Article

Dehydroepiandrosterone supplementation improves ovarian response and cycle outcome in poor responders



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Abstract

The effect of dehydroepiandrosterone (DHEA) supplementation on cycle outcome was assessed in patients with poor ovarian response. In total, 19 poor responder patients who were scheduled to undergo a second intracytoplasmic sperm injection (ICSI)/embryo transfer cycle were enrolled and first ICSI/embryo transfer cycles were taken as the control group. All subjects were given DHEA supplementation (25 mg t.i.d.) for at least 3 months prior to their second ICSI/embryo transfer cycle. In both cycles a fixed dose of rFSH (300 IU/day) and human menopausal gonadotrophin (HMG) (75 or 150 IU/day) along with a flexible gonadotrophin-releasing hormone (GnRH) antagonist protocol were administered. A favourable decrease was noted in mean day 3 serum oestradiol concentrations after DHEA supplementation (75.14 ± 28.93 versus 43.07 ± 11.77; $P < 0.01$). Increased number of >17 mm follicles (3 ± 0.7 versus 1.9 ± 1.3; $P < 0.05$), MII oocytes (4 ± 1.8 versus 2.1 ± 1.8; $P < 0.05$), top quality day 2 (2.2 ± 0.8 versus 1.3 ± 1.1; $P < 0.05$) and day 3 embryos (1.9 ± 0.8 versus 0.7 ± 0.6; $P < 0.05$) were achieved in DHEA-supplemented cycles. Cycle cancellation rates were reduced (5.3% versus 42.1%; $P < 0.01$), and the pregnancy rate per patient and clinical pregnancy rate per embryo transfer (47.4% versus 10.5%; $P < 0.01$ and 44.4% versus 0%; $P < 0.01$) were improved after DHEA supplementation. DHEA supplementation might enhance ovarian response, reduce cycle cancellation rates and increase embryo quality in poor responders.

Keywords: *DHEA supplementation, GnRH antagonist, ICSI, poor ovarian response*

Introduction

Poor ovarian response (POR), which reflects diminished ovarian reserve, still remains among the foremost challenging problems in reproductive medicine. Despite several proposed strategies such as increasing gonadotrophin dose or using different down-regulation protocols, the oocyte yield is usually low and the quality is poor, resulting in unfavourable embryo quality and low pregnancy rates.

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEA-S) are ubiquitous steroids of primarily adrenocortical reticularis zonal origin (Casson *et al.*, 1998a; Hugues and Durnerin, 2005). DHEA is proposed to act as a steroid prehormone for ovarian follicular sex steroidogenesis that circulates in high amounts in female reproductive life, with concentrations falling progressively with advancing age (Orentreich *et al.*, 1984; Haning *et al.*, 1993; Hugues and Durnerin, 2005). Casson et al. first speculated that exogenous administration of

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DHEA could restore ovarian follicular sex steroidogenesis in elderly women (Casson *et al.*, 1998b). Thereafter, beneficial effects of DHEA supplementation prior to ovulation induction have been reported by some authors in IVF or ICSI cycles of patients with POR using microdose flare-up down regulation (Casson *et al.*, 2000; Barad and Gleicher, 2005, 2006). In the current study, the presumed beneficial effects of DHEA supplementation were investigated in ICSI/embryo transfer cycles of patients using a multidose flexible gonadotrophin-releasing hormone (GnRH) antagonist protocol with a previous history of poor ovarian response.

Materials and methods

Subjects

Nineteen patients who were diagnosed with POR scheduled to undergo their second intracytoplasmic sperm injection (ICSI)/embryo transfer cycle were enrolled in the study. The study was approved by the ethical committee of Ankara University. All patients were thoroughly informed about the novelty and unknown efficacy of the dietary supplement DHEA to improve ovarian response. All enrolled patients had undergone their first ICSI/embryo transfer cycle at least 4 months prior to the current study. POR was determined as having at least one of the following criteria regarding the outcomes of previous ICSI/embryo transfer and ovarian stimulation cycles: (i) cycle cancellation due to low oestradiol concentrations; on day 6 of the cycle (<130 pg/ml), or on the day of human chorionic gonadotrophin (HCG) administration(<450 pg/ml), (ii) <4 retrieved oocytes despite vigorous ovarian stimulation.

Ovarian stimulation, patient monitoring and follow-up

The first cycle outcomes of the patients were used as their own control group. After the first failed IVF cycle, DHEA (25 mg capsules, 21st Century; AZ, USA) was administered 25 mg t.i.d. for at least 90 days (range 90–180 days) to all patients. Ovarian stimulation, as similar to the previous cycle, was performed with a fixed dose of recombinant FSH (Gonal F, Merck-Serono, Turkey and Puregon, Schering Plough, Turkey) (300 IU/day) and human menopausal gonadotrophin (HMG, Menogon, Ferring Pharmaceuticals, Turkey) (75 or 150 IU/day) started on cycle day 2–3. Antral follicle count was measured in each patient and noted on day 2–3 of the cycle prior to gonadotrophin stimulation. Flexible multiple dose GnRH antagonist protocol (cetrotide, 0.25 µcg/day s.c.; Merck-Serono, Turkey) was started when the leading follicle was >13–14 mm and/or serum oestradiol concentration was >350 pg/ml. Final oocyte maturation was triggered using 10,000 IU urinary HCG (Pregnyl®; Organon-Turkey). Follicular development was monitored by transvaginal ultrasonography using a 4–8 MHz vaginal probe (HD 11 Ultrasound System; Philips Medical Systems, Turkey), serum oestradiol and LH concentrations. Oocyte retrieval (OR) was performed at 35–36 h after HCG administration under local anaesthesia with double lumen oocyte retrieval needle (Embryo Genesis, California, USA). Embryo transfer was performed on day 2 or 3 of OR with a soft intrauterine transfer catheter (Swemed; Vitrolife, Kungsbacka, Sweden) under the guidance of abdominal ultrasonography (Shimatzu; Logic 3500, South Korea). A trial soft catheter was introduced before the transfer procedure, and cervical mucus and/or blood contact was noted. The luteal phase was supported using natural micronized progesterone vaginal capsules 200 mg t.i.d. (Progestan; Koçak Pharmacy, Turkey), which was continued throughout 12 weeks of gestation. Pregnancy was confirmed by a positive serum HCG concentration >20 IU/ml measured on day 12 of embryo transfer, and clinical pregnancy was defined by observation of fetal heart beat(s) using transvaginal ultrasonography in gestational weeks 5–6.

Criteria for cycle cancellations

Ovulation induction and ICSI/embryo transfer cycles were cancelled when one of the following criteria was encountered: (i) serum oestradiol concentrations <130 pg/ml on day 3 of gonadotrophin induction; (ii) an increase in serum oestradiol concentration <25% on the 5th or the subsequent follow-up days performed at 48-h intervals from gonadotrophin induction, regardless of follicular development; (iii) no follicular development and an increase in serum oestradiol concentration of <50% on day 5 or the subsequent follow-up days mentioned above; (iv) total fertilization failure; and (v) total arrest of embryonic development.

Immunoassay of hormones

Serum concentrations of LH and FSH were measured using two-site chemiluminescent sandwich immunoassay system (ACS:180; Bayer Diagnostics Corporation, Tarrytown, NY, USA). All samples were assayed in duplicate. The LH and FSH values were expressed in terms of the reference standards [World Health Organization (WHO) 2nd International Standard (IS) 94/632 and WHO 2nd IS 80/552, respectively]. Assay sensitivity for FSH was 0.3 mIU/ml and for LH was 0.07 mIU/ml. Oestradiol concentrations were assayed by a fully automated enzyme-linked fluorescence assay system (Vidas; bioMerieux, Marcy l'Etoile, France). The minimum detection limit was 9 pg/ml. The intra- and inter-assay coefficients of variation were 3.46% and 4.82% for FSH, 4.4% and 5.6% for LH and 4.2% and 5.2% for oestradiol, respectively.

Main measurement and outcome(s)

Antral follicle count, mean number of follicles >14 and >17 mm on the day of HCG administration, mean serum oestradiol concentration on the day of HCG administration, mean number of retrieved oocytes, mean number of mature oocytes (MII), mean number of transferred embryos and rates of fertilization, implantation, pregnancy, and clinical pregnancy were assessed. In addition, mean cumulative gonadotrophin dose and duration of ovarian stimulation were compared between first and second IVF cycles.

Statistics

Statistical comparisons were performed using Student's *t*-test, Mann–Whitney *U*-test, significance test for comparing two proportions (two-sided *z*-test) and Fisher's exact test, as applicable. A *P*-value of 0.05 was considered to be statistically significant.

Results

The mean age and mean BMI of the patients were 32.92 ± 3.76 years (range 29–43 years) and 25.04 ± 3.99 kg/m^2 (range 18.75–35.26 kg/m^2), respectively (**Table 1**). All of the patients were diagnosed with POR in their preceding cycle; four of the enrolled patients met the first POR criteria detailed in Materials and methods, whereas the remaining 15 patients met the second criteria. Of the enrolled patients, 10 patients had severe endometriosis/bilateral tubal factor and two had diminished ovarian reserve along with severe–mild male factor infertility, and the remaining seven patients had only diminished ovarian reserve. In the first cycle, all patients had either FSH >12 IU/ml or oestradiol >75 IU/ml on days 2 or 3 of the menstrual cycle. Mean day 3 FSH concentration was not altered after DHEA pretreatment (9.72 ± 2.19 versus 11.35 ± 2.66; $P > 0.05$); however, mean day 3 oestradiol concentrations were found to be significantly lower after DHEA pretreatment compared with basal serum concentrations in the preceding cycle (43.07 ± 11.77 versus 75.14 ± 28.93 pg/ml; $P < 0.05$) (**Table 1**). In the first cycle, two patients had fertilization failure (one with severe male factor).

The outcomes of ovarian stimulation and total gonadotrophin consumption are presented in **Table 2**. Mean number of follicles >17 mm were higher in DHEA pretreated cycles compared with the preceding cycles (3.0 ± 0.7 versus 1.9 ± 1.3; $P < 0.05$), whereas peak serum oestradiol concentrations were similar among DHEA pretreated and non-pretreated cycles (1282 ± 513 versus 875 ± 642 pg/ml, **Table 2**). Mean numbers of total retrieved oocytes (4.9 ± 2.3 versus 2.5 ± 1.6; $P < 0.05$) and MII oocytes

Table 1. Patient characteristics and basal ovarian reserve assessment before and after dehydroepiandrosterone (DHEA) supplementation.

Parameter	*Before DHEA*	*After DHEA*	P-*value*
Age (years)	32.92 ± 3.76 (range 29–43)	–	NA
BMI (kg/m^2)	25.04 ± 3.99 (range 18.75–35.26)	–	NA
FSH (mIU/ml)	9.72 ± 2.19	11.35 ± 2.66	NS
LH (mIU/ml)	5.36 ± 1.52	4.5 ± 1.38	NS
Oestradiol (pg/ml)	75.14 ± 28.93	43.07 ± 11.77	<0.01
Antral follicle count	4.2 ± 1.8	5.1 ± 2.3	NS

BMI, body mass index; NA, not available; NS, not statistically significant.

Table 2. Ovarian stimulation outcomes and total gonadotrophin consumption before and after dehydroepiandrosterone (DHEA) supplementation.

Parameter	*Before DHEA*	*After DHEA*	P-*value*
Serum oestradiol concentrations on HCG day (pg/ml)	875 ± 642	1282 ± 513	NS
Endometrial thickness on HCG day (mm)	10 ± 1.7	10 ± 1.3	NS
Total rFSH dose (IU)	3047 ± 928	3565 ± 1000	NS
Total HMG dose (IU)	1348 ± 618	1604 ± 500	NS
Stimulation days	8.9 ± 1.7	9.9 ± 1.63	NS
Follicles <17 mm (*n*)	3.1 ± 1.4	3.3 ± 1.9	NS
Follicles >17 mm (*n*)	1.9 ± 1.3	3 ± 0.7	<0.05
Oocytes retrieved (*n*)	2.5 ± 1.6	4.9 ± 2.3	<0.05
MII oocytes (*n*)	2.1 ± 1.8	4 ± 1.8	<0.05
Day 2 cleavage embryos (*n*)	1.8 ± 1.2	2.4 ± 0.8	NS
Day 2 grade A embryos (*n*)	1.3 ± 1.1	2.2 ± 0.8	<0.05
Day 3 grade A embryos (*n*)	0.7 ± 0.6	1.9 ± 0.8	<0.05
Transferred embryos (*n*)	2.2 ± 1.6	2.4 ± 0.8	NS
Fertilization rate (%)	53 ± 32	56 ± 17	NS

Values are means ± SD.

HCG, human chorionic gonadotrophin; HMG, human menopausal gonadotrophin; MII, metaphase II; NS, not statistically significant; rFSH, recombinant FSH.

(4 ± 1.8 versus 2.1 ± 1.8; $P < 0.05$) were also statistically higher in DHEA pretreated cycles than in DHEA non-pretreated cycles. The ratio of mature oocytes to retrieved oocytes was favourable in DHEA pretreated cycles compared with non-pretreated cycles ($90 \pm 13\%$ versus $69 \pm 22\%$; $P < 0.05$; data not given in **Table 2**). Mean number of cleavage stage embryos was similar among both cycles, whereas mean number of day 2 and day 3 grade A embryos was found to be higher in DHEA pretreated cycles compared with non-treated cycles (**Table 2**).

Fertilization rates (53 ± 32 and $56 \pm 17\%$) and mean number of transferred embryos (2.2 ± 1.6 and 2.4 ± 0.8) were similar in non-pretreated and DHEA pretreated cycles (**Table 2**). In the DHEA non-pretreated cycles, ovarian stimulation was cancelled in six patients due to POR, whereas no cancellation occurred in DHEA pretreated cycles (**Table 3**). Of importance to mention is that one patient who had severe male factor infertility and encountered total fertilization failure in the first ICSI/embryo transfer cycle achieved pregnancy after DHEA supplementation. Notably, rates of pregnancy per embryo transfer (50.0% and 18.2%; $P < 0.05$) and per patient (47.4% and 10.5%; $P < 0.01$), as well as rates of clinical pregnancy per embryo transfer (44.4% and 0%; $P < 0.01$) and implantation (23.9% and 0%; $P < 0.05$), were all found to be significantly superior in DHEA pretreated cycles compared with non-pretreated ones (**Table 3**). Of these pregnancies achieved in DHEA pretreated cycles, two resulted in a healthy live birth and six are ongoing with one >12 weeks, two >20 weeks and three >30 weeks of gestational age. One of the clinical pregnancies diagnosed in DHEA pretreated cycles ended in a missed abortion at 9 weeks of gestational age.

Discussion

Although various stimulation protocols and down regulation strategies have been proposed to overcome POR, a group of women still remains refractory to ovarian stimulation leading to limited success by modifications of ovarian stimulation protocols. However, as the physiology of in vivo oocyte development and steroidogenesis has been understood, novel challenges to supplement physiological mechanisms are being undertaken.

Androgens, essential substrates for steroidogenesis, have been acknowledged as playing critical roles in follicular development. In the early in-vitro studies on primates, granulosa cell stimulation by FSH has been shown to be an androgen-modulated process wherein androgens may influence the responsiveness of ovaries to gonadotrophins (Hillier and De Zwart, 1981; Harlow *et al.*, 1986; Hugues and Durnerin, 2005). Subsequently, testosterone and dihydrotestosterone augmentation have been reported to increase follicular FSH receptor expression in granulosa cells (Weil *et al.*, 1998), promote initiation of primordial follicular growth, and increase the number of pre-antral and small antral follicles in primate studies (Vendola *et al.*, 1998, 1999). Studies on women with polycystic ovarian syndrome (PCOS) demonstrated that elevated androgen concentrations both in serum and follicular fluid were associated with increased number of pre-antral and small antral follicles, along with increased responsiveness to gonadotrophin induction (de Leo *et al.*, 1998). Besides, morphological features of PCOS were observed in women with long term exposure to exogenous androgens. Conversely, it has been demonstrated that serum 17-hydroxyprogesterone, testosterone and dihydrotestosterone concentrations were markedly increased after FSH administration, while serum DHEA and inhibin B concentrations were stable and unchanged similar to serum hormone concentrations in normal women (Wachs *et al.*, 2008).

DHEA functions as the prehormone for up to 48% of follicular testosterone, which is the prehormone for oestradiol. In a preliminary study, oral administration of physiological DHEA resulted in marked augmentation of serum IGF-I concentrations (Casson *et al.*, 1998b). Based on this initial study, Casson et al. subsequently demonstrated a mild improvement in ovarian response after DHEA supplementation (25 mg t.i.d.) for 2 months in five POR cases with documented basal FSH concentrations <20 mIU/ml in intrauterine insemination cycles (Casson *et al.*, 2000). Five years after this small preliminary study, a continuous and dramatically improved ovarian response was reported in a 43-year-old patient with severely decreased ovarian reserve with concomitant DHEA supplementation (Barad and Gleicher, 2005). On the basis of their initial findings, Barad and Gleicher demonstrated significant increases in the number of fertilized oocytes, normal day 3 embryos, and transferred embryos, along with improved average embryo scores per oocyte after DHEA supplementation in cases with significantly diminished ovarian reserve and repeated IVF failure (Barad and Gleicher, 2006). Similarly, Gleicher and Barad reported excellent pregnancy rates after DHEA pretreatment in patients undergoing IVF with diminished ovarian reserve

Table 3. IVF cycle outcomes and cycle cancellation rates.

Parameter	*Before DHEA* (n = *19*)	*After DHEA* (n = *19*)	P-*value*
Total cycle cancellation	42.1 (8/19)	5.3 (1/19)	<0.01
Cycle cancellation due to POR	31.6 (6/19)	0.0 (0/19)	<0.01
Pregnancy/embryo transfer	18.2 (2/11)	50.0 (9/18)	<0.05
Pregnancy/patient	10.5 (2/19)	47.4 (9/19)	<0.01
Clinical pregnancy/per embryo transfer	0.0 (0/11)	44.4 (8/18)	<0.01
Implantation	0.0 (0/11)	23.9 (11/46)	<0.05

Values are % (number/total).

DHEA, dehydroepiandrosterone; NS, not statistically significant; POR, poor ovarian response.

according to age-based ovarian reserve assessment (Gleicher and Barad, 2006). Based on the findings from their studies and case report, Gleicher and Barad suggested that at least 4 months of DHEA supplementation is required to achieve an optimal ovarian response. In a recent case control study, IVF cycle outcomes were assessed in 89 DHEA pretreated and 101 DHEA non-pretreated cycles in patients with POR. In that study, a significant increase in cumulative pregnancy rates, more than doubling of rates with a 3.8 relative ratio of achieving pregnancy, was observed in DHEA pretreated cycles compared with non-pretreated control ones (28.4% versus 11.9%, respectively) (Barad *et al.*, 2007).

A more recent study has shown promising results (Mamas and Mamas, 2009). The study presented five successful pregnancies in five premature ovarian failure cases wherein four of the cases were conceived naturally and one of them conceived by intrauterine tubo-peritoneal insemination after at least 2 months of DHEA supplementation. Of these pregnancies, one resulted in a live birth, one ended with a spontaneous abortion at 7 weeks of gestation and the other three were ongoing pregnancies >11 gestational weeks at the time of the correspondence. Interestingly, four of these cases responded to DHEA supplementation (25 mg d.i.d.), which led to regular periods along with decreased serum FSH concentrations and increased serum oestradiol concentrations. However, due to inadequate response with 50 mg per day DHEA supplementation for 2 months, in one case daily supplementation dose was increased to 75 mg per day (25 mg t.i.d.) and improved ovarian response was achieved in 1 month. Surprisingly, in two of these cases, who conceived naturally, serum FSH concentrations regressed from extremely elevated concentrations >100 mIU/ml to concentrations <18.5 mIU/ml and one gave a birth to a healthy child.

In particular, pretreatment DHEA supplementation prior to IVF cycles has been reported to reduce aneuploidy rates among an older population (Gleicher *et al.*, 2007). It has also been reported that DHEA supplementation seems to be associated with lower miscarriage rates (Gleicher *et al.*, 2008). Of interest to note is that the likelihood of bearing male offspring is increased in patients who received DHEA to improve ovarian response (Ryan *et al.*, 2008).

The current study is the only one that assesses the effectiveness of DHEA supplementation in POR patients undergoing GnRH antagonist down-regulated ICSI/embryo transfer cycles. In the current study, DHEA supplementation dramatically improved ovarian reserve as well as many of the IVF success parameters in patients with POR. There were remarkable improvements in oocyte retrieval rates, production of mature oocytes and good quality embryos, leading to notably increased pregnancy rates with acceptably low cancellation rates. The gonadotrophin start dose was not changed, and total gonadotrophin consumption was similar among the DHEA pretreated and non-pretreated IVF cycles. Therefore, based on the results of the current study and the previous ones, one can assume that while ovarian reserve is restored, IVF success parameters can be improved by supplementation of DHEA prior to IVF or ICSI/embryo transfer cycles in POR patients.

The limitations of the current study, including the small study population and not monitoring serum concentrations of DHEA and testosterone (Barad and Gleicher, 2006, 2007; Gleicher and Barad, 2006), are acknowledged. However, albeit with small size, the clear difference between the groups seems to be convincing, along with the previous strong data. After the initial positive clinical effects of DHEA were observed, it was routinely recommended to all patients diagnosed with POR, hence it was not possible to increase the sample size. In addition, finding such eligible patients with POR is quite difficult to enlarge the study population. Second, as the serum concentrations of DHEA and testosterone were precisely documented in the earlier studies, these hormones were not measured in the current study, in order to prevent augmented treatment costs and unnecessary blood samplings. None of the previous studies noted any complications associated with DHEA supplementation for at least 4 months. In the present sample, almost all of the patients received DHEA for more than 3 months, wherein the interval between two cycles was at least 4 months. In previous large studies, 3 months of DHEA supplementation was presumed to be adequate for restoring unfavourable IVF outcomes in POR cases as well. Although no difference was found in cumulative gonadotrophin consumption, it was higher in DHEA pretreated cycles, mainly due to lower cancellation rates and a longer stimulation period. It is unlikely that stimulation with a higher cumulative gonadotrophin dose confounded results, since using a gonadotrophin start dose >450 IU/l/day usually has no or only minimal effect on ovarian response in patients with POR (Siristatidis and Hamilton, 2007).

In conclusion, the current study, which should be considered as a preliminary one, shows that DHEA supplementation in POR patients undergoing GnRH antagonist down-regulated IVF or ICSI/embryo transfer cycles prior to stimulation protocols augments ovarian response and enhances IVF success rates. Although it was not possible to assess miscarriage rates after DHEA supplementation due to low pregnancy rates in the first IVF attempt, the promisingly low aneuploidy and miscarriage rates reported by recent studies deserves further evaluation. Another question that should also be clarified is the implication of unbalanced sex ratio favouring male sex that was indicated by a recent presentation. Although more prospective, randomized and large studies are still essential to strengthen the findings of the current and previous observational studies, patients with POR should be encouraged to use DHEA in the light of the available data.

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