Quantitative Determination of Estradiol Fatty Acid Esters in Human Pregnancy Serum and Ovarian Follicular Fluid

Veera Vihma, Herman Adlercreutz, Aila Tiitinen, Paula Kiuru, Kristiina Wåhälä, and Matti J. Tikkanen

Background: Lipophilic estradiol derivatives carried by lipoprotein particles in blood may mediate antioxidant or endocrine effects. We developed a new quantitative method to determine the concentration of circulating lipophilic estradiol fatty acid esters in human early- and late-pregnancy serum and in ovarian follicular fluid.

Methods: After extraction from serum or follicular fluid, estradiol fatty acid esters were separated from nonesterified estradiol by Sephadex LH-20 column chromatography. The estradiol ester fraction was hydrolyzed by saponification and further purified by several chromatographic steps. The hydrolyzed estradiol esters were measured by time-resolved fluoroimmunoassay.

Results: The average estradiol fatty acid ester concentration in serum increased 10-fold during pregnancy, from 40.4 pmol/L (expressed as pmol/L estradiol; range, 25.0 – 64.2 pmol/L) in early pregnancy (n = 8) to 404 pmol/L (196 – 731 pmol/L) in late pregnancy (n = 10). The ratio of estradiol ester to nonesterified estradiol remained relatively constant during pregnancy, at 0.4 – 0.6%. In 10 follicular fluid samples, the mean estradiol ester concentration was 106 nmol/L (56.9 – 262 nmol/L). Compared with serum, a greater proportion of estradiol in follicular fluid (3.0 – 10%) was in the esterified form.

Conclusion: The new method provides a means to measure circulating estradiol fatty acid ester concentrations in human pregnancy serum.

Estradiol fatty acid esters constitute a heterogeneous family of mainly unsaturated fatty acid esters at the C-17 hydroxyl (1–3). They are synthesized by various animal tissues (2, 4) and are present in several human tissues, including blood (5). Compared with free 17β-estradiol, estradiol fatty acid esters are metabolized more slowly (6, 7) and are more potent estrogens (8–10). In theory, estradiol fatty acid esters could function as a storage form of estradiol in tissues and be hydrolyzed to free hormone when needed (11). Results from in vitro studies indicate that the lipophilic estradiol fatty acid esters bind to lipoproteins in blood (12–15). Interestingly, esterification of physiologic concentrations of estradiol added to blood has been shown to protect LDL from oxidation in vitro (16).

There have been few quantitative studies on concentrations of estradiol fatty acid esters in human tissues. Endogenous estradiol esters have been detected in human blood, muscle tissue, breast cyst fluid, and, in larger amounts, in fat tissue (5). The highest concentration of estradiol esters has been detected in ovarian follicular fluid, on average ~91.8 nmol/L (25.0 µg/L expressed as µg/L estradiol) (17). The estradiol esters circulating in human blood were first described in 1983 (18), but the RIA method used was not quantitative. The same group later measured circulating estradiol ester concentrations of 47.7–91.8 pmol/L (13–25 ng/L estradiol) by a gas chromatographic-mass spectrometric method in three women whose ovaries had been stimulated by gonadotropin injections (5). In the same study, estradiol esters were detected in the blood of four cycling women, but the concentrations were too low to be quantified accurately. In men, estradiol esters were not detectable.

In the present study, we set out to measure the concentrations of estradiol esters in early- and late-pregnancy serum. Despite the increased production of estrogens, no reports have been published concerning estradiol ester concentrations during pregnancy. We also deter-
minded the estradiol ester concentrations in ovarian follicular fluid samples of 10 women undergoing gonadotropin treatment for infertility.

We developed a quantitative method for the isolation and analysis of estradiol fatty acid esters. After separation of esterified estradiol from the free steroid, hydrolysis of the esters by saponification and several chromatographic purification steps, the concentration of estradiol originating from the estradiol ester fraction was measured by time-resolved fluoroimmunoassay (TR-FIA).

**Materials and Methods**

**SUBJECTS AND SAMPLES**

Blood was obtained from 20 pregnant women. Ten early-pregnancy samples were obtained from women who had received treatment for infertility by ovulation induction at the Helsinki University Central Hospital. The blood samples were obtained at 7–10 weeks of gestation when the pregnancy was confirmed by ultrasonography. Ten late-pregnancy samples were obtained at 39–42 weeks of gestation from women with uncomplicated pregnancies. Blood was centrifuged within 1 h and stored at −20 °C until analyzed. Pooled sera from 50–60 male donors (complement inactivated at 56 °C for 30 min; serum stored at −20 °C), purchased from SPR, was used as control. Ovarian follicular fluid was obtained from 10 women undergoing ovarian stimulation for in vitro fertilization at the Helsinki University Central Hospital. Ovarian stimulation was induced by a combination of gonadotropin-releasing hormone analog and gonadotropins. Each sample consisted of fluid aspirated from several ovarian follicles of the same subject. Follicular fluid samples were centrifuged twice and stored at −80 °C. The study was approved by the Ethics Committee of the Department of Obstetrics and Gynecology, and written informed consent was obtained from patients.

**ESTROGENS AND PREPARATION OF CONTROL SAMPLES**

Nonradioactive estrogens (17β-estradiol and estradiol-17β-stearate) were purchased from Steraloids Inc. and stored at 4 °C. 3H-labeled estradiol-3,17β-dioleate was synthesized from [2,4,6,7-3H]-17β-estradiol (2.7 × 1012 Bq/mmol; NEN Lifescience Products) using an excess of oleyl chloride (99%; Sigma), dimethylaminopyridine, and dry pyridine under nitrogen at 55 °C for 20 h. The reaction was monitored with thin-layer chromatography. After the reaction was completed, the reaction mixture was acidified with 6 mol/L HCl, extracted with ethyl acetate, and washed with 100 g/L NaHCO3 and water. The crude product was purified by Sephadex LH-20 column chromatography (hexane–chloroform, 1:1 by volume) and stored at 4 °C. Before every assay the labeled estradiol-3,17β-dioleate was again purified by Sephadex LH-20 column chromatography. 3H-labeled estradiol-3,17β-dioleate was used as an internal standard in the estradiol ester quantitative method. Because the endogenous estradiol fatty acid esters are monoesters, the extraction and copurification of estradiol dioleate with estradiol-17β-stearate was examined. The diester and the monoester were extracted and eluted from the Sephadex LH-20 column [Fig. 1, Sephadex LH-20 column chromatography (I)] similarly.

To prepare the low, medium, and high control samples, we added estradiol-17β-stearate in tetrahydrofuran to the male serum pool (SPR), at concentrations corresponding to free estradiol concentrations of 110, 257, and 598 pmol/L (30, 70, and 163 ng/L), respectively. The concentration of the estradiol-17β-stearate solution was confirmed by spectrophotometry and by gas chromatography–mass spectrometry before it was added to the serum pool (19). After careful mixing, the control sera were divided to 1.1-mL fractions and stored at −20 °C.

**SOLVENTS AND REAGENTS**

Methanol, hexane, and ethyl acetate were HPLC grade, and diethyl ether was glass-distilled grade (Rathburn Chemicals Ltd). Chloroform (Uvasol) was purchased from Merck, and tetrahydrofuran from Romil Ltd. Sephadex LH-20 was purchased from Amershams Pharmacia Biotech AB, Sep-Pak C18 cartridges from Waters Corp., and Lipidex-5000 (in methanol suspension, stored at 4 °C) from Packard Instrument Company, Inc. The Sep-Pak C18 cartridges and Lipidex-5000 were washed before use. In the estradiol TR-FIA, Tris-HCl buffer, pH 7.8 [50 mmol/L Tris, 8.78 g/L NaCl, 0.5 g/L sodium azide, 5.0 g/L bovine serum albumin (Sigma), and 0.1 mL/L Tween 40 in sterile water] was used as the assay buffer. Commercial estradiol TR-FIA reagent sets were obtained from Wallac Oy.

**INSTRUMENTATION**

The estradiol TR-FIA was performed with the DELFIA Platewash, DELFIA Plateshake, and the VICTOR 1420 multilabel counter with the software version for time-resolved fluorescence measurements, all purchased from Wallac Oy. Radioactivity was measured by liquid scintillation counting (Rack-beta; Wallac Oy).

**EXTRACTION**

Serum (1 mL for late-pregnancy, 2 mL for early-pregnancy samples) or ovarian follicular fluid samples (0.015 or 0.5 mL); the low, medium, and high control samples (1 mL); and the male control serum sample (1 mL for late-pregnancy, 2 mL for early-pregnancy assays) were pipetted into disposable extraction tubes. Follicular fluid samples were diluted to a final volume of 1 mL with distilled water. [3H]estradiol-3,17β-dioleate (3500–4000 dpm in 10 μL of hexane) was added to each tube to measure recovery (Fig. 1). The samples were extracted with 2.5 volumes of diethyl ether–ethyl acetate (1:1 by volume) by mixing for 3 min. The water phase was frozen in a dry ice–ethanol mixture, and the ether phase was decanted into a glass tube. After the extraction was
repeated four times, the combined ether phases were evaporated to dryness under nitrogen in a 41 °C water bath.

**SEPARATION OF ESTERIFIED FROM NONESTERIFIED ESTRADIOL**

Sephadex LH-20 column chromatography [Fig. 1, Sephadex LH-20 column chromatography (I)] was carried out as described previously (20). Briefly, disposable Pasteur pipettes (145 mm; Volac) plugged with cotton were packed to a height of 5 cm with a suspension of Sephadex LH-20 in hexane–chloroform (1:1 by volume). After the columns were washed with fresh solvent, the dry samples were dissolved in 0.3 mL of hexane–chloroform (1:1 by volume) and applied to the columns in two 0.3-mL aliquots of the same solvent. The estradiol ester fraction was eluted with 6 mL of hexane–chloroform (1:1 by volume). The nonesterified estradiol fraction was then eluted with 5 mL of methanol. Both fractions were evaporated to dryness under nitrogen (Fig. 1). The nonesteri-
fied estradiol fraction was stored at 4°C in 1 mL of methanol.

HYDROLYSIS OF THE ESTERS
The estradiol ester fraction was saponified at 60°C for 2 h in 1 mL of 1 mol/L KOH in methanol (Fig. 1). After incubation, the samples were neutralized by the addition of 1 mL of distilled water and 0.26 mL of 4 mol/L HCl and evaporated under nitrogen until ~1 mL of the water phase was left in the tubes.

CHROMATOGRAPHIC PURIFICATION STEPS
After 10 mL of water was added, the samples were applied to prewashed Sep-Pak C18 cartridges for further purification (Fig. 1). The samples were desalted by washing the cartridges with 15 mL of water. The estradiol fraction was eluted with 6 mL of methanol and evaporated to dryness under nitrogen. Cholesterol was then removed from the samples by Lipidex-5000 reversed-phase column chromatography (21). Briefly, Pasteur pipettes (145 mm) plugged with cotton were packed to a height of 5 cm with Lipidex-5000 in a mixture of methanol–water–chloroform (8:2:2 by volume). The samples were applied to the columns in two 0.2-mL aliquots of the solvent and eluted with 4 mL of the same solvent.

After evaporation to dryness, the samples were subjected to a second Sephadex LH-20 column chromatography to remove lipophilic impurities that might interfere with the immunoassay [Fig. 1, Sephadex LH-20 chromatography (II)]. The samples were first dissolved in 0.15 mL of chloroform, and 0.15 mL of hexane was then added; after mixing, the samples were applied to the Sephadex LH-20 columns. The lipophilic impurities were removed with 6 mL of hexane–chloroform (1:1 by volume). The nonesterified estradiol remaining in the column was eluted with 5 mL of methanol.

TR-FIA
After evaporation, the samples were dissolved in 1 mL of the assay buffer and mixed carefully. Two 0.4-mL aliquots were taken for liquid scintillation counting to determine the recovery. The ovarian follicular fluid samples were diluted >100-fold with buffer for the analysis of esterified estradiol and >6000-fold for the analysis of nonesterified estradiol. Two 25-μL aliquots of the sample were pipetted into the precoated microtitration wells for duplicate determination by estradiol TR-FIA. Samples giving a value outside the range of the calibration curve were diluted with the assay buffer and the measurement was repeated.

The calibrators for TR-FIA [11.5–1836 pmol/L (3.13–500 ng/L)] were made from an estradiol stock solution in methanol by serial dilutions with the assay buffer. The concentration of estradiol in the stock solution was confirmed by spectrophotometry and by gas chromatography–mass spectrometry in the selected ion monitoring mode (19). The protocol for the quantitative assay was similar to the protocol for the commercial TR-FIA reagent set except that the estradiol antiserum and europium tracer solution were 50% more diluted.

Results

ASSAY CHARACTERISTICS
Sensitivity. The lower limit of detection of the estradiol ester method, defined as the mean + 3 SD concentration (22) of 15 determinations of 1 mL of male control serum (pooled male serum), was 44.1 pmol/L (12 ng/L). Instead of distilled water, pooled male serum was used in the determination of the detection limit to take into consideration the effect of possible interfering serum matrix components. The precision profile of the commercial estradiol TR-FIA was evaluated because of the changes made to the assay protocol. The precision profile was calculated from nine replicates of each calibrator. When a CV of 10% was taken as the discrimination limit, the working range of the quantitative TR-FIA was 55.1–1840 pmol/L (15–500 ng/L).

Imprecision. Table 1 shows the imprecision of the quantitative estradiol ester method. The within-run CVs were calculated from the determinations of the low, medium, and high control samples. No corrections for recovery or blank, defined as the value of the male serum sample with the labeled internal standard, were performed in the calculation of the within- and between-run CVs. If the results were corrected for both recovery and blank, the between-run CV of the low control sample would drop from 13% to 5.4%.

Analytical recovery and linearity. Recovery of known amounts of estradiol-17β-stearate added to male control serum samples was determined (Table 2). The results were corrected for blank and extraction recovery.

After isolation and hydrolysis of estradiol esters and after the chromatographic purification steps, ovarian follicular fluid and serum samples were diluted serially with the assay buffer and analyzed by estradiol TR-FIA. Dilution curves showed good linearity (Table 3).

<table>
<thead>
<tr>
<th>Table 1. Imprecision of estradiol ester quantitative method.</th>
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<tbody>
<tr>
<td><strong>Assay</strong></td>
</tr>
<tr>
<td><strong>Within-run</strong></td>
</tr>
<tr>
<td>Low control b</td>
</tr>
<tr>
<td>Medium control b</td>
</tr>
<tr>
<td>High control b</td>
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<tr>
<td><strong>Between-run</strong></td>
</tr>
<tr>
<td>Low control b</td>
</tr>
<tr>
<td>Medium control b</td>
</tr>
<tr>
<td>High control b</td>
</tr>
</tbody>
</table>

a Results are expressed as pmol/L estradiol.
b Low, medium, and high control samples are pooled male sera to which estradiol-17β-stearate in tetrahydrofuran has been added.
and estriol (9:91 by volume) to separate free estradiol from estrone. LH-20 column chromatography in methanol–toluene lar fluid samples were subjected to additional Sephadex cartridges (Fig. 1), three late-pregnancy and three follicu-
esters by saponification and after purification by Sep-Pak determined. After isolation and hydrolysis of the estradiol occurred when low concentrations of estradiol esters were high concentrations of free estrone and estriol in preg-
Specificity. According to manufacturer (Wallac Oy), the cross-reactivity of the antiserum of estradiol TR-FIA was low, 0.75% for estrone and 0.40% for estriol. Because of the high concentrations of free estrone in estradiol and estriol in pregnancy serum, we had to confirm that no cross-reaction occurred when low concentrations of estradiol esters were determined. After isolation and hydrolysis of the estradiol esters by saponification and after purification by Sep-Pak cartridges (Fig. 1), three late-pregnancy and three follicu-
Table 2. Analytical recovery of estradiol-17β-stearate added to male serum samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added</th>
<th>Observed</th>
<th>Recovery, %</th>
</tr>
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<tbody>
<tr>
<td>1c</td>
<td>110</td>
<td>122</td>
<td>111</td>
</tr>
<tr>
<td>2c</td>
<td>177</td>
<td>183</td>
<td>103</td>
</tr>
<tr>
<td>3d</td>
<td>257</td>
<td>272</td>
<td>106</td>
</tr>
<tr>
<td>4d</td>
<td>598</td>
<td>712</td>
<td>119</td>
</tr>
</tbody>
</table>

a Concentration of estradiol-17β-stearate is expressed as pmol/L estradiol.
b Calculated as (observed/added) × 100.
c Assayed in duplicate.
d Single determination.

Table 3. Dilution linearity of TR-FIA with follicular fluid and serum samples containing hydrolyzed estradiol fatty acid esters.

| Sample      | Dilution factor | Estradiol ester, pmol/L | % of expected
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Follicular fluid 1</td>
<td>2</td>
<td>844</td>
<td>914</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>940</td>
<td>914</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>925</td>
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</tr>
<tr>
<td></td>
<td>20</td>
<td>907</td>
<td>914</td>
</tr>
<tr>
<td>Follicular fluid 2</td>
<td>2</td>
<td>2305</td>
<td>2254</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2452</td>
<td>2254</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2258</td>
<td>2254</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2276</td>
<td>2254</td>
</tr>
<tr>
<td>Serum 1c</td>
<td>2</td>
<td>977</td>
<td>859</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1028</td>
<td>859</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>932</td>
<td>859</td>
</tr>
<tr>
<td>Serum 2d</td>
<td>2.5</td>
<td>1377</td>
<td>1465</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1402</td>
<td>1465</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1413</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>40</td>
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</tr>
<tr>
<td></td>
<td>80</td>
<td>1483</td>
<td>1465</td>
</tr>
</tbody>
</table>

a Results are expressed as pmol/L estradiol.
b Calculated as (observed/expected) × 100.
c Late-pregnancy serum (2 mL).
d Pooled male serum to which estradiol-17β-stearate has been added.

The mean concentrations of estradiol esters and nonest-
erified estradiol in early- and late-pregnancy samples and ovarian follicular fluid samples are shown in Table 4. The samples were analyzed in duplicate or triplicate except for the early-pregnancy samples, which were single determinations. The low, medium, and high control samples were analyzed in every assay. If one control measurement exceeded the ± 3 SD control limits, the assay was rejected. The results were calculated by subtracting the value of the male control serum sample considered as blank in the individual assays. The blank in the assays of the serum samples was 24.6 pmol/L (range, 19.1–32.7 pmol/L) in all but two of the late-pregnancy sample assays, which suffered from higher blanks because of impurities in the reagents. Pooled male serum was thought to contain no measurable amounts of estradiol esters because the mean value of 24.6 pmol/L was too low to be qualitatively detected by the estradiol TR-FIA. In the analysis of follicular fluid samples, distilled water instead of serum was used as the blank for the assay. The value obtained for the water blank in the assays of the follicular fluid samples was 0 pmol/L. The results were also corrected for recovery according to the labeled internal standard in the samples to take into account the losses during purification. The mean recovery was 67% (range, 58–74%).

Two of the 10 first-trimester serum samples analyzed
the ratio of estradiol ester to nonesterified estradiol, increased 10-fold between the first and third trimesters, though the mean serum concentration of estradiol ester in esterified estradiol in blood during pregnancy. Al-
hexane–chloroform.
retained in the column during the initial elution with
tive purification of the nonesterified estradiol that was
LH-20 column chromatography (II), which allowed effec-
chromatography on Sephadex LH-20 [Fig. 1, Sephadex
substances was necessary. This was achieved by a second
chromatography [Fig. 1, Sephadex LH-20 column chromatogra-
that might interfere with the assay. Large amounts of
these lipids derived from lipoproteins were present in the
organic extract of serum and were eluted in the lipophilic
estradiol ester fraction during Sephadex LH-20 chroma-
tography [Fig. 1, Sephadex LH-20 column chromatogra-
layer could anchor these molecules to the lipoprotein
theory, protect LDL against oxidation in vivo. We specu-
peroxidation in vitro. Thus, estradiol ester molecules
known to be powerful antioxidants that inhibit LDL

Discussion
We developed a reproducible method for determining
estradiol fatty acid esters in serum and ovarian follicular
fluid. Two important problems had to be solved to
achieve adequate specificity and sensitivity. The first
challenge was that it was of crucial importance to achieve
good separation of estradiol esters from nonesterified
estradiol. This was accomplished by hydrophobic chro-
matography on Sephadex LH-20, which allowed elution
of the ester fraction in the void volume while retaining
the nonesterified estradiol in the column; the nonesterified
estradiol was then eluted by switching the elution solvent
from hexane–chloroform to methanol. Adequate separa-
tion was particularly important when late-pregnancy se-
rum samples containing high concentrations of free estra-
diol and small amounts of estradiol esters were analyzed.
The second challenge was to remove lipid contaminants
that might interfere with the assay. Large amounts of
these lipids derived from lipoproteins were present in the
organic extract of serum and were eluted in the lipophilic
estradiol ester fraction during Sephadex LH-20 chroma-
tography [Fig. 1, Sephadex LH-20 column chromatogra-
philic characteristics of these molecules, they are carried exclusively in lipoprotein
particles in the bloodstream (12–15). One can only
speculate about their physiologic role, if any. Although
present in very small amounts, esterified estrogens are
known to exert powerful estrogenic effects (8–10). Fur-
ther studies are needed before an endocrine role for
 estradiol esters can be confirmed or ruled out. In theory,
estradiol esters could also serve as antioxidants protecting
the lipoprotein particles. Previous studies have indicated
that estradiol forms esters with a variety of fatty acids at
C-17 of the molecule (11). We have demonstrated by in
vitro studies that estradiol esters produced by HLDA-
associated lecithin:cholesterol acyltransferase can be
transferred to LDL in a process catalyzed by cholesteryl
ester transfer protein (24). Estrogenic substances (25–27),
including phytoestrogens (28), which contain free hy-
droxyl groups attached to aromatic ring structures, are
known to be powerful antioxidants that inhibit LDL
peroxidation in vitro. Thus, estradiol ester molecules
retaining their free aromatic 3-hydroxyl group could, in
theory, protect LDL against oxidation in vivo. We specu-
late that the fatty acid carbon chain sticking into the lipid
layer could anchor these molecules to the lipoprotein
surface. This might constitute another antiatherogenic
mechanism, explaining the low cardiovascular morbidity
in fertile women in addition to the favorable pattern of
serum lipoprotein risk factor concentrations (29, 30).

In agreement with a previous report (17), the mean
concentration of estradiol esters in ovarian follicular fluid
was high, 106 nmol/L. In contrast, the ratio of estradiol
ester to nonesterified estradiol (3.0–10%) was many times
lower than the ratio reported by others (20–165%) (17).
We analyzed pooled follicular fluid samples from the
same subject, not fluid from individual follicles, which
could partly explain the difference in the ratios. We
previously demonstrated that labeled estradiol was con-
verted to a lipophilic derivative during incubation with
follicular fluid in a process catalyzed by lecithin:choles-

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Estradiol ester</th>
<th>Nonesterified estradiol</th>
<th>Estradiol ester/estradiol ratio, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, first trimester</td>
<td>8</td>
<td>40.4 (12.1)</td>
<td>6540 (2650)</td>
<td>0.6 [0.5–0.8]</td>
</tr>
<tr>
<td>Serum, third trimester</td>
<td>10</td>
<td>404 (171)</td>
<td>113 000 (34 300)</td>
<td>0.4 [0.2–0.6]</td>
</tr>
<tr>
<td>Ovarian follicular fluid</td>
<td>10</td>
<td>106 (60.9)</td>
<td>1 900 (1 230)</td>
<td>6.0 [3.0–10]</td>
</tr>
</tbody>
</table>

* Results are expressed as the mean (SD) [range].
* Calculated as (estradiol ester/nonesterified estradiol) × 100.
* Concentration expressed as pmol/L estradiol.
* Concentration expressed as nmol/L estradiol.
terol acyltransferase (24). This derivative behaved identically to estradiol-17β-stearate in various chromatographic systems and was incorporated in HDL, the only lipoprotein present in follicular fluid.

In conclusion, our results demonstrate that estradiol esters can be quantitatively determined in early- and late-pregnancy serum. Further investigations and methodologic development are needed to determine estradiol fatty acid ester concentrations in nonpregnant females and during hormone replacement therapy and to clarify the physiologic role of these lipoprotein-bound hormone derivatives.

This work was supported financially by the Sigrid Jusélius Foundation and by the Helsinki University Central Hospital (EVO Grants TYH 0062 and TYH 0337). We thank Terhi Hakala, Aila Heikkinen, Anja Koskela, and Adile Samaletdin for technical advice and helpful discussions.

References