Circulating concentrations of placenta protein 14 during the natural menstrual cycle in women significantly reflect endometrial receptivity to implantation and pregnancy during successive assisted reproduction cycles

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Placenta protein 14 (PP14), which is the most abundant product of the secretory endometrium, has been proposed as the best biochemical marker of endometrial function in women. In this study, 19 normogonadotrophic women of infertile couples were monitored with serial measurements of concentrations of PP14, gonadotrophins and sex steroids and ultrasound scanning of endometrial thickness throughout three consecutive cycles. The first two of these were natural, unstimulated cycles (cycles 1 and 2), while ovarian stimulation with clomiphene and human menopausal gonadotrophin combined with assisted reproduction (intrauterine insemination in four cases and in-vitro fertilization in 15) was performed in the third cycle (cycle 3). A newly developed enzyme-linked immunosorbent assay was used to measure serum PP14 concentrations. In cycle 3, seven women became pregnant (group A) and 12 did not (group B). Circulating concentrations of PP14 were significantly lower in group A than in group B throughout all three cycles and in all cycle phases with exception of the late luteal phase of cycle 3, during which PP14 concentrations in group A were significantly higher than in group B. Statistical analyses showed no significant correlations between serum concentrations of PP14 and follicle stimulating hormone, luteinizing hormone and progesterone, and endometrial thickness. By contrast, serum oestradiol concentrations during the pre-ovulatory phase were significantly correlated with PP14 concentrations during the mid-luteal phase of the cycle. It is concluded that circulating PP14 is a most reliable biochemical marker of endometrial function in women and that relatively low concentrations in serum during the natural, unstimulated cycle are significantly correlated to implantation and pregnancy during successive assisted reproduction cycles. Measurement of PP14 in serum may thus be useful as a method of screening endometrial function in women, before commencing troublesome and costly treatment for infertility. However, further studies in a much larger number of women are needed to confirm this observation and to elucidate the as yet undefined physiological functions of PP14 in women.

Key words: assisted reproduction/circulating PP14/endometrial function/implantation/natural cycle

Introduction

Optimal conditions for successful implantation and establishment of pregnancy in connection with assisted reproduction in humans are still incompletely known. Previous studies have mostly focused on defining the optimal conditions for ovarian stimulation in the follicular phase and those for the gametes in vitro in relation to implantation and pregnancy. Studies that increase the knowledge of the conditions for optimal receptivity of the endometrium at the time of implantation are few in number and hampered by the difficulty in evaluating the endometrium simultaneously with the actual in-vitro fertilization (IVF) treatment. Ultrasonography studies on the thickness and echogenic appearance of the endometrium have been performed. However, the prognostic value of measurements of endometrial thickness and/or the appearance of the endometrium in conception and non-conception IVF cycles remains controversial (Gonen et al., 1989; Brooks et al., 1996; Rinaldi et al., 1996). Biochemical studies of the many proteins produced in the human endometrium and measurable in the circulation indicate that placenta protein 14 (PP14) may provide the most promising indicator of endometrial function (Chard et al., 1996). PP14, which has many synonyms, including pregnancy-associated α2-globulin (α2-PEG) and progestagen-associated endometrial protein (PEP), is the most abundant protein product of the late secretory endometrium (Wahlström et al., 1985). It appears to be exclusively a glandular marker of the endometrium (Tornehave et al., 1989; Seppälä et al., 1994) and is detected only in ‘reproductive tissues’ (Chard and Olajide, 1994). The exact biological function of this protein in relation to implantation remains to be defined, but PP14 has been found to inhibit dose-dependently sperm–zona pellucida binding in a hemi-zona assay, suggesting that it may play a role in the fertilization process (Oehninger et al., 1995). In addition, it has been demonstrated recently that primary cultures of tubal epithelial cells secrete PP14 in large amounts into the culture media, though without variations correlated to the phase of the menstrual cycle (Sarodigan et al., 1997).

In women, circulating concentrations of PP14 are lowest at mid-cycle, start to rise one week after ovulation coinciding with the peri-implantation period, and reach maximum concentrations at the onset of the menstrual period. In a conception cycle, concentrations of PP14 reach maximum within 4 weeks,
remain for 8–10 weeks, and decline thereafter in parallel with human chorionic gonadotrophin (HCG) in serum (Joshi et al., 1982).

Whether circulating PP14 concentrations during the luteal phase of the menstrual cycle predict the implantation window has been addressed in a number of studies, though these have failed to show significant differences in PP14 concentrations between conceptual and non-conceptual cycles (Edwards, 1988; Wood et al., 1990; Check et al., 1993). However, in a former study comparing serum PP14 concentrations between conceptual and non-conceptual cycles after IVF, significantly lower concentrations during the follicular phase of the cycles resulting in pregnancy were found, suggesting that conditions controlling PP14 secretion in the previous cycle may influence the likelihood of conception in the succeeding IVF cycle (Yding Andersen et al., 1992).

In the present study, 19 women seeking treatment for infertility by monitored biochemical measurement of serum concentrations of PP14, sex steroids and gonadotrophins, and ultrasonographically by measurement of endometrial thickness throughout three consecutive cycles. The first two cycles were natural, unstimulated cycles, while ovarian stimulation with clomiphene citrate and human menopausal gonadotrophin (HMG) combined with assisted reproduction either as intrauterine insemination (IUI) or IVF were performed in the third cycle. The aim of the study was to compare the biochemical and ultrasonographic parameters between unstimulated and stimulated cycles with each woman serving as her own control, and to evaluate the ability of these parameters to predict a successful/unsuccessful outcome of the treatment cycle.

Materials and methods

Patients

Nineteen infertile couples admitted to the Fertility Clinic, Odense University Hospital for treatment, were included in this study. The inclusion criteria for the women were: (i) regular menstrual cycles with intervals between 26 and 32 days; (ii) normal health and endocrinology with concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) <10 IU/l on cycle day 2; and (iii) no hormonal medication within 6 months before the start of the study. Women with abnormal endocrinology or with uterine (e.g. myoma) or ovarian (e.g. cysts) pathology were excluded from the study.

The couples included in this study were selected so as to represent tubal factor as being the sole cause of infertility in half of the cases (10 couples) and other causes in the remaining (male factor, seven couples; idiopathic infertility, two couples). Of the 10 patients with tubal infertility, two had unilateral hydrosalpinx as observed by ultrasound. No patient had endometriosis according to anamnesis and ultrasound investigations. Laparoscopy was not done routinely before treatment. The diagnosis of unexplained infertility was based on the finding of normal ovulatory function (i.e. normal ultrasound and normal basal concentrations of gonadotrophins and luteal phase progesterone), normal hysterosalpingography and normal semen quality only.

Before inclusion, all couples were informed both verbally and in writing about the aims and procedures of the study and all agreed in writing to participate. The study was approved by the Ethics Committee of the counties of Fyn and Vejle.

Circulating PP14 reflects endometrial function

### Table 1. Study design

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Cycle day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>2 8 12 14</td>
</tr>
<tr>
<td>PP14</td>
<td>x x x x</td>
</tr>
<tr>
<td>Luteinizing hormone</td>
<td>x x x x</td>
</tr>
<tr>
<td>Follicle stimulating hormone</td>
<td>x</td>
</tr>
<tr>
<td>Progesterone</td>
<td>x x x x</td>
</tr>
</tbody>
</table>

*Blood sample taken for analysis.

### Study design

The 19 women were monitored by vaginal ultrasound scans of the ovaries and uterus, and by blood sampling for hormone analysis on seven fixed cycle days throughout three consecutive cycles according to the scheme shown in Table I. This scheme was designed to cover the early follicular phase (cycle day 2), the mid-follicular phase (day 8), the pre-ovulatory phase (days 12 and 14), the mid-luteal phase (days 20 and 24) and the late luteal phase (day 28) of the cycle.

In the pre-ovulatory phase, the cycle day (either day 12 or 14) with the highest serum LH concentration was selected as the basis for the pre-ovulatory values of PP14 concentration and endometrial thickness. Likewise in the mid-luteal phase, the cycle day (either day 20 or 24) with the highest serum progesterone concentration was selected as the basis for the mid-luteal value of PP14 concentration and endometrial thickness.

### Hormonal treatment and assisted reproduction

The first two cycles were unstimulated, natural cycles. The patients were monitored with vaginal ultrasound scanning and blood sampling, as described above. In the third cycle—the assisted reproduction cycle—the women were treated with ovarian stimulation, ovulation induction and assisted reproduction.

Four couples (two idiopathic infertility, two moderate male factor infertility) were treated with clomiphene citrate (Serono Nordic, Copenhagen, Denmark), 100 mg per day on cycle days 3–7 followed by HMG (Serono) injections, 75 IU i.m. on cycle days 8 and 9 and ovulation induction with HCG (Serono) injection, 10 000 IU i.m. on the day that the diameter of the leading follicle exceeded 17 mm, followed by intrauterine insemination 36 h later.

Fifteen couples (10 tubal factor and five severe male factor infertility) were treated with IVF–embryo transfer after ovarian stimulation with clomiphene citrate (100 mg/day), cycle days 2–6 and HMG 150 IU, i.m. daily from cycle day 5 until at least four follicles with diameter >16 mm were seen. Then 10 000 IU HCG was given by i.m. injection and transvaginal ultrasound-guided oocyte retrieval performed 36 h later. A maximum of three pre-embryos was transferred back to the uterine cavity after 2–3 days in culture. Details of the procedures for IVF and embryo culture have been described previously (Westergaard et al., 1996).

### Vaginal ultrasound scanning

The ovaries and uterus were scanned ultrasonographically (Aloka Echo Camera SSD 650 scanner; 5 MHz probe) on the above seven fixed cycle days throughout three consecutive cycles. In the ovaries, the number and diameter of all follicles and size and appearance (cystic/solid) of the corpus luteum were recorded. In the uterus, the thickness and morphological appearance of the endometrium was measured and characterized according to criteria described by Gonen et al. (1989), with echo patterns being recorded in the A- (characteristic
proliferative phase), B- (characteristic pre-ovulatory phase) or C-
(characteristic luteal phase) patterns throughout all three cycles in
all patients.

Enzyme-linked immunosorbent assay (ELISA) of PP14

The rabbit anti-PP14 antiserum used for antibody purification was
identical to that described previously (Fay et al., 1988). Antibody
preparations were prepared as immunoglobulin G (IgG) fraction and
as immunospecifically purified anti-PP14.

For production of the IgG fraction, (NH4)2SO4 was added to the
monospecific rabbit anti-PP14 antiserum at the final concentration of
25% (w/v). Following incubation at room temperature for 30 min the
mixture was centrifuged at 5000 g for 15 min. The precipitate was
washed with 22% (w/v) (NH4)2SO4 and after recentrifugation was
dissolved in H2O followed by dialysis overnight against phosphate-
buffered saline (PBS), pH 7.3.

For the immunospecific purification of PP14 antibodies, purified
PP14 was coupled to CNBr-activated Sepharose (Pharmacia, Uppsala,
Sweden) in accordance with the manufacturer’s recommendations,
and this matrix was used for affinity purification of anti-PP14
antibodies. The monospecific rabbit anti-PP14 antiserum was
diluted 1:2 with PBS + 1 M NaCl, pH 7.3 and applied to the PP14–
Sepharose matrix. The column was washed to baseline with PBS
containing 0.5 M NaCl and eluted with citric acid (0.5 M), pH 2.8.

The antibody-containing fractions were identified by line immuno-
assays (Fay et al., 1988) and dialysed against PBS, pH 7.3.

Biotinylation of immunospecifically purified antibodies was perfor-
mained as follows. The antibodies were dialysed against 0.1 M
NaHCO3, pH 8.0 overnight. A stock solution of biotin succinimide
ester (BNHS; Sigma), 40 mg/ml in dimethylsulphoxide (DMSO) was
mixed gently with the antibody solution for 4 h at room temperature.
The amount of BNHS in the mixture was one-sixth of the amount of
protein, as estimated by measurement of optical density at 280 nm.

Unbound BNHS and DMSO was removed by dialysis against PBS,
pH 7.3 and NaCl3 added to a final concentration of 0.15 mM.

The ELISA was performed using Maxisorp flat bottom microtitre
plates (Nunc, Roskilde, Denmark). Coating (100 µl/well) was per-
fomed with the IgG fraction of anti-PP14 diluted to a concentration of
40 µg/ml in carbonate buffer (15 mM Na2CO3, 34.9 mM NaHCO3),
pH 9.6, overnight at 4°C. All washing procedures were performed in
PBS + 0.37 M NaCl, 0.05% Tween 20, pH 7.3. Calibrator, quality
controls and samples were diluted in PBS containing 1% normal
rabbit serum and 0.05% Tween 20 (dilution buffer) and incubated
(100 µl/well, duplicates) overnight at 4°C.

The biotinylated, affinity-purified anti-PP14 antibody (diluted to
0.3 µg/ml in dilution buffer) was added (100 µl/well) and incubated at
room temperature for 1 h. Streptavidin-labelled horseradish peroxidase
(Zymed Laboratories Inc., San Francisco, USA; 100 µl/well, diluted
1:1000) was added. After incubation (25 min at room temperature) and
washing, the H2O2-ortho-phenylene-diamine (OPD) substrate
indicator solution (0.4 µl H2O2 and 0.4 mg OPD/ml 0.1 M citrate
buffer, pH 5.0) was added (100 µl/well) followed by incubation (in the
dark at room temperature) for 15 min. The colour reaction was
stopped by addition of 150 µl of 1 M H2SO4 to each well. All
chemicals, unless otherwise stated, were purchased from Sigma, St
Louis, MO, USA.

Assays of FSH, LH, oestradiol and progesterone

FSH and LH were analysed using a Microparticle Enzyme Immuno
Assay (MIEA) technique (Imx; Abbott Laboratories, USA). The intra-
and interassay coefficients of variation for FSH and LH were
4.4, 3.6, and 7.6, 5.0%, respectively.

Table II. Characteristics of 19 women (couples) monitored with hormone
analysis and ultrasound scanning throughout three consecutive cycles of
which cycles 1 and 2 were spontaneous and cycle 3 was hormone-
stimulated for assisted reproduction treatment

<table>
<thead>
<tr>
<th>No. of women</th>
<th>Median (range) age (years)</th>
<th>Median (range) duration of infertility (years)</th>
<th>Primary infertility rate (%)</th>
<th>Median (range) menstrual cycle interval (days)</th>
<th>Cause of infertility (%)a</th>
<th>Tubal</th>
<th>Male</th>
<th>Idiopathic</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>31 (23–36)</td>
<td>4 (3–17)</td>
<td>79</td>
<td>28 (26–32)</td>
<td></td>
<td>53 (10)</td>
<td>37 (7)</td>
<td>11 (2)</td>
</tr>
</tbody>
</table>

aValues in parentheses are numbers of patients.

Oestradiol was measured by fluorimunnoassay (Auto DELFIA; Wallac Denmark A/S, Allerød, Denmark) and progesterone by chemi-
iluminescence assay (Immulight-DPC; Kingo Diagnostics, Praesø,
Denmark). The intra- and interassay coefficients of variation for oestradiol were <4.2 and 3.6% and for progesterone <5 and 10%,
respectively.

Statistical methods

Differences in serum concentrations of hormones, PP14 and endomet-
trial thickness between groups—pregnant/not pregnant; unstimulated/
stimulated cycles—were tested statistically using the Wilcoxon signed
rank sum test for non-parametric data. Spearman rank order correlation
coefficients were calculated using the Statview 4.51 package (Abacus
Concepts Inc., CA, USA). P values <0.05 were considered significant.

Results

The pretreatment characteristics of the 19 women (couples)
included in this study are shown in Table II. Two of four
women treated with IUI and five of 15 treated with IVF–
embryo transfer became pregnant in cycle 3. The distribution of
pregnancies according to cause of infertility was as follows:
idiopathic, two pregnancies and two deliveries (one singleton
and one twin); male infertility, one pregnancy and one spon-
taneous abortion (week 23); tubal infertility, four pregnancies,
three deliveries (two singleton and one twin) and one bio-
chemical pregnancy.

Validation of the PP14 ELISA

Samples of amniotic fluid were obtained from women under-
going diagnostic amniocentesis in week 16; samples of endo-
metrial extracts came from women undergoing induced abortion
during weeks 6–12. The PP14 assay was designed
using a pool (n = 65) of second trimester amniotic fluid as
calibrator. Amniotic fluid was arbitrarily assigned to concentra-
tion of 1 IU/l of PP14. The calibration curve was a dilution
series of amniotic fluid (11 steps; duplicates) ranging from
104.2 µIU/l to 3.3 µIU/l. Sera (n = 3, cycle day 28), amniotic
fluid and PP14 purified from endometrial extract revealed
parallel titration curves in the ELISA (Figure 1). Moreover,
normal male serum did not give a signal, when applied
undiluted in the assay (n = 6), indicating that the assay was
not influenced by ‘normal’ circulating antigens. Two quality
control samples with a PP14 concentration of 10 µIU/l and 80
µIU/l were included on each plate throughout the study. The

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Figure 1. Comparison of the optical density (OD) at 492 nm signal of 2-fold dilution series of amniotic fluid starting at 69.4 µIU/ml (○), PP14 purified from endometrial extracts starting at 58.9 µIU/ml (□), and sera from three patients at cycle day 28 starting at 46.6 µIU/ml (▲), 74.6 µIU/ml (●) and 116 µIU/ml (×), respectively.

Figure 2. Serum PP14 profiles (mean concentration ± SEM) in 19 women throughout three consecutive cycles of which cycles 1 and 2 were natural, unstimulated and cycle 3 a hormone-stimulated, assisted reproduction cycle. ○, pregnant, i.e. women who conceived in cycle 3; ●, not pregnant, i.e. women who did not conceive in cycle 3. EF, early follicular phase; MF, mid follicular phase; PO, pre-ovulatory phase; ML, mid-luteal phase; LL, late luteal phase.

intra- and interassay coefficients of variation were <5% (n = 20), and all samples from one patient were analysed in duplicate on the same microtitre plate.

Variations of serum PP14

Serum PP14 concentrations did not differ between IUI- and IVF-treated patients; therefore, the groups were combined. The variations of serum PP14 concentrations (mean ± SEM) throughout the three consecutive cycles and their relation to cycle phase and occurrence of pregnancy in cycle 3 are shown in Figure 2. Mean concentrations of PP-14 in the two first, unstimulated cycles (cycles 1 and 2) are compared with those in cycle 3, the hormone-stimulated, assisted reproduction cycle, in Table III.

Serum concentrations of PP14 are lower in all three cycles and in all cycle phases in the seven women who became pregnant in cycle 3 (group A) compared with the 12 women who did not (group B). The only exception to this was the late luteal phase of cycle 3, during which significantly higher concentrations of PP14 were found in group A compared with group B. Differences between group A and B were statistically significant in most cases (Table III).

Comparing PP14 concentrations in cycles 1 and 2 with cycle 3 for group A, no significant differences were found in the proliferative phases of the cycle, whereas concentrations in the mid- and late luteal phases of cycle 3 were significantly higher than those in the same phases of cycles 1 and 2. By contrast, no significant differences were found in the luteal phases between cycles 1 and 2 and cycle 3 in group B. Also, no significant difference was found between pre-pregnant concentrations of PP14 in viable and non-viable pregnancies.

Gonadotrophins and steroids

In the early follicular phase, mean concentrations of FSH were similar between groups A and B, as well as between cycles 1 and 2 and cycle 3. By contrast, LH concentrations were significantly lower in group A than in group B in cycles 1 and 2, as well as in cycle 3 (Table IV).

Progesterone concentrations were similar between groups A and B during the pre-ovulatory, mid- and late luteal phases in cycles 1 and 2, but significantly higher in group A than group B during the late luteal phase of cycle 3, when pregnancy was established in the former group. Comparing cycles 1 and 2 with cycle 3, progesterone concentrations in the pre-ovulatory, mid- and late luteal phases in groups A and B were significantly higher than those in cycles 1 and 2. The only exception was that the progesterone concentration in the late luteal phase of cycle 3 in group B was similar to that in cycles 1 and 2 (Table IV).

Concentrations of oestradiol in the pre-ovulatory phase of cycle 3 were significantly higher than those in cycles 1 and 2 in both groups A and B. Moreover, in cycle 3, oestradiol concentrations in group A were significantly lower than those in group B. In the mid-luteal phase, oestradiol concentrations were significantly higher in group A than group B in cycle 3, and significantly higher than both of those in both groups in cycles 1 and 2.

Endometrial thickness

Ultrasound measurements throughout cycles 1, 2 and 3 showed that the endometrium in group A was thicker in all three cycles and in all cycle phases than that in group B (Figure 3). However, these differences were statistically significant only
significant positive correlation was found between PP14 in the endometrial thickness (all phases) and the following: FSH (early follicular phase), LH statistically significant correlation between PP14 (any cycle phase) and serum gonadotrophins, steroids and endometrial thickness showed no conception and non-conception groups, and concentrations of serum in the different phases of the three cycles in the Statistical correlation analysis between PP14 concentrations in Correlations between serum PP14 and other parameters

Table III. Variations in serum concentrations of placenta protein 14 (PP14) through three consecutive cycles (cycles 1 and 2, unstimulated; cycle 3, hormone-stimulated, assisted reproduction) in 19 women related to cycle phase and to whether pregnancy resulted (group A, n = 7) or not (group B, n = 12) in cycle 3. PP14 concentrations are expressed in µIU; values are mean ± SEM

<table>
<thead>
<tr>
<th>Cycle phase</th>
<th>Cycles 1 and 2 (Unstimulated)</th>
<th>Cycles 3 (Assisted reproduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>No. of samplesa</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Early follicular</td>
<td>196 ± 34</td>
<td>273 ± 40</td>
</tr>
<tr>
<td>Mid-follicular</td>
<td>40 ± 3b</td>
<td>74 ± 12b</td>
</tr>
<tr>
<td>Pre-ovulatory</td>
<td>17 ± 4d</td>
<td>32 ± 4d</td>
</tr>
<tr>
<td>Mid-luteal</td>
<td>35 ± 4th</td>
<td>58 ± 7f</td>
</tr>
<tr>
<td>Late luteal</td>
<td>169 ± 32i</td>
<td>227 ± 40</td>
</tr>
</tbody>
</table>

In cycles 1 and 2 the PP14 values represent the mean of the two PP14 concentrations found in each phase in cycles 1 and 2, respectively. There were no significant differences between values in cycle 1 and cycle 2. 

<table>
<thead>
<tr>
<th>Cycle phase</th>
<th>Cycles 1 and 2 (Unstimulated)</th>
<th>Cycles 3 (Assisted reproduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>No. of samplesa</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Cycle phase</td>
<td>FSH (IU/l)</td>
<td>LH (IU/l)</td>
</tr>
<tr>
<td></td>
<td>7.7 ± 0.5</td>
<td>4.7 ± 0.4b</td>
</tr>
<tr>
<td>Early follicular</td>
<td>7.9 ± 0.4</td>
<td>6.4 ± 0.5b</td>
</tr>
<tr>
<td>Mid-luteal</td>
<td>993 ± 162</td>
<td>2.4 ± 0.3b</td>
</tr>
<tr>
<td>Late luteal</td>
<td>3.0 ± 0.5f</td>
<td>573 ± 51d</td>
</tr>
<tr>
<td>Progesterone (nmol/l)</td>
<td>44.6 ± 2.7i</td>
<td>42.9 ± 3.1k</td>
</tr>
<tr>
<td>Progesterone (nmol/l)</td>
<td>12.6 ± 3.1l</td>
<td>13.6 ± 2.7</td>
</tr>
</tbody>
</table>

In cycles 1 and 2 the PP14 values represent the mean of the two PP14 concentrations found in each phase in cycles 1 and 2, respectively. There were no significant differences between values in cycle 1 and cycle 2.

Discussion
Placenta protein 14 (PP14) represents the most abundant protein product of the human endometrium, and maximal concentrations are seen in the late luteal and early follicular phases of the menstrual cycle, as also demonstrated in this study. PP14 has been proposed as the most reliable biochemical marker of endometrial function in women (Chard and Olajide, 1994). This view is supported by the present results, which furthermore demonstrate that concentrations of PP14 in serum throughout the menstrual cycle are likely to reflect the implantation potential of the secretory endometrium. In three consecutive cycles—the first two being natural menstrual cycles and the third a hormone-stimulated, assisted reproduction cycle—

during the late luteal phase of cycle 3, during which the endometrium was significantly thicker in group A than in group B and thicker than in cycles 1 and 2 (Table V). No significant correlations were found between these echo-patterns and serum PP14 concentrations.

Correlations between serum PP14 and other parameters

Statistical correlation analysis between PP14 concentrations in serum in the different phases of the three cycles in the conception and non-conception groups, and concentrations of gonadotrophins, steroids and endometrial thickness showed no statistically significant correlation between PP14 (any cycle phase) and the following: FSH (early follicular phase), LH (early follicular, pre-ovulatory, mid- and late luteal phases), progesterone (pre-ovulatory, mid- and late luteal phases), endometrial thickness (all phases); $P > 0.05$. By contrast, a significant positive correlation was found between PP14 in the mid-luteal phase and oestradiol in the pre-ovulatory phase ($r = 0.46$; $P = 0.0005$).
Table V. Variations in endometrial thickness (mm) through three consecutive cycles (cycles 1 and 2, unstimulated; cycle 3, hormone-stimulated assisted reproduction) in 19 women related to cycle phase and to whether pregnancy resulted (group A, n = 7) or not (group B, n = 12) in cycle 3 (treatment cycle). All values are mean ± SEM

<table>
<thead>
<tr>
<th>Cycle phase</th>
<th>Group A</th>
<th>Group B</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>14</td>
<td>24</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Early follicular</td>
<td>3.7 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td>3.3 ± 0.5</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Mid-follicular</td>
<td>7.4 ± 0.6</td>
<td>6.6 ± 0.4</td>
<td>6.7 ± 0.6</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>Pre-ovulatory</td>
<td>10.9 ± 0.5</td>
<td>9.8 ± 0.4</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mid-luteal</td>
<td>12.4 ± 0.7</td>
<td>10.9 ± 0.6</td>
<td>12.9 ± 0.6</td>
<td>11.2 ± 1.2</td>
</tr>
<tr>
<td>Late luteal</td>
<td>9.6 ± 1.4d</td>
<td>9.0 ± 0.9</td>
<td>14.3 ± 1.0d</td>
<td>10.0 ± 1.3c</td>
</tr>
</tbody>
</table>

aIn cycles 1 and 2 the PP14 values represent the mean of the two PP14 concentrations found in each phase in cycles 1 and 2, respectively. There were no significant differences between values in cycle 1 and cycle 2. bNot assayed; cP < 0.05; dP < 0.05.

Figure 3. Variations of endometrial thickness (mean ± SEM) in 19 women throughout three consecutive cycles of which cycles 1 and 2 were natural, unstimulated and cycle 3 a hormone-stimulated, assisted reproduction cycle. ○, pregnant, i.e. women who conceived in cycle 3; ●, not pregnant, i.e. women did not conceive in cycle 3. Abbreviations of cycle phases are as for Figure 2.

In the present study, basal concentrations of gonadotrophins, i.e. on cycle day 2, in all women and in all three cycles were within the normal reference range, i.e. <10 IU/l, confirming the normogonadotrophic status of these women. Interestingly, however, cycle day 2 concentrations of LH in cycles 1, 2 and 3 were significantly higher in the non-conception group compared with the conception group, whereas FSH concentrations were similar in the two groups. Thus, these limited data confirm that increased basal concentrations of LH, though within normal limits, may affect fertility negatively, as reported previously in studies on much larger numbers of patients (Regan et al., 1990). It should be stressed, however, that
in this study no statistically significant correlation between concentrations of LH and PP14 was found.

In the present study, serum concentrations of progesterone and oestradiol during the follicular and luteal phases of cycles 1 and 2 were similar between the conception and non-conception groups. Not surprisingly, during cycle 3 the concentrations of both steroids were significantly increased, reflecting the effects of ovarian stimulation with multifollicular (and corpus luteum) development in both groups, and during the mid- and late luteal phases the establishment of pregnancy in the conception group.

In contrast to progesterone, oestradiol concentrations during the pre-ovulatory phase of cycle 3 were significantly correlated to whether or not pregnancy was achieved, being significantly lower in the conception than in the non-conception group. This finding confirms earlier observations in which clomiphene citrate with or without HMG was used for ovarian stimulation (Shoham et al., 1990; Yding Andersen, 1997), and indicates that excessive follicular oestradiol production resulting from ovarian stimulation may adversely affect fertility either through a more or less direct effect on the intrafollicular oocyte or indirectly via the circulation through an effect on the endometrium, probably mediated by PP14. Thus, in the present study a positive, statistically significant correlation between circulating oestradiol concentrations during the pre-ovulatory phase and PP14 concentrations during the mid-luteal phase in cycles 1, 2 and 3 was found. This finding, which confirms previous observations by Seppälä et al. (1989), emphasizes the importance of a proper pre-ovulatory follicular maturation and oestradiol priming in the follicular phase to subsequent optimal maturation of the endometrium in the luteal phase and supports the notion that the concentration of circulating PP14 in the mid-luteal phase may serve as a reliable marker of endometrial conditions that are favourable or unfavourable for implantation and establishment of pregnancy, as demonstrated by the present results.

In contrast to the above-observed correlation between oestradiol and PP14 production, and in agreement with previous reports (for review, see Chard and Olajide, 1994) no significant correlation between luteal phase concentrations of progesterone and PP14 was found in this study. This indicates that the effects of progesterone and PP14 on the endometrium may be parallel, but not necessarily interdependent.

Measurements of endometrial thickness throughout three consecutive cycles in this study revealed that women conceiving in cycle 3 had thicker endometrium in all cycle phases during the unstimulated cycles 1 and 2 than women in the non-conception group. However, this difference was not statistically significant and, during cycle 3—in which all women were treated with clomiphene citrate combined with HMG for ovarian stimulation—endometrial thicknesses were similar in the two groups. This finding, indicating that endometrial thickness during the natural, unstimulated cycle more accurately reflects endometrial function than during hormone-stimulated cycles, may offer some explanation for the conflicting results reported in the literature on the correlation between endometrial thickness and probability of implantation and pregnancy (Brooks et al., 1996; Rinaldi et al., 1996).

As the exact physiological function of PP14 is not known, an explanation of the significant correlation between relatively low concentrations of circulating PP14 and the chance of pregnancy can only be speculative. Theoretically, PP14 may affect conception/implantation at two levels: in the Fallopian tube during fertilization, and in the endometrium during implantation, either directly or indirectly. The report of Öhnineter et al. (1995) indicates that high local concentrations of PP14 in the Fallopian tube may impair the fertilization process. Conversely, low PP14 concentrations would facilitate fertilization. As demonstrated in this study, circulating concentrations of PP14 reach a nadir in the periovulatory phase of the cycle, i.e. at the time when fertilization takes place. PP14 is actually secreted in large amounts by tubal epithelial cells in vitro, but a cyclical variation in this secretion has not been demonstrated (Sarodigan et al., 1997). Logically, such an effect of PP14 would apply only to fertilization in the tube, i.e. in vivo. Also, in the present study no correlation was found between the rate of fertilization in vitro and circulating concentrations of PP14 during the pre-ovulatory phase in the patients treated with IVF. However, in IVF patients high concentrations of PP14 in the endometrium might interfere with implantation of the pre-embryo by an as yet unknown mechanism of action. It could be postulated that the local concentration of PP14 in the endometrium is dependent on the quality/thickness of the endometrium, so that an optimal and thick endometrium would consume more PP14, thus keeping concentrations low both locally and in the general circulation. Although a statistically significant correlation between circulating PP14 concentrations and endometrial thickness could not be demonstrated in the present study, the results indicate that this theory merits further investigation on a larger scale.

In conclusion, the results of this study suggest that measurement of serum PP14 during the natural, unstimulated cycle using the ELISA assay described, may for the first time permit reliable biochemical and non-invasive assessment of endometrial receptivity in women before commencing troublesome and costly treatment for infertility. However prospective studies, including a much larger number of patients than in the present study and different stimulation regimens, are required in order to define precisely threshold levels of serum PP14 with a sufficiently high predictive value. Such studies, which may furthermore elucidate some of the yet undefined physiological functions of PP14, are in progress in our department.

References


Circulating PP14 reflects endometrial function


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