Cortisol concentrations in follicular fluid of ‘low responder’ patients

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The study was undertaken to examine any differences existing in total cortisol concentrations in the follicular fluid (FF) of pre-ruptured follicles between ‘low responder’ patients (group 1, n = 20) and ‘good responder’ patients (group 2, n = 15). The groups were defined according to how many oocytes had been retrieved during the previous in-vitro fertilization procedure (group 1: three or fewer; group 2: more than three) and total oestradiol concentration at previous in-vitro fertilization (IVF) (group 1: ≤500 pg/ml; group 2: >500 pg/ml). All patients were aged 36–43 years (group 1 mean ± SD: 36.2 ± 4.7; group 2: 32.1 ± 3.8 years) and were diagnosed with tubal or unexplained infertility. The total FF cortisol concentrations obtained in conjunction with an IVF procedure were assayed and related to oocyte fertilization. Follicular fluid was analysed for total cortisol content. Only follicles between 19 and 20 mm diameter were analysed in both groups. After aspiration of blood-free FF, total cortisol concentrations were measured by radioimmunoassay, designed for the quantitative measurement of cortisol, and related to oocyte fertilization. Total cortisol concentration in FF from fertilized oocytes was 9.7 ± 0.6 µg/ml (mean ± SD) in group 1 compared to 9.2 ± 4.4 µg/ml in group 2 (not statistically significant). Total cortisol concentrations were not associated with oocyte fertilization and no difference between the groups was found in total cortisol concentrations in the FF of unfertilized oocytes or empty follicles.

Key words: cortisol/follicular fluid/in-vitro fertilization

Introduction

The presence of cortisol and corticosteroid-binding globulin in follicular fluid (FF), as well as a large proportion of free cortisol, together with the observation that glucocorticoids possess receptors in the ovaries, demonstrates that adrenal steroids can affect ovarian function (Mahajan and Little, 1978; Schreiber et al., 1982; Fateh et al., 1989). Cortisol has been shown to stimulate aromatase activity effectively and to have a direct effect on human luteinized granulosa cells (GC) in vitro (Cleland et al., 1985; Ben-Rafael et al., 1988). A few authors (Ben-Rafael et al., 1988; Fateh et al., 1989) have demonstrated that cortisol concentrations in stimulated FF cycles were correlated with oocyte maturation and fertilization, whereas others have not (Andersen and Hornnes, 1994).

In this study, total cortisol concentrations in serum and FF were measured in ‘low’ and ‘good’ responder patients, during in-vitro fertilization (IVF) cycles. In both groups, total cortisol concentrations were assessed in relation to oocyte fertilization and overall IVF outcome. Empty follicles were also analysed.

Materials and methods

Patients with tubal or unexplained infertility who were enrolled in the IVF–embryo transfer programme were divided (according to their previous IVF cycle outcomes) into two groups: ‘low’ responders (group 1, n = 20), and ‘good’ responders (group 2, n = 15). The mean ages ± SD of patients in groups 1 and 2 were 38.2 ± 4.7 and 32.1 ± 3.8 years respectively. The low responders were defined as those who had three or fewer oocytes retrieved during their previous IVF procedure and whose total oestradiol concentrations were >500 pg/ml.

The protocol of ovulation induction comprised 3.2 mg of gonadotrophin releasing hormone analogue (GnRHa; d-TRP6, Decapeptyl depot controlled release; Ferring, Malmö, Sweden) given on day 1 of the cycle, followed 2 weeks later by 225 IU of human menopausal gonadotrophin (HMG; Teva Pharmaceuticals, Kfar Saba, Israel) per day. After serum oestradiol concentrations reached >500 pg/ml in normal responders and at least two follicles >8 mm in diameter were observed on vaginal ultrasound, 10 000 IU of human chorionic gonadotrophin (HCG, Chorigon; Teva Pharmaceuticals, Petah Tikva, Israel) was injected. Oestradiol was determined by radioimmunoassay using direct solid phase kits provided by Diagnostic Products Corp. (Los Angeles, CA, USA). The sensitivity of the oestradiol assay was 10 pg/ml and the intra- and interassay coefficients of variation were 7 and 10%, respectively. Oocytes were retrieved by ultrasonographically-guided vaginal puncture and were cultured and maintained in human tubal fluid medium (Irvine Scientific, Irvine, CA, USA), supplemented with 10% scientific serum (Irvine Scientific). The oocytes were inseminated in a concentration of 100 000 motile spermatozoa/ml. Only follicles of between 19 and 20 mm were analysed in both groups.

Thirty-five blood sera, and 48 FF samples that were entirely free of blood, were analysed after they had been frozen at −20°C and subsequently thawed. The Coat-A-Count® cortisol procedure (Diagnostic Products Corp.) was used. This is a solid-phase radioimmunoassay, wherein 125I-labelled cortisol competes for a fixed time with cortisol in the patient sample for antibody sites. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabelled cortisol. The reliability of the Coat-A-Count® cortisol procedure was assessed by examining its reproducibility on samples selected to represent a range of cortisol concentrations. Results are expressed as µg/dl. The
The measurement of cortisol concentrations in FF of patients enrolled in the study is summarized in Table I. The values were shown to be normally distributed and not significantly different in patients who had previously responded to ovulation induction inadequately (group 1), and those who were known to be ‘good’ responders (group 2). The mean ages of the patients were 36.1 ± 0.6 and 30.6 ± 1.1 years in groups 1 and 2 respectively. Mean serum cortisol concentrations were 15.8 ± 3 and 13.9 ± 0.6 respectively.

Furthermore, we had the opportunity to study three patients with an unstimulated cycle, whose mean cortisol concentration in FF was 8.2 ± 1.4 µg/ml and in serum was 11.5 ± 1.1 µg/ml. This value was lower than that found in the FF of aspirated follicles from stimulated cycles, but because of the small number of follicles analysed in the natural cycles, no comment can be made on the differences between stimulated and unstimulated cycles.

The overall oocyte fertilization and pregnancy rates were 72 and 19% respectively in the ‘low’ responders, compared to 93 and 29% in the group of ‘good’ responders.

### Discussion

The presence of cortisol and cortisol-binding globulin (CBG) in FF in humans and domestic animals (Short, 1960; Mahajan and Little, 1978) provides some evidence that adrenal steroids may influence ovarian function. Moreover, receptors to glucocorticoids in GC have been demonstrated in domestic animals, supporting the concept that cortisol plays a role in follicular development, oocyte maturation, and fertilization (Schreiber et al., 1982; Fateh et al., 1989).

The present study demonstrates that cortisol, a steroid hormone produced by the adrenals, accumulates in the FF of HMG/HCG-stimulated follicles. The cortisol concentration in serum is close to that of FF (not significantly different by Student’s t-test), suggesting that cortisol bound to CBG may have penetrated the FF from the circulation.

In this study, it was demonstrated that total cortisol concentrations did not differ in the FF of ‘low’ and ‘good’ responder patients receiving ovarian stimulation during IVF treatment. In addition, we observed the same cortisol concentrations in the FF of empty follicles. Moreover, despite the small number of patients examined, during oocyte retrieval in natural cycles their FF cortisol concentrations did not exceed those of ‘low’ or ‘good’ responders found in our study.

These observations did not support the notion that cortisol plays a major role in the pre-ruptured follicle regarding oocyte maturation and fertilization. However, from the literature, it has been shown that cortisol is essential for GC culture in vitro (Mahajan and Little, 1978; Savion et al., 1981; Orly et al., 1982), and for effective stimulation of aromatase activity in other tissues (Schreiber et al., 1982; Jimena et al., 1992).

Anovulation occurs in patients who demonstrate hyperactivity of adrenal steroids, the most common anovulatory disorder being polycystic ovarian disease (Yen, 1986). It is not clear whether cortisol has a local effect at the ovarian level. It has been shown that high concentrations of glucocorticoids may inhibit follicle stimulating hormone (FSH)-induced oestradiol production, and stimulate progesterone secretion by GC in vitro (Hsueh and Erickson, 1978). In humans, luteinized GC (stimulated by gonadotrophins), responds to cortisol by increasing oestradiol and progesterone secretion. In addition, it has been suggested that cortisol can directly affect GC through mechanisms other than FSH–receptor interaction (Cleland et al., 1985; Fateh et al., 1989).

This study, however, demonstrates that cortisol concentrations did not vary in the different groups enrolled in the IVF programme. It would appear that total cortisol accumulates in all pre-ruptured follicles, despite the fact that oocytes may or may not be present. Furthermore, it is likely that glucocorticosteroids play a role in the pre-ovulatory follicle, especially through aromatase enzyme activity, and their concentration in the FF does not signify their role in the ovarian cycle.

The observation that three patients had total cortisol concentrations that were equal in their serum and FF in unstimulated cycles provides further evidence that, in the last stages of oocyte maturation, cortisol is not essential. Other studies (Harlow et al., 1996; Kerdelhué et al., 1997) have shown a correlation between cortisol and stress conditions in IVF cycles, but no influence on the outcome was observed.

In summary, it has been shown in the literature that glucocorticoids play a role in the early stages of follicular development by their interaction with aromatase activity and GC functions. Further studies with larger numbers of patients and follicles are required, however, to confirm whether or not

<table>
<thead>
<tr>
<th>Table I</th>
<th>Total cortisol levels in follicular fluid of ‘low’ and ‘good’ responder patients</th>
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<tbody>
<tr>
<td>Follicular fluid total cortisol concentration (µg/ml)</td>
<td>Low responders</td>
</tr>
<tr>
<td>n</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
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<tr>
<td>Fertilized oocytes</td>
<td>18</td>
</tr>
<tr>
<td>Unfertilized oocytes</td>
<td>4</td>
</tr>
<tr>
<td>Empty follicles</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
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</table>

There were no significant differences.
glucocorticoids play a major role in the development of the pre-ruptured follicle, or in the final stage of oocyte maturation or fertilization, as has been suggested by the observation in this study that no major differences existed in total cortisol concentrations in FF from low and good responder patients.

References


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