Adrenal hormones in human follicular fluid

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Considerable evidence indicates that adrenal hormones may affect gonadal function. To assess the role of some adrenal hormones in human follicular fluid and their relationship with the ability of the oocyte to be fertilized and then to cleave in vitro, cortisol and dehydroepiandrosterone sulfate were measured in follicular fluid obtained at the time of oocyte recovery for in vitro fertilization from cycles stimulated by clomiphene citrate, human menopausal gonadotropin and human choriionic gonadotropin. Thirty-six follicular fluid containing mature oocyte-corona-cumulus complexes and free of visible blood contamination were included in this study. There was no significant difference in follicular fluid dehydroepiandrosterone sulfate concentration between follicles with oocytes which did or did not fertilize (5.1 ± 1.1 vs 5.8 ± 2.0 μmol/l). However, follicular fluid from follicles whose oocytes were not fertilized had levels of cortisol significantly higher than those in follicular fluid from follicles containing successfully fertilized oocytes (406.0 ± 75.9 vs 339.2 ± 37.0 nmol/l; p<0.005). No significant correlations were found between rates of embryo cleavage and cortisol and dehydroepiandrosterone levels in follicular fluid. We conclude that cortisol levels in follicular fluid may provide an index of fertilization outcome, at least in stimulated cycles by clomiphene citrate, human menopausal gonadotropin and human chorionic gonadotropin.

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Inhibition of normal ovarian function has been observed during periods of adrenal hyperactivity (1–3), but the question as to how the hypothalamic-pituitary-adrenal axis affects fertility remains to be answered. An adrenal hormone-mediated decrease in pituitary responsiveness to gonadotropin-releasing hormone and a centrally mediated inhibition of GnRlH release (4) have been suggested. However, Suter and Schwartz (5) have suggested that the adverse effects of glucocorticoids on reproduction in vivo are not exerted on the pituitary.

Another possibility is that adrenal hormones may alter normal reproductive function by acting at the ovarian level (6–9). Cortisol is the main glucocorticoid secreted by the adrenal cortex. Cortisol and cortisol-binding protein (CBP) are present in follicular fluid (FF) (10–13). It is generally accepted that the actions of glucocorticoids are mediated through specific receptors, and glucocorticoid receptors have been shown to exist in ovaries (14).

The main androgen secreted by adrenal gland is dehydroepiandrosterone sulfate (DHEA-S). More than 90% of DHEA-S is of adrenal origin. DHEA-S is present in FF (15–17) and serves as an ovarian prehormone (18, 19). Evidence of the possible importance of DHEA-S in ovarian function has recently been published (20, 21).

This study was designed to determine whether adrenal hormones play a role in follicular physiology. We correlate cortisol and DHEA-S FF levels from cycles stimulated by clomiphene citrate (CC), human menopausal gonadotropin (hMG) and human chorionic gonadotropin (hCG) with the FF levels of the gonadal steroids, estradiol (E₂), progesterone (P) and testosterone (T), and gonadotropins, LH and FSH, and with the ability of oocyte from the same follicle to be fertilized and then to cleave in vitro.

Materials and methods

Follicular fluid (N = 36) was aspirated laparoscopically from 12 women (ages 26–37 years) who were participating in our in vitro fertilization (IVF) program because of tubal infertility (22). All patients had regular monthly bleeding. Follicular development was induced with 100 mg CC (Omifin, Merrell Dow, Madrid, Spain) for five consecutive days beginning on the third day of the menstrual cycle, and hMG (Pergonal, Serono, Madrid, Spain) from the seventh day in decreasing doses (from 225 to 75 IU/day). Follicular growth was monitored by serum LH and E₂ levels and ultrasonographic development of follicles. When the serum E₂ level reached 1100 to 1500 pmol/l per follicle (> 18 mm diameter), 5000 IU hCG (Profasi, Serono, Madrid, Spain) was given, and
follicle aspiration under general anesthesia for oocyte retrieval was performed 35 h later. Ten millilitres of blood was obtained from an antecubital vein before anesthesia between 08.00 and 09.00 for cortisol and DHEA-S serum concentrations.

Oocytes were identified and immediately classified as immature or mature according to the morphologic appearance of the oocyte-corona-cumulus complex. Only FF containing mature oocyte-corona-cumulus complexes and free from visible blood contamination were used for further analysis. FF was centrifuged and the cell-free supernatant was kept frozen until assay.

After 5 h of preincubation, insemination was performed with spermatozoa at a concentration of 50–100 × 10⁶/mL. All semen samples satisfied the WHO criteria of normality (sperm counts, motility and morphology) (23) and to avoid an unknown male factor all semen samples used had fertilized at least one oocyte. Attached corona cells were removed, 15–18 h after insemination, by repeated transfer through a hand-drawn glass pipette. Fertilization was assessed microscopically by the appearance of two pronuclei and two polar bodies.

The embryo development stage was assessed by the rate of cleavage 36–40 h after insemination. The FF samples were divided into three groups according to oocyte development after IVF as follows: follicles containing non-fertilized oocytes (N = 12), follicles yielding ova that were fertilized and cleaved to less than four cells (slow cleavage rate, N = 12) and follicles yielding ova that were fertilized and cleaved to four or more cells (fast cleavage rate, N = 12).

**Hormone assays**

E₂, P and T concentrations were analyzed with commercial RIA kits (Coat-a-Count kits, Diagnostic Products Corporation, Los Angeles, CA). Respective intra- and interassay coefficients of variation were 6.8 and 9.9% for E₂, 8.0 and 8.7% for P, and 6.3 and 9.1% for T. Cortisol and DHEA-S determinations were performed with RIA (Innuchem, ICN Biomedicals, USA), as described previously (13, 24). Intra- and interassay coefficients of variation were 7.9 and 9.5% for cortisol and 8.0 and 9.1% for DHEA-S. LH and FSH concentrations were measured with commercial enzyme linked immunosorbent assay (ELISA) kits (Terumo Medical Corporation, Elkton), used according to the manufacturer’s instructions. Respective intra- and interassay coefficients of variation were 7.2 and 8.3% for LH and 6.1 and 8.9% for FSH.

Cross-reactivities of 17-hydroxyprogesterone (17-OH-P), E₂, P, T and cortisol with the DHEA-S antiserum were 0.35, <0.1, 0.85, 2.9 and <0.1% respectively. Cross-reactivities of the cortisol antiserum with 17-OH-P, E₂, P, T and DHEA-S were 0.20, <0.1, <0.1, <0.1, and <0.1% respectively.

**Table 1. Cortisol and DHEA-S concentrations in serum and follicular fluid (FF).**

<table>
<thead>
<tr>
<th></th>
<th>DHEA-S (µmol/l)</th>
<th>Cortisol (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Serum</td>
<td>(N = 12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.94–9.53</td>
<td>5.38 ± 2.70</td>
</tr>
<tr>
<td>FF</td>
<td>2.71–9.07</td>
<td>5.33 ± 1.47</td>
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</tbody>
</table>

*p < 0.002.

**Table 2. Cortisol and DHEA-S levels in follicular fluid yielding unfertilized and fertilized oocytes.**

<table>
<thead>
<tr>
<th></th>
<th>DHEA-S (µmol/l)</th>
<th>Cortisol (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Unfertilized</td>
<td>(N = 12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.98–9.07</td>
<td>5.81 ± 2.01</td>
</tr>
<tr>
<td>Fertilized</td>
<td>(N = 24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.71–8.00</td>
<td>5.08 ± 1.08</td>
</tr>
</tbody>
</table>

*Significantly lower than FF from follicles whose oocytes were not fertilized (p < 0.005).

**Table 3. Simple linear correlation coefficients between cortisol and DHEA-S concentrations, and gonadotropins and gonadal steroid hormone levels in FF (N = 36).**

<table>
<thead>
<tr>
<th></th>
<th>LH</th>
<th>FSH</th>
<th>E₂</th>
<th>P</th>
<th>T</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>-0.405*</td>
<td>-0.030</td>
<td>-0.385*</td>
<td>-0.328</td>
<td>0.373*</td>
<td>—</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>-0.164</td>
<td>-0.327</td>
<td>-0.195</td>
<td>-0.191</td>
<td>0.183</td>
<td>0.013</td>
</tr>
</tbody>
</table>

*p < 0.05.

**Statistical analyses**

All values are expressed as the mean ± SD. After multiple analysis of variance, comparisons were made with Scheffe’s and Tukey’s tests. Significance was defined as p < 0.05. Simple linear regression analysis was used to show the degree of linear association between cortisol and DHEA-S concentrations and gonadotropins and gonadal steroid hormone levels. To predict cortisol and DHEA-S levels in follicular fluid, stepwise multiple regression was used. In the stepwise multiple regression analysis, a variable was included if its partial regression coefficient was significant at the 0.05 level, and was eliminated if its partial regression coefficient failed to reach significance at the 0.10 level. Statistical analyses were performed using the BMDP statistical package (BMDP Statistical Software, Los Angeles, CA).

**Results**

Cortisol and DHEA-S levels measured by RIA in FF ranged from 287.0 to 574.1 nmol/l and 2.71 to 9.07
μmol/l, respectively. No significant differences in DHEA-S concentrations were found between serum and FF. However, the mean serum cortisol levels at the time of oocyte retrieval were significantly higher than the levels in FF (Table 1).

There was no significant difference between FF DHEA-S concentration in follicles with oocytes which did or did not fertilize (5.08 ± 1.08 vs 5.81 ± 2.01 μmol/l). FF from follicles whose oocytes were not fertilized had levels of cortisol significantly higher than those in FF from follicles containing successfully fertilized oocytes (406.0 ± 75.9 vs 339.2 ± 37.0 nmol/l, p < 0.005, Table 2). No significant correlations were found between rates of embryo cleavage and DHEA-S and cortisol levels in FF.

The relationship between cortisol and DHEA-S concentrations, and gonadotropins and gonadal steroid hormone levels in FF was examined (Table 3). We found a significant direct relationship between cortisol and T levels, and inverse correlations between cortisol and E2, and cortisol and LH. However, when we used stepwise multiple regression, only LH (coefficient: −2.068; r = 0.405; p < 0.001) was included in the model to predict cortisol levels in FF. No candidate variable was included in the model to predict DHEA-S concentrations.

Discussion

Little is known about the role of adrenal androgens in follicular development and differentiation in vivo and in vitro. The ovary has been shown to be capable of metabolizing DHEA-S to dehydroepiandrosterone (DHEA) and androstenedione in vitro (19) and in vivo (18). For DHEA-S to serve as a prehormone in the ovary, DHEA must be released from the sulfates prior to serving as a substrate for the 3β-hydroxysteroid dehydrogenase-isomerase enzyme. The enzyme responsible for hydrolyzing the sulfate from DHEA-S is steroid sulfatase, which is present within the ovary (25, 26). Other possible functions of adrenal androgens in ovarian physiology are: to adjust the insulin sensitivity of the follicle (27) or to control follicular development (20, 21). The present study shows that DHEA-S levels are similar in preovulatory follicles of women stimulated by CC/hMG/hCG regardless of fertilization outcome. We observed no relationship between FF DHEA-S and other gonadal steroid levels.

The FF DHEA-S concentrations in the present study were similar to those reported by Dehenni et al. (17), and higher than those observed by de Jong et al. (15). These discrepancies are probably due to methodological differences (17). Different authors have shown a correlation between serum DHEA-S and testosterone-free index during induction of ovulation with menotropins, before the administration of hCG (28, 29). Tracer studies in normal women during the follicular phase have shown that DHEA-S is used as an ovarian prehormone (18). We examined FF from preovulatory ovarian follicles exposed to hCG. It remains possible that after the hCG/LH surge, the ability of the human follicle to use DHEA-S as an ovarian prehormone is impaired or lost. This hypothesis rests upon the fact that sulfatase activity in the corpus luteum is significantly lower than in the follicle (26).

Cortisol in FF from human ovaries has been reported previously as one among many steroid components (11–13). Possible sources of cortisol detected in FF are local synthesis, sequestration from serum, blood contaminating the FF, or a combination of them all. Although our data do not allow speculation as to the exact source of the FF cortisol, the contribution from contaminating blood should be minimal since none of our samples was bloody. Thus, it appears likely that FF cortisol is derived from local synthesis, sequestration from serum, or both. Based on the finding that serum levels were greater than FF levels, Fateh et al. (13) suggested that FF cortisol was most likely sequestered from serum. Our results confirm this finding. However, the higher serum cortisol levels may be due to the stress of laparoscopy (30) or to rapid diffusion of the cortisol present in FF into surrounding tissues (13). Furthermore, Dehenni et al. (11) have shown the presence in the human ovarian follicle of 21-hydroxylase and 11β-hydroxylase activity, suggesting local synthesis, and corroborating the findings of Aebi et al. (31). It seems, therefore, that FF cortisol is probably derived from both mechanisms.

Regardless of the source, our results indicate that an oocyte is more likely to fertilize when the total cortisol concentration in FF is low, in agreement with other authors (13). Cortisol that is bound to CBP is not available for biological action, while those portions of the hormone that are free or albumin-bound can readily enter responsive cells. Nearly all of the cortisol in FF of preovulatory follicles is either free or albumin-bound, and therefore biologically available. The difference in the distribution of cortisol between blood and FF can be attributed to the high concentration of P in FF, which prevents the binding of cortisol to CBP (9). A protective role for CBP in the developing follicle is possible. Recently, Andersen (10) has shown that high levels of CBP in FF are associated with successful IVF. The negative effect of cortisol on the follicle may be exerted directly on the oocytes or on the process of granulosa cell differentiation (6, 7, 32). Ben-Raphael et al. (9) have shown that cortisol can directly affect granulosa cells through mechanisms other than FSH-receptor interaction.

In our study, FF cortisol was predicted statistically by a negative regression coefficient for LH concentrations. The interpretation of this relationship is unclear, but it may mean that cortisol concentrations in FF fall as luteinization progresses. However, considering the parameters investigated, we have obtained no information about the temporal changes of cortisol during follicular maturation. It would be interesting to measure levels in the smaller aspirated follicles, but it is questionable whether the content of immature follicles exposed to hCG truly reflects intrafollicular events.
In conclusion, DHEA-S levels in preovulatory FF containing mature oocytes were poor predictors of oocyte fertilizability. However, although a rationale for the inverse relationship between cortisol and the outcome of IVF is lacking, these preliminary results do raise the possibility that cortisol could have a direct action on the ovary. These findings also emphasize that differences in the hormonal milieu surrounding the oocyte may have profound effects on the ultimate success of IVF.

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