then stimulate PEP synthesis during the mid- and late-luteal phases. Furthermore, the accumulation of PEP in endometrial tissue and blood might be related to a slow turnover rate (Joshi *et al.*, 1986).

The progressive rise in PEP in the late luteal phase, similar to the advancing of the progesterone-stimulated endometrium, led to further speculation as to whether the serum PEP level could replace or improve the diagnosis of luteal phase defects (LPD), traditionally reserved for the endometrial biopsy. Indeed, there was one study which demonstrated that a greater proportion of serum PEP levels fell outside the 95% confidence limits in women with out-of-phase endometrial biopsies (Joshi *et al.*, 1986; Joshi, 1989). Furthermore, since an occasional patient was found who did not demonstrate a raised level of late luteal phase serum PEP, despite adequate corpus luteal function, this assay was considered to be a possible method for determining endometrial glandular insensitivity to progesterone (P) (Joshi *et al.*, 1986).

The study presented here was aimed at determining in a much larger series whether there is a correlation between the serum PEP and both the endometrial biopsy and the serum P level. Furthermore, if the serum PEP level was outside the 95% confidence limit, then additional P therapy would be given in the luteal phase to determine whether raising the serum P level would in turn, increase low serum PEP levels to within the 95% range of confidence limits.

Materials and methods

A total of 176 patients and 793 cycles were evaluated. In each cycle, a late luteal phase PEP level was obtained as well as mid-luteal phase serum oestradiol (E₂), P, and 17-hydroxyprogesterone (17-OHP) levels. The serum PEP levels were performed by Sharad Joshi using non-equilibrium conditions (Joshi *et al.*, 1982). Results for E₂, P, and 17-OHP were obtained using radioimmunoassay procedures from Diagnostic Products, Amersham and Pentex respectively.

An endometrial biopsy was performed, using the Pipelle endometrial suction curette (Check *et al.*, 1989a), in the initial cycle of evaluation on the same day the PEP was obtained, and dated according to the criteria of Noyes *et al.* (1950). The

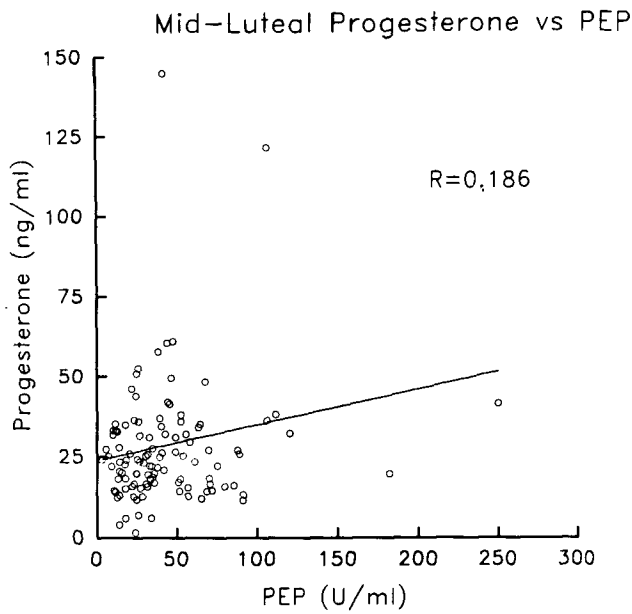


Fig. 1. Relationship between mid-luteal progesterone levels and late luteal phase PEP levels.

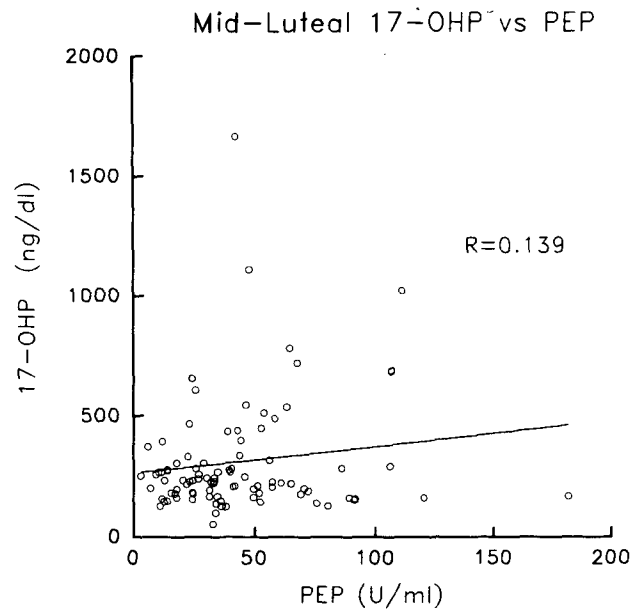


Fig. 3. Relationship between mid-luteal phase 17-OHP levels and PEP levels in the late luteal phase.

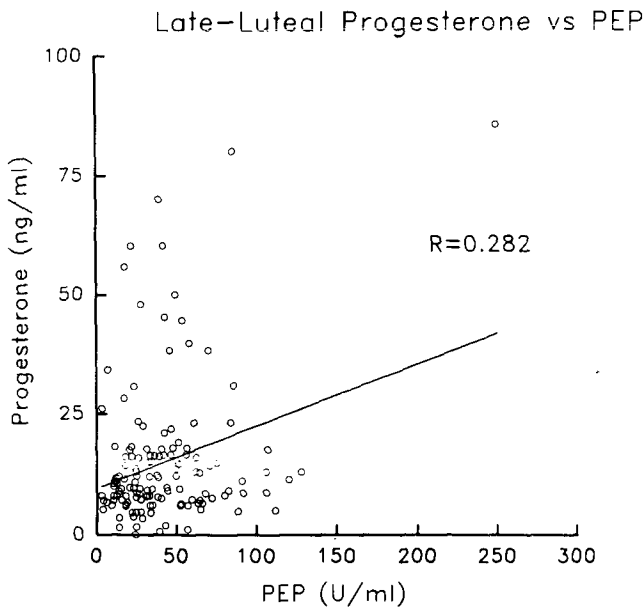


Fig. 2. Relationship between late luteal phase serum progesterone and PEP levels.

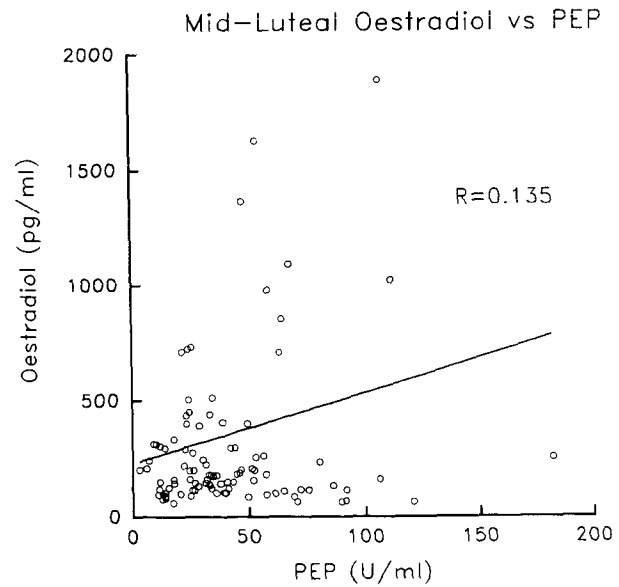


Fig. 4. Relationship between mid-luteal phase oestradiol levels and late luteal phase PEP levels.

Table I. Serum PEP levels as related to the use of ovulation-inducing drugs

Fertility drug	PEP level (U/ml)	Number of patients
None	39.6 ± 27.3*	85
Clomiphene	39.8 ± 33.7	65
HMG	51.8 ± 46.2	20
Bromocriptine	36.9 ± 8.0	6

*There were no statistically significant differences (using ANOVA) in PEP values (U/ml). Values are presented as mean ± SD.

expected dating was based on the timing of ovulation employing sonography and serum E₂ and P levels as previously described (Check *et al.*, 1988). If the biopsy was > two days out of phase,

it was repeated in the next cycle if an adjustment in therapy was made.

Only ovulatory patients with a mid-luteal phase P level

≥ 12 ng/ml were included in the study. If the follicle was not mature (maturity defined as minimum diameter of 18 mm associated with a serum oestradiol ≥ 200 pg/ml) then a follicle-maturing drug was employed, starting with clomiphene citrate and raising the dosage until follicular maturation was achieved. If hostile cervical mucus developed, the patient was switched to human menopausal gonadotrophins (HMG) (Check *et al.*, 1988). Follicular immaturity associated with hyperprolactinaemia prompted treatment with bromocriptine 2.5 mg twice daily (Check *et al.*, 1989b). If the follicle was mature, only luteal phase P support with vaginal suppositories was given (Check and Adelson, 1987).

Serum PEP levels were not obtained unless the follicle was mature. The PEP levels for a given cycle were not included if conception occurred that month. If the PEP level was outside the 95% confidence limits, then the patient was given P therapy (25 mg twice daily) after follicular collapse was demonstrated by sonography, and the dosage was increased by 25 mg each cycle. If no significant improvement was observed when 100 mg/day had been reached, then the increments were increased by 50 mg/day. If there was failure to raise the PEP level to within the 95% confidence limits by 200 mg/day of P, then follicular stimulation with clomiphene or HMG was given. Or, if hyperprolactinaemia was present, even if the follicle was mature, then bromocriptine was given.

Results

No significant correlation was found between the late luteal serum PEP level and the mid-luteal phase P level ($r = 0.186$) (Figure 1), the late luteal phase P level ($r = 0.282$) (Figure 2), the mid-luteal phase 17-OHP level ($r = 0.139$) (Figure 3) and the mid-luteal phase E_2 level ($r = 0.135$) (Figure 4).

Furthermore, no significant correlation was found between the late luteal phase PEP level and the dosage of P used in therapy (Figure 5). Similarly the use of follicle-maturing drugs failed to

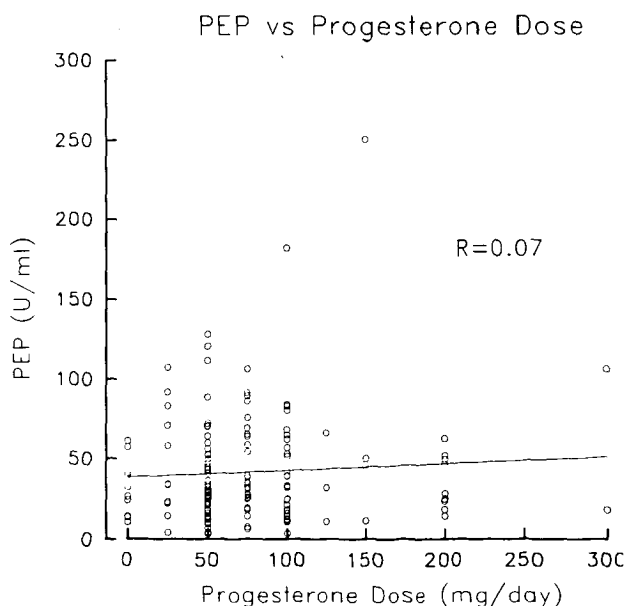


Fig. 5. Relationship between progesterone dosage (mg/day) in the luteal phase and serum PEP levels in the late luteal phase.

cause any significant differences in serum PEP levels compared to no follicle-maturing drugs (Table I). Finally, no correlation was found between late luteal phase PEP levels and the endometrial biopsies ($P = 0.127$) using the Kruskal-Wallis one-way analysis of variance. PEP values were also grouped by the number of days by which the endometrial biopsy was out of phase, the latter being a discontinuous variable and representing non-parametric values. The correlation coefficient was 0.17 by linear regression.

Discussion

Seppälä *et al.* (1987) found in five cases that oral micronized progesterone could raise the circulating level of endometrial PP14/ β -lactoglobulin homologue in the late luteal phase. Although these women were infertile, they were apparently ovulatory without known luteal phase defects. It is not apparent why we were not able to demonstrate any appreciable relationship between increasing dosages of P therapy and an increase of the serum PEP level. Since we only employed the P therapy for women with serum PEP outside the 95% confidence limit, it is possible that some other regulating factor was missing which would have provided some permissive role for P to cause a rise in the serum PEP level. There is, in fact, some evidence that oestrogen and progesterone alone, without ovaries, are not sufficient to stimulate a rise in PP14 levels, strongly suggesting that some other ovarian factor(s) besides P is needed to control PP14 or PEP levels (Critchley *et al.*, 1990). The possibility exists that increasing P causes a rise in the tissue content of PEP, but some other factor inhibits the secretion of it into the circulation. In fact, the highest secretion rates of this protein were found in first trimester pregnancy endometrium (Bell *et al.*, 1985). Furthermore, in short-term explant studies, the secretion rate of PP14 was not influenced by exogenous P, in contrast to the other endometrial proteins (Bell *et al.*, 1986).

A discrepancy between the late luteal phase endometrial biopsy, which measures for the most part the structural integrity of the stroma, and the serum PEP level is not surprising, since the latter is more a measure of the functional integrity of the glandular endometrium. Thus our data do not support the use of the late luteal phase serum PEP level as a non-invasive replacement for the endometrial biopsy. Further clinical studies are needed to determine whether a late luteal phase PEP level below the 95% confidence limit has any relationship to infertility. There are some data suggesting that purified PP14 may have immunosuppressive activity (Bolton *et al.*, 1987). However, the present data are consistent with the possibility that this protein is more important physiologically, early in the first trimester of pregnancy.

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