Assessment of the follicular cortisol:cortisone ratio

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Cortisol and cortisone concentrations in serum and follicular fluid (FF) from women undergoing in-vitro fertilization (IVF) treatment were monitored. Four groups were included: group 1, women in their natural menstrual cycle having an endogenous mid-cycle surge of gonadotrophins; group 2, women in their natural menstrual cycle receiving human chorionic gonadotrophin (HCG) for ovulation induction; group 3, women receiving exogenous gonadotrophins for ovarian stimulation and HCG for ovulation induction; and group 4, women receiving exogenous gonadotrophins for ovarian stimulation, follicles being aspirated immediately before administration of HCG. In this study, 12 follicles contained oocytes which resulted in clinical pregnancy after IVF. Cortisone concentrations were significantly higher in FF compared with that of matched serum samples, while the opposite was observed for cortisol, resulting in cortisol:cortisone ratios being significantly lower in FF compared with serum. FF from group 4 showed significantly higher cortisone concentrations than FF from each of the other three groups. FF from group 1 showed significantly higher cortisol concentrations and significantly lower cortisol:cortisone ratios in comparison with groups 2 and 3. None of the observed parameters pinpointed any of the follicles containing oocytes which resulted in a clinical pregnancy. The intrafollicular concentrations of cortisol and cortisone suggest that pre-ovulatory follicles actively convert cortisol to cortisone. Neither FF concentrations of cortisol and cortisone nor the cortisol:cortisone ratio seem to reflect implantation potential of the derived pre-embryos.

Key words: cortisol/cortisone/follicle/oocyte/ovary

Introduction

It is now well recognized that glucocorticoids affect female reproductive function by a direct effect on the ovary. Cortisol and cortisol-binding protein (CBP) are present in the follicle (Fateh et al., 1989; Yding Andersen and Hornnes, 1994; Bider et al., 1998) and the proposed mechanisms include a direct effect on granulosa cells affecting steroidogenesis and an influence on oocyte quality (Michael et al., 1993a; Michael and Cooke, 1994).

In addition, follicle cells seem to actively participate in the regulation of glucocorticoid action expressed by the ability to metabolize cortisol. Interconversion of the biological active cortisol (F) to the inactive cortisone (E) is catalysed by the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD), which is expressed in granulosa cells (Michael et al., 1993b) and in the oocyte (Benediktsson et al., 1992). The expression of 11βHSD in granulosa cell cultures has been linked to oocyte quality and pregnancy potential, since achievement of pregnancy in patients undergoing in-vitro fertilization (IVF) treatment shows a significant negative correlation with the ability of lutein-granulosa cells to convert F to E in vitro (Michael et al., 1993b, 1995). These results have, however, been questioned by a recent study (Thomas et al., 1998). At least two isoforms of 11βHSD are found in various human tissues including granulosa cells (Michael et al., 1997; Tetsuka et al., 1997); the 11βHSD type 1, which predominantly converts E to F, while the 11βHSD type 2 reverses this action by predominantly inactivating F to E (Monder and Lakshmi., 1989; Mercer et al., 1993; Michael et al., 1997). Furthermore, another study demonstrated that the expression of the two types of 11βHSD is developmentally regulated (Tetsuka et al., 1997). Non-luteinized granulosa cells obtained before the mid-cycle surge of gonadotrophins express relatively high concentrations of 11βHSD type 2 but not 11βHSD type 1. The opposite picture prevails in luteinized granulosa cells, where abundant expression of 11βHSD type 1 occurs without concomitant type 2 expression.

Taken together, there is now good circumstantial evidence to suggest that glucocorticoids acting directly on the ovary exert physiological functions in the regulation of follicular development and oocyte maturation. However, no studies have yet addressed whether any measurable effects of 11βHSD activity are reflected in the concentrations of F and E and the F:E ratio in the follicular compartment, and the intrafollicular concentration of E has not yet been determined.

The aims of the present study, therefore, were to monitor concentrations of F and E in follicular fluid (FF) and to examine how the F:E ratio relates to the available information of the expression of the two isoforms of 11βHSD. FF from women collected before the onset of the mid-cycle surge of gonadotrophins was included, as well as fluid from pre-ovulatory follicles obtained from women in their natural
menstrual cycle, who either receive human chorionic gonadotrophin (HCG) for ovulation induction or experienced a natural mid-cycle surge of gonadotrophins. In addition, FF from a group of women who underwent ovarian stimulation with exogenous gonadotrophins and received HCG for ovulation induction was also included.

Materials and methods

Follicular fluid samples

FF samples representing four different types of pre-ovulatory FF were included.

Group 1: women in their natural cycle having an endogenous mid-cycle surge of gonadotrophins to induce ovulation. A total of 11 women (aged 34 ± 1 years, range 28–37) suffering from tubal infertility received treatment in their natural menstrual cycle without administration of any exogenous gonadotrophins or medications. They were monitored by daily transvaginal ultrasound measurements from the time when the follicle measured ~14 mm in diameter until oocyte retrieval. Ovulation was predicted by measuring the luteinizing hormone (LH) surge in the urine. All patients had normal ovulatory cycles and luteal phase, based on basal body temperature curves and serum progesterone measurements. One pre-ovulatory follicle developed in each woman, and transvaginal ultrasound-guided follicle puncture resulted in the recovery of eight oocytes. Four of the oocytes fertilized, cleaved and were replaced. One woman became clinically pregnant and delivered a healthy child. FF from one follicle from each of the 11 women was included in this study.

Group 2: women in their natural cycle, where HCG was used to induce ovulation. Seven women (aged 34 ± 1 years, range 29–38) underwent 10 treatment cycles (three women underwent two cycles and four women one cycle each). They were in their natural menstrual cycle and were monitored by transvaginal ultrasound measurements from the time when the follicle measured ~14 mm in diameter until the follicle measured ≥18 mm in diameter, when ovulation was induced with HCG (10 000 IU). Infertility was caused by tubal factors (n = 5) and idiopathic infertility (n = 2). All patients had normal ovulatory cycles and luteal phase, based on basal body temperature curves and serum progesterone measurements. One oocyte was retrieved from each of the 10 treatments, seven fertilized and cleaved and reassembled into at least a 2-cell pre-embryo after 48 h culture. The seven pre-embryos were replaced and two women conceived and delivered one healthy child each.

Group 3: women after ovarian stimulation and ovulation induction with HCG. Six women (aged 32 ± 1 years, range 26–36) underwent IVF treatment after multiple follicular development was induced according to the flare-up protocol with a gonadotrophin-releasing hormone (GnRH) analogue and treatment with exogenous human menopausal gonadotrophin (HMG) (Macnamee et al., 1989; Yding Andersen et al., 1992). Ovulation was induced with HCG (10 000 IU) when at least two follicles were ≥17 mm. Infertility was caused by tubal factors. All patients had normal ovulation and luteal phase, based on basal body temperature curves and serum progesterone measurements. The women were selected from a cohort of women undergoing IVF treatment because they all became clinically pregnant. In addition, the number of pre-embryos replaced equalled the number of concepti. None of the women had any pre-embryos which were not transferred and the fate of every oocyte was known. The group included four single, one twin and one triplet clinical pregnancies. Fluid from a total of 16 follicles was studied.

Group 4: women just before they received HCG (i.e. 36 h before expected ovulation). Six women (aged 27 ± 1 years, range 25–29) with clomiphene-resistant anovulation due to polycystic ovary syndrome (PCOS) received ovarian stimulation with increasing doses of exogenous HMG (Ingerslev, 1991). When the leading follicle measured ≥17 mm in diameter all follicles ≥10 mm in diameter were aspirated the following day leaving the two or three largest follicles. HCG was then administered and the couple advised to have intercourse 24 and 48 h later. The two largest of the aspirated follicles from each woman were included in this study. No attempts were made to recover oocytes from the aspirated follicles. All the women had polycystic ovaries on ultrasound and showed elevated LH:follitell stimulating hormone (FSH) ratio with increased androgen concentrations and decreased sex-hormone binding globulin concentrations. Tubal patency was confirmed. This procedure has been shown to result in a clinical pregnancy rate of 33% per started cycle (Ingerslev, 1991).

In all cases, the FF was collected into a syringe by manual suction. Using a fresh syringe the follicle was thereafter flushed with Ham’s F-10 medium (Life Technologies, Paisley, UK). After isolation of the oocyte, each FF aspirate was immediately centrifuged at 1500 g and the supernatant stored at −20°C until required for analysis. FF in which more than one oocyte was found or FF which contained visual evidence of blood contamination were excluded. The first time an FF was thawed, it was divided into small aliquots to avoid repeated freezing and thawing.

A small serum sample was taken at the time of oocyte recovery, except in the group of women undergoing natural cycles with an endogenous mid-cycle surge of gonadotrophins. After coagulation, the serum was isolated and stored at −20°C until analysis. FF in which more than one oocyte was found or FF which contained visual evidence of blood contamination were excluded. The first time an FF was thawed, it was divided into small aliquots to avoid repeated freezing and thawing.

Measurement of steroids

Oestradiol and progesterone were measured using a commercially available direct radioimmunoassay kit (Orion, Turku, Finland). The inter-assay variation was 6–8% and results were only considered valid when two internal standards were in the range given by the manufacturer. These assays showed a good correlation with an extraction assay (Schioel and Thode, 1988). Due to the high steroid content in FF, samples were diluted 1:500 or 1:1000 in steroid-free serum prior to measurement.

Cortisol was measured in two different ways: (i) by a commercially available direct radioimmunoassay kit (Orion, Turku, Finland) that employs antibodies which according to the manufacturer’s specifications showed a low cross-reactivity to other steroids found in FF, such as 17α-hydroxyprogesterone (0.3%), oestradiol and progesterone (0.1%); and (ii) by an extraction method which was used to detect F and E after purification. A surplus of dichloromethane was used to extract F and E. The organic phase was dried and redissolved in % dichloromethane/iso-octane and F and E were purified into separate fractions on a Celite/ethylene glycol column. The purified F was measured using a commercially available radioimmunoassay kit obtained from Incstar (Sorin Biomedica, France) and the purified E samples were measured using a radioimmunoassay with specific antibodies against E, as described previously (Morineau et al., 1997). The antibody employed for measurement of E showed minimal cross-reactivity towards other steroids: F (0.42%), 17α-hydroxyprogesterone (0.05%) and progesterone (<0.01%). The intra-assay variation of the F assay was <9% and the inter-assay variation of an FF pool having an F concentration of 106 nmol/l was 15% (n = 8). The intra-assay variation of the E assay was <7% and the inter-assay variation of an
Table I. Follicular fluid oestradiol and progesterone concentrations and follicular diameter and volume. Values are shown as mean ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of follicles</th>
<th>Volume (ml)</th>
<th>Diameter (mm)</th>
<th>Oestradiol (µmol/l)</th>
<th>Progesterone (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural cycle (LH surge)</td>
<td>1 11</td>
<td>2.7 ± 0.4a</td>
<td>19.3 ± 0.5a</td>
<td>5.6 ± 0.9a</td>
<td>39.8 ± 3.7a</td>
</tr>
<tr>
<td>Natural cycle (+ HCG)</td>
<td>2 10</td>
<td>2.7 ± 0.3a</td>
<td>ND</td>
<td>6.2 ± 0.5a</td>
<td>42.9 ± 2.4a</td>
</tr>
<tr>
<td>Ovarian stimulation (+ HCG)</td>
<td>3 16</td>
<td>3.6 ± 0.3b</td>
<td>18.9 ± 0.6b</td>
<td>2.5 ± 0.2b</td>
<td>29.7 ± 2.3b</td>
</tr>
<tr>
<td>Ovarian stimulation (before HCG)</td>
<td>4 12</td>
<td>2.2 ± 0.2b</td>
<td>14.8 ± 0.5b</td>
<td>16.6 ± 1.3b</td>
<td>12.1 ± 2.4b</td>
</tr>
</tbody>
</table>

Within each column, values having a different letter are significantly different (P < 0.02).

LH = luteinizing hormone; HCG = human chorionic gonadotrophin; ND = not determined.

Table II. Concentration of cortisol (F), cortisone (E) and the F:E ratio in follicular fluid. Values are shown as mean ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of follicles</th>
<th>Cortisol (F) extraction assay (nmol/l)</th>
<th>Cortisol (F) direct assay (nmol/l)</th>
<th>Cortisone (E) extraction assay (nmol/l)</th>
<th>Cortisone (E) direct assay (nmol/l)</th>
<th>F:E ratio extraction assay</th>
<th>F:E ratio direct assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural cycle (LH surge)</td>
<td>1 11</td>
<td>55 ± 8b</td>
<td>192 ± 17b</td>
<td>52 ± 5b</td>
<td>1.3 ± 0.3b</td>
<td>4.2 ± 0.7b</td>
<td></td>
</tr>
<tr>
<td>Natural cycle (+ HCG)</td>
<td>2 10</td>
<td>104 ± 11a</td>
<td>248 ± 19a</td>
<td>39 ± 3a</td>
<td>2.9 ± 0.3a</td>
<td>7.0 ± 1.0a</td>
<td></td>
</tr>
<tr>
<td>Ovarian stimulation (+ HCG)</td>
<td>3 16</td>
<td>121 ± 8a</td>
<td>254 ± 13a</td>
<td>40 ± 3a</td>
<td>3.2 ± 0.2a</td>
<td>6.7 ± 0.3a</td>
<td></td>
</tr>
<tr>
<td>Ovarian stimulation (before HCG)</td>
<td>4 12</td>
<td>42 ± 6a</td>
<td>164 ± 22b</td>
<td>84 ± 7c</td>
<td>0.5 ± 0.1c</td>
<td>2.0 ± 0.3c</td>
<td></td>
</tr>
</tbody>
</table>

Within each column, values having a different letter are significantly different (P < 0.02).

LH = luteinizing hormone; HCG = human chorionic gonadotrophin.

FF pool having an E concentration of 31.5 nmol/l was 9% (n = 8). The observed F:E ratio showed a mean of 3.52 with an inter-assay variation of 9%.

Statistical analysis
Statistical evaluations were carried out using Student’s t-test. Results are expressed as mean ± SEM. Correlations between measured hormones were examined by least-square linear regression analysis.

Results
The FF volume and the follicular diameter were similar in women of groups 1, 2 and 3 following the mid-cycle surge of gonadotrophins or administration of HCG, whereas they were significantly lower in women before the administration of HCG (group 4) (Table I). FF concentrations of oestradiol and progesterone were similar in women in their natural menstrual cycle irrespective of whether or not HCG was given. In contrast, these figures were significantly lower in FF from women undergoing ovarian stimulation after HCG stimulation for ovulation induction. Concentrations of oestradiol were significantly higher and concentrations of progesterone significantly lower in FF obtained before administration of HCG (group 4) than after (group 3) (Table I).

FF concentrations of F and E and the F:E ratio are shown in Table II. The mean concentration of E varies from 39 to 84 nmol/l, being highest in FF obtained before administration of HCG (group 4). FF concentrations of E were similar in women who received HCG for ovulation induction irrespective of whether ovarian stimulation with exogenous gonadotrophins occurred or not, and these, in turn, were significantly lower than those of follicles from natural menstrual cycles with an endogenous mid-cycle surge of gonadotrophins.

Cortisol was measured with two different assays; the assay employing an organic extraction gave significantly lower values than the direct assay. However, results from the two assays showed a highly significant linear correlation (r = 0.96 P < 0.001), results from the direct assay being related to the extraction assay through the equation: direct = 0.94 extraction + 144. The observed differences between the groups were similar with the two assays. As for E, the concentrations of F were similar in FF from women who received HCG for ovulation induction (groups 2 and 3). These values were significantly higher than those observed in FF from women with an endogenous mid-cycle surge of gonadotrophins (group 1) and in FF obtained before the administration of HCG (group 4). F:E ratios were also similar among groups 2 and 3, whereas the F:E ratios were significantly higher (~2-fold) than those observed in FF from women in their natural menstrual cycle and ~6-fold higher (extraction assay) than in group 4 women, i.e. before the onset of the mid-cycle surge of gonadotrophins or administration of HCG (P < 0.02).

However, compared with the F:E ratios observed in serum (Table III) the FF ratios were significantly lower (P < 0.01) being only half or one third of those measured in serum. The concentration of E in serum was ~30 nmol/l, whereas the concentration of F was ~10-fold higher. The serum concentration of E was constantly and significantly lower than that observed in FF (P < 0.001).

Nine of the follicles from women in group 3 (ovarian stimulation and HCG), and two of the follicles from women in group 2 (natural menstrual cycle receiving HCG) and one follicle from women in group 1 (natural mid-cycle surge of gonadotrophins) contained pregnancy-related oocytes. Upon fertilization, pre-embryo development and replacement to the uterus they resulted in the birth of a child. However, there was no difference in concentrations of F, E, oestradiol and progesterone or any of the measured parameters for the follicles which contained an oocyte which later on implanted and those
Within each column, values having a different letter are significantly different ($P < 0.02$).

*Only one sample contained enough serum for measurements of F and E after extraction.

LH = luteinizing hormone; HCG = human chorionic gonadotrophin; ND = not determined.

Figure 1. Cortisol:cortisone ratio (F:E ratio) in individual follicular fluids from women in the four different groups. Filled circles indicate follicles which contained an oocyte which subsequent to fertilization, pre-embryo development and replacement into the uterus developed into a clinical pregnancy.

Serum concentrations of oestradiol and progesterone at the time of FF collection are also shown in Table III. Concentrations of oestradiol were significantly higher and concentrations of progesterone significantly lower in serum before administration of HCG (group 4 women) than 36 h after the injection of HCG in women undergoing ovarian stimulation with exogenous gonadotrophins (group 3). As expected, women in their natural menstrual cycle, where HCG was used to induce ovulation, exhibited a more modest concentration of both steroids (group 2).

**Discussion**

This study provides new quantitative information on the concentrations of circulating and intrafollicular F and E. The intrafollicular concentration of E in all the monitored FF samples was significantly higher than that found in the matched serum samples. The contrary was observed for F, where all FF concentrations were lower than the corresponding serum values. Consequently, the F:E ratio was significantly lower in FF, compared with the corresponding serum values. Taken together, this suggests that the pre-ovulatory follicle actively converts F to E.

Before the onset of the mid-cycle surge of gonadotrophin the follicle seems to convert F to E most effectively. Here the FF concentration of E is around twice as high as the corresponding F concentration (extraction assay) and almost twice as high as the concentration of E found in FF just before ovulation. This observation fits with the observed expression pattern of 11βHSD in human granulosa cells obtained before the onset of the mid-cycle surge of gonadotrophins. In non-luteinized follicles obtained before the onset of mid-cycle surge of gonadotrophins, a relatively high expression of the type 2 isoform is found (Tetsuka et al., 1997) which converts F to E with high affinity, whereas type 1, which predominantly converts E to F, showed low expression. Conversely, in luteinized granulosa cells, the type 1 isoform was predominantly expressed. These results suggest that conversion of F to E occurs less effectively in luteinized granulosa cells. In the present study, concentrations of E declined from before the onset of the mid-cycle surge of gonadotrophins until shortly before ovulation, matching the enzyme activities and the observed expression pattern of the two types of 11βHSD (Tetsuka et al., 1997). However, it cannot be excluded that the decrease in the concentration of E and the rise of F in FF close to follicular rupture merely reflects a dilution due to an inflow of a transudate from plasma, with a low concentration of E and a high concentration of F, occurring as a consequence of the mid-cycle surge of gonadotrophins.

Ovulation has been characterized as a controlled inflammatory reaction (Espey, 1994; Espey and Lindner, 1994). We have previously reported that the concentrations of free biological active F found in FF close to follicular rupture are very high (Yding Andersen and Hornnes, 1994); this has recently been confirmed (Harlow et al., 1997). It was suggested that these high concentrations of non-protein bound F may serve locally to reduce and confine the inflammatory reactions occurring in the pre-ovulatory follicle as it prepares for ovulation (Yding Andersen and Hornnes, 1994). The observed expression pattern of 11βHSD (Tetsuka et al., 1997) and the increased F:E ratio

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### Table III. Serum concentrations of oestradiol and progesterone, cortisol (F), cortisone (E) and the F:E ratio. Values are shown as mean ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of serum samples</th>
<th>Oestradiol (nmol/l)</th>
<th>Progesterone (nmol/l)</th>
<th>Cortisol (F) extraction assay (nmol/l)</th>
<th>Cortisol (F) direct assay (nmol/l)</th>
<th>Cortisone (E) (nmol/l)</th>
<th>F:E ratio extraction assay</th>
<th>F:E ratio direct assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural cycle (+ HCG)</td>
<td>2</td>
<td>0.21 ± 0.03</td>
<td>2.4 ± 0.2</td>
<td>271 ± 38a</td>
<td>412 ± 38a</td>
<td>28 ± 2.3a</td>
<td>9.6 ± 1.1a</td>
<td>14.8 ± 0.8a</td>
</tr>
<tr>
<td>Ovarian stimulation (+ HCG)</td>
<td>3</td>
<td>2.3 ± 0.4a</td>
<td>20.8 ± 3.3b</td>
<td>283a</td>
<td>285 ± 26</td>
<td>47b</td>
<td>6.0a</td>
<td>5.6b</td>
</tr>
<tr>
<td>Ovarian stimulation (before HCG)</td>
<td>4</td>
<td>13.7 ± 2.0b</td>
<td>11.2 ± 2.8b</td>
<td>594 ± 145b</td>
<td>712 ± 115b</td>
<td>31 ± 5a</td>
<td>20 ± 5b</td>
<td>25 ± 9b</td>
</tr>
</tbody>
</table>

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Within each column, values having a different letter are significantly different ($P < 0.02$).
in FF close to follicular rupture as observed in this study support a physiological role of F as a modulator of some of the reactions involved in ovulation.

FF concentrations of F, E and the ratio of F:E were similar in cycles in which ovarian stimulation was performed and in natural menstrual cycles where HCG was used to induce ovulation. Interestingly, however, this study shows significant differences in these values compared with FF from natural menstrual cycles in which ovulation was induced by an endogenous mid-cycle surge of gonadotrophins. Follicles exposed to an endogenous mid-cycle surge of gonadotrophins have significantly lower concentrations of F and higher concentrations of E, and consequently a more efficient conversion of F to E seems to take place. There are at least two implications of this finding: (i) a large bolus of HCG as used for ovulation induction seems to provide a supra-physiological luteinizing signal to the follicle(s) causing an increased in-flow/accumulation of F in the follicular compartment; and (ii) as previously demonstrated (Tetsuka et al., 1997), the granulosa cells change expression of 11\(\beta\)HSD from isoform type 2 to isoform type 1 as a consequence of the administration of HCG. It can therefore be speculated that the large bolus of HCG alters the expression pattern of 11\(\beta\)HSD in a way other than the natural mid-cycle surge of gonadotrophins.

Furthermore, these results may show that the physiological impact of other studies (Michael et al., 1993b, 1995; Tetsuka et al., 1997) may be masked by their use of a large bolus of HCG for ovulation induction. It was reported that patients undergoing IVF treatment only conceived provided that their menstrual cycles in which ovulation was induced by an endogenous mid-cycle surge of gonadotrophins. Follicles may therefore reflect that a proportion of the aspirated follicles contained an oocyte which was known to implant after IVF and replacement to the uterus, showed no difference from those follicles in which the oocyte failed to cleave in vitro or implant after replacement.

In this study F was monitored by a direct assay and by an assay after organic extraction and purification. The two assays were significantly correlated but the direct assay measured a surplus of F of ~144 nmol/l as indicated by the intercept on the y axis of the regression curve. This illustrates the problems of measuring F in FF, an environment with concentrations of chemically-related progestins, 3 orders of magnitude higher than that observed in circulation. Although the antibody preparations employed show only minor cross-reactivity to progestins, this could still significantly alter the F concentration measured (Dehennin, 1989), and is suggested by the present results. Indeed, the ratio of F measured by the direct non-extraction assay to the extraction assay is ~1.2–1.5 for serum samples (where sex-steroid concentrations are relatively low). In contrast, in FF this ratio varied from 2.1 to 3.9, showing that the direct assay is likely to measure higher values in a solution rich in sex-steroids. The cross-reactivity of the antibody preparation used for the direct assay towards sex-steroids like 17\(\alpha\)-hydroxyprogesterone, progesterone and oestradiol as designated by the manufacturer does in fact (due to the high concentrations of these steroids) account for ~50 nmol/l of the measured F concentration in FF. However, even serum samples from women in their natural menstrual cycle with normal concentrations of steroids showed higher values of F by the direct assay (~1.5) indicating that other factors may be of importance as well.

The serum concentrations of F and E relate well to those previously published (Morineau et al., 1997) and FF concentrations before and after administration of HCG relate well with previous reports (Yding Andersen and Hornnes, 1994; Harlow et al., 1997). However, serum concentrations of F in the group of women with PCOS (group 4) were significantly higher than in the other groups, although this was not reflected in the corresponding serum E concentrations. Furthermore, FF concentrations of F were low in this group. One previous study also found elevated serum F concentrations in PCOS women, compared with normal women (Luppa et al., 1995), whereas another failed to do so (Invitti et al., 1991), and the presence of PCOS has been linked to increased urinary excretion of cortisol metabolites (Invitti et al., 1991; Rodin et al., 1994; Luppa et al., 1995). However, it has been shown (Harlow et al., 1996) that women who experience stress in relation to the IVF procedure have elevated serum F values, and the high serum F concentrations in women with PCOS are also likely to result from extra stress experienced by patients undergoing the seldom-used procedure of having surplus follicles aspirated before administration of HCG.

In conclusion, new information on the intrafollicular concentrations of F and E and the F:E ratio is presented, suggesting that the follicle actively converts F to E. The observed concentrations of F and E relate well with the available information on the expression of 11\(\beta\)HSD before the onset of mid-cycle surge of gonadotrophins or HCG administration, whereas data are less clear after the onset of the mid-cycle surge. In addition, the present study did not allow the
pinpointing of pre-embryos which would later implant and result in a clinical pregnancy.

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References


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