Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF

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BACKGROUND: The aim of this study was to investigate the effect of treatment with dehydroepiandrosterone (DHEA) on fertility outcomes among women with diminished ovarian reserve. MATERIALS AND METHODS: This is a case–control study in an academically affiliated private infertility centre. Twenty-five women with significantly diminished ovarian reserve had one IVF cycle before and after DHEA treatment, with otherwise identical hormonal stimulation. Women received 75 mg of DHEA daily (25 mg three times daily) for an average of 17.6 ± 2.13 weeks. We performed a comparison of IVF outcome parameters, before and after DHEA treatment, including peak estradiol (E2) levels, oocyte and embryo numbers, oocyte and embryo quality and embryo transfer statistics. RESULTS: Paired analysis of IVF cycle outcomes in 25 patients, who underwent cycles both before and after DHEA supplementation, demonstrated significant increases in fertilized oocytes (P < 0.001), normal day 3 embryos (P = 0.001), embryos transferred (P = 0.005) and average embryo scores per oocyte (P < 0.001) after DHEA treatment. CONCLUSION: This study confirms the previously reported beneficial effects of DHEA supplementation on ovarian function in women with diminished ovarian reserve.

Key words: dehydroepiandrosterone/embryo score/IVF/ovarian reserve/polycystic ovary syndrome

Introduction

The ability of women to respond to ovulation-inducing medications declines with age (Navot et al., 1991; Scott et al., 1993; Scott and Hofmann, 1995; Scott, 1996). With IVF cycles, older women produce few oocytes (Chuang et al., 2003; Orvieto et al., 2004) and yield few normal embryos (Terriou et al., 2001; Ziebe et al., 2001) when exposed to maximal gonadotrophin stimulation (Scott, 1996; Karande et al., 1997; Schoolcraft et al., 1997; Surrey et al., 1998; Orvieto et al., 2004). This change in ovarian responsiveness is known as diminished ovarian reserve (Toner and Flood, 1993). A long-standing dogma of fertility care has been that the ageing process of ovaries could not be affected. Indeed, the irreversibility of decreased ovarian reserve has been one of the most cherished caveats of modern infertility care.

We recently have challenged this caveat after having had the unique opportunity to observe an older woman, with overwhelming evidence of greatly decreased ovarian reserve. This woman succeeded to rejuvenate her ovarian function to an extraordinary degree after prolonged treatment with dehydroepiandrosterone (DHEA). This patient, recently reported elsewhere in detail (Barad and Gleicher, 2005), over 9 months of consecutive treatment, increased her oocyte yield from one, before DHEA use, to a peak of 18 oocytes. This extraordinary response to DHEA treatment led us to an extended investigation of this drug in women with proven decrease in ovarian reserve.

The aim was to compare IVF treatment outcomes between pre- and post-treatment cycles among a cohort of women with known decreased ovarian reserve, using DHEA supplementation.

Materials and methods

All 25 women included in this study had convincing evidence of diminished ovarian reserve. Most of these patients presented after repeated IVF failures at other centres that were attributed to diminished ovarian reserve. A majority reported a prior recommendation to become donor oocyte recipients. They all had experienced a prior IVF cycle, with age-appropriate ovarian stimulation, and had produced less than four oocytes and uniformly poor embryo quality, defined as slow dividing embryos, with none having exceeded 4-cell stage on day 3 after retrieval or no embryo beyond grade 3 and abnormal baseline ovarian function tests, defined as FSH levels >10 mIU/ml or estradiol (E2) levels >75 pg/ml (275.3 pmol/l). The upper limit of baseline FSH to enter an ovulation induction cycle, after DHEA treatment, in our centre was 40 mIU/ml.

Patients who agreed to be studied as part of this case-series signed an informed consent. This study was reviewed and approved by our institutional review board.
Patients who agreed to participate in the study began taking micronized DHEA by prescription, compounded by a single pharmacy, 25 mg orally, three times a day. Because our prior reported experience suggests that DHEA achieves maximal effectiveness on ovarian function after approximately 4 months’ use (Barad and Gleicher, 2005), if clinically possible, patients received approximately 16 weeks of DHEA treatment before any post-treatment IVF cycle.

The patients experienced both their pre- and post-DHEA treatment IVF cycles at our centre. Patients who did conceive were taken off DHEA supplementation once a normal rise in pregnancy hormone was observed over two measurements. The previously reported patient (Barad and Gleicher, 2005) was excluded from the present analysis.

Ovulation induction was accomplished using norethindrone acetate tablets (10 mg) for 10 days, starting on day 2 of menses, followed 3 days later by 50 μg of leuprolide acetate, twice daily, and, after another 3 days, by 450 IU of recombinant FSH and 150 IU of HMG. When at least two lead follicle diameters reached 18 mm, follicular maturation was triggered with the injection of 10 000 IU HCG, with oocyte retrieval taking place 34–35 h later. The same hormonal stimulation was used both before and after DHEA treatment.

The variables analysed for all IVF cycles included peak E2 levels, total number of oocytes retrieved, embryo numbers and average cumulative embryo scores, rate of cycle cancellations, positive pregnancy tests and ongoing clinical pregnancies. A positive pregnancy test was defined as a serum hCG level of >25 IU/l, at ≥14 days post embryo transfer.

Fertilization was determined by the presence of two pronuclei on the first day after insemination. We considered embryos with equal to, or greater than, four cells on day 3 to be normal. Cell counts and embryo grades were determined on the third day of incubation. Embryos were graded from one to five, based on per cent fragmentation and expected cell counts: grade 5 equal-sized symmetrical blastomeres with no fragmentation; grade 4 equal-sized symmetrical blastomeres with <10% fragmentation; grade 3, uneven blastomeres with <10% fragmentation; grade 2, 10-50% fragmentation; and grade 1, >50% fragmentation. For embryos that ceased growth on day 2 of incubation, the day 2 grade and cell counts were carried forward to day 3. Oocytes that did not fertilize were counted as single cells and the grade was carried forward from scoring at the day of retrieval.

Cumulative embryo scores were calculated by multiplying the cell number and grade of each embryo, on its day 3 of development, and then summing the scores for embryos produced by each patient in each cycle of treatment (Steer et al., 1992). For example, an 8-cell grade 4 embryo would be assigned a score of 32. Average cumulative embryo scores were calculated by dividing the cumulative embryo score by the total number of oocytes retrieved. Assays of E2 and FSH were performed using the Automated Chemiluminescence System (ACS: 180® Bayer Health Care LLC, Tarrytown, NY, USA).

Analysis involved the paired evaluation of 25 study subjects who underwent both pre- and post-DHEA IVF cycles. In these patients, outcomes of cycles were compared between pre- and post-DHEA IVF cycles by univariate testing, with paired t-test.

All statistical calculations were performed using SPSS for Windows, Standard version 10.0.7 (SPSS Co., Chicago, IL, USA). Outcomes are presented as mean ± 1 SE.

Results

The 25 women in this study used DHEA for an average of 16 weeks before entry into their IVF cycles. The cycle day 2 FSH levels in these patients ranged between 3 and 20 mIU/ml. In all cases, patients with baseline FSH <10 mIU/ml had baseline E2 >75 pg/ml.

Paired IVF cycle characteristics of the 25 patients, before and after DHEA treatment, are summarized in Table I. After treatment with DHEA, patients produced an average of 1.04 ± 0.46 more oocytes (P < 0.05), had higher fertilization rates (P < 0.001), had higher average day 3 blastomere counts (P = 0.01) and had higher grade embryos on day 3 (P = 0.02). The average cumulative embryo score per oocyte retrieved was significantly higher among patients after DHEA treatment (P < 0.001) (Table I).

The cycle cancellation rate was 8/25 cycles (32%) in pre-treatment and 1/25 (4%) in post-DHEA treatment (P = 0.02). There was a trend towards higher peak E2 levels in the post-DHEA treatment cycles, but it failed to reach significance. Paired comparison of fertilized oocytes and normal day 3 embryo count are shown in Figure 1.

The medication was well tolerated by all patients. No patient dropped out of treatment because of side effects attributed to DHEA use. Some patients reported increased sebum production and a few developed transitional acne. No patient reported hirsutism. Several patients reported an improved feeling of well-being and two reported increased libido while using DHEA.

Discussion

This study confirms that DHEA treatment increases oocyte and embryo numbers. The new observation that DHEA treatment increased the fertilization rate, day 3 blastomere counts, embryo grade and average cumulative embryo scores per oocyte retrieved allows us to infer that DHEA may lead to improved embryo quality.

### Table I. Comparison of results of IVF before and after treatment with dehydroepiandrosterone (DHEA)

<table>
<thead>
<tr>
<th></th>
<th>Pre-DHEA</th>
<th>Post-DHEA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.9 ± 0.8</td>
<td>40.4 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Weeks of DHEA</td>
<td>–</td>
<td>17.6 ± 2.13</td>
<td></td>
</tr>
<tr>
<td>Cancellation</td>
<td>8/25 (32%)</td>
<td>1/25 (4.3%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Peak estradiol (pmol/l)</td>
<td>3493 ± 512</td>
<td>4065 ± 589</td>
<td>Not significant</td>
</tr>
<tr>
<td>Oocytes</td>
<td>3.4 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fertilized oocytes</td>
<td>1.4 ± 0.3</td>
<td>3.0 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage of fertilized oocytes</td>
<td>39</td>
<td>67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day 3 embryo blastomeres</td>
<td>3.4 ± 0.4</td>
<td>4.7 ± 0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Day 3 embryo grade</td>
<td>2.9 ± 0.1</td>
<td>3.4 ± 0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Cumulative embryo score per oocyte retrieved</td>
<td>8.4 ± 1.5</td>
<td>16.1 ± 1.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Transferred embryos</td>
<td>1.4 ± 0.2</td>
<td>2.4 ± 0.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Normal day 3 embryos</td>
<td>1.2 ± 0.2</td>
<td>2.7 ± 0.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>
All of these observations, of course, raise the question, how DHEA might effect these outcome improvements? A number of different pieces of circumstantial evidence may point into certain directions and allow us to speculate on how DHEA exerts its effect(s): a first reasonable assumption is that DHEA, based on the two-cell-2-hormone theory (McNatty et al., 1979), is an essential substrate for steroidogenesis. If DHEA is abnormally low, substrate for the production of androstenedione, testosterone and E2 is lacking and these hormones can be expected to be low.

Androgens can, however, influence ovarian follicular growth not only by acting as metabolic precursors for steroid production (Dorrington et al., 1975) but also by serving as ligands for androgen receptors (Hillier et al., 1994) or by other non-classical mechanisms. During ovulation induction with exogenous gonadotrophins, DHEA is the prehormone for up to 48% of follicular fluid testosterone (Haning et al., 1993), which, of course, is the prehormone for E2. Androgens act, together with FSH, to stimulate follicular differentiation (Hillier, 1985; Daniel and Armstrong, 1986; Gore-Langton and Armstrong, 1988). Androgens are also known to promote steroidogenesis (Harlow et al., 1986), to promote follicular recruitment and to increase insulin-like growth factor (IGF-1) in the primate ovary (Vendola et al., 1999).

Casson et al. (2000), who were the first to report the beneficial effects of DHEA on ovaries with diminished reserve, demonstrated a transient increase in IGF-1 in patients undergoing exogenous gonadotrophin ovulation induction after pretreatment for only 8 weeks of DHEA (Casson et al., 1998). Such a transient increase in IGF-1 may have been due to either increased production or androgen effect on the liver producing decreased IGF-1-binding hormone.

Others reported higher baseline testosterone levels associated with improved IVF outcomes (Frattarelli and Peterson, 2004), and higher serum testosterone level has been correlated with higher oocyte numbers retrieved at IVF (Barbieri et al., 2005). Some authors have suggested that improved outcomes in women with diminished ovarian reserve, after co-treatment with an aromatase inhibitor during cycle stimulation, may be the consequence of induction of FSH receptors on granulosa cell by androgens (Mitwally and Casper, 2002; Goswami et al., 2004). The resultant ovarian response then leads to improved follicular survival, increased follicle numbers and higher E2 levels during stimulation, as classically also observed in polycystic ovary syndrome (PCOS) (MacDougall et al., 1993).

It is possible that DHEA treatment may create PCOS-like characteristics in the ageing ovary. Human polycystic ovaries have been described as representing a ‘stock-piling’ of primary follicles, secondary to an alteration at the transition from primordial to primary follicle (Maciel et al., 1993). Possible mechanisms that have been suggested for this observation are abnormal levels of growth factor, abnormally increased LH levels or increased ovarian androgens. Normal ovarian theca cells of the pre-antral follicle produce androstenedione, DHEA and testosterone. Women with polycystic ovaries have higher serum testosterone, androstenedione and DHEA levels compared with controls and higher ovarian venous levels of DHEA, androstenedione and testosterone (Amirikia et al., 1986; van der Westhuizen and van der Spuy, 1996). Long-term exogenous androgen exposure can induce PCOS-like histological and sonographic changes in normal ovaries similar to PCOS (Amirikia et al., 1986; Pache et al., 1991), which also became a prominent feature in our index patient (Barad and Gleicher, 2005).

Androgens have also been reported to suppress apoptosis (Billig et al., 1993; Kaipia and Hsueh, 1997). Patients in the present study demonstrated better oocyte and embryo numbers as well as overall improved quality in the cohort of embryos...
produced in each cycle, as reflected in the significantly improved embryo scores. Moreover, the peak effect of DHEA treatment appears to coincide with the normal time period for follicular recruitment cycle (Gougeon, 1998).

DHEA is a mild male hormone which in the USA can be purchased without prescription. Adverse effects are uncommon at a DHEA dose of <100 mg per day. In this study, a DHEA dose of 75 mg per day was used because our index patient had utilized this dosage (Barad and Gleicher, 2005). Possible adverse effects of DHEA include acne, deepening of the voice and facial hair growth (Kroboth et al., 1999). Long-term effects of DHEA supplementation remain unknown. The safety issue of most concern is that DHEA—as a precursor of sex steroids—may increase the risk of estrogen- or androgen-dependent malignancies (Kaaks et al., 2005). Pregnancy, in itself, is a high androgen/DHEA state (McClamrock and Adashi, 1992), and women with PCOS, also a high androgen/ DHEA state (Sir-Petermann et al., 2002), do not deliver daughters with masculinized external genitalia. This suggests that low-dosage use of DHEA should be safe.

The most obvious limitation of this study is lack of randomization. It is, therefore, possible that other, yet unknown, factors might have been responsible for the here observed effects. Patients were recruited for this study because of a history of poor response to prior ovulation induction. The observed increase in oocytes yield could, therefore, simply represent the ‘regression to the mean’, with a more normal response to maximal stimulation in a subsequent cycle.

A strong point of this study is that all patients received the same maximal gonadotrophin stimulation before and after DHEA treatment. There is also a logical consistency to the observed improvement in fertilization rates, numbers of transferable embryos and overall embryo scores.

It is noteworthy that the observation on improving oocyte numbers has now been made independently three times: first by Casson et al. (2000), second by us in a longitudinal report of one patient (Barad and Gleicher, 2005) and now, albeit modestly, for the third time, here, with the exclusion of that initial patient from analysis.

In summary, the preliminary data of this ongoing investigation confirm the previously reported beneficial effect of DHEA on oocyte yield (Barad and Gleicher, 2005) and, in addition, now also suggest that DHEA may beneficially affect oocyte and embryo quality. The findings reported here thus provide a foundation for further studies which, preferably, will include the completion of a randomized controlled trial. Such a trial is currently underway at our Centre.

Conflict of interest
Amongst other parties, Dr Barad and Dr Gleicher have applied for a patent for the use of DHEA as co-treatment to improve ovarian function.

References


Chuang CC, Chen CD, Chao KH, Shen SU, Ho HN and Yang YS (2003) Age is a better predictor of pregnancy potential than basal follicle-stimulating hormone levels in women undergoing in vitro fertilization. Fertil Steril 79,63–68.


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