Dehydroepiandrosterone supplementation within a gonadotropin-releasing hormone antagonist protocol in patients with poor ovarian response

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ABSTRACT

Objective: The aim of this study was to compare IVF treatment outcomes between pre- and post-treatment cycles among a cohort of women with known decreased ovarian reserve, using dehydroepiandrosterone (DHEA) supplementation.
Materials Methods: A prospective cohort study in a private assisted reproduction centre. Twenty-five women with significantly diminished ovarian reserve had IVF cycle before and after 50 mg/day DHEA for 2 months, with otherwise identical hormonal stimulation.
Results: After treatment with DHEA, patients demonstrated a significant improvement in day 3 FSH (7.1 ± 0.5 vs. 9.9 ± 1.5 mIU/ml) (p< 0.05), day 3 E2 (325.46 ± 9.7 vs. 227.37 ± 16.95 pmol/L) (p< 0.05), day 3 testosterone (79.84 ±10.93 vs. 54. 64 ±10.31 µg/dl) (p< 0.05) , day 3 DHEA-S (99.94 ± 15.4 vs. 62 .54 ± 16.3 µg/dl) (p < 0.05), number of follicles demonstrated by ULS on the day of hCG administration[Total (9 ±1.5 vs.6 ± 1.5) (p=0.01), ≥ 14 mm (4.5±1.5 vs.3.5 ± 1.5) (p = 0.01) and ≥ 17 mm(4.5 ±1.5 vs. 2.5 ±1.5) (p = 0.01)], serum E2 level on the day of hCG administration (4760±1524 vs. 3800±1684 pmol/L)(p = 0.001), number of oocytes retrieved (7.5 ± 0.5 vs. 5.5 ± 0.5) (p = 0.01) and number of mature oocytes (Metaphase II) (5.5± 0.5 vs. 3.5± 0.5)(p = 0.01). Although there were significant differences in fertilization rate (72.6 % vs. 45.8 %) (p<0.005), percentage of grade I/II embryos (73.6 % vs. 50.4%) (p= 0.005), cumulative embryo score per oocyte retrieved (18.5±1.2 vs. 10.5±1.2) (p = 0.001) and cancellation rate (15% vs. 40%) (p = 0.001).These were not reflected on biochemical pregnancy, clinical pregnancy rate/cycle or clinical pregnancy rate/embryo transfer.
Conclusions: DHEA supplementation for poor responder patients could have beneficial effects on ovarian follicular function, improvement in hormonal profile and embryological data. However, large prospective randomized placebo controlled trials are waited to demonstrate the improvement in clinical pregnancy rate after DHEA supplementation.
Key Words: DHEA, GnRH antagonist, poor responders, IVF.

INTRODUCTION

Despite considerable advances in assisted reproductive techniques (ART), management of poor responder patients is still a challenge. Although there is lack of uniform definitions, poor response to controlled ovarian hyperstimulation (COH) can be generally defined as unsatisfactory ovarian response in terms of low number of follicles developed, low serum E2 levels, and low number of oocytes retrieved despite adequate ovarian stimulation. However the cutoff points for these parameters that define poor response vary between studies 1, 2.

Poor response to ovarian hyperstimulation is a complication in 5% to 18% of all in vitro fertilization (IVF) cycles. Poor responders have significantly worse IVF outcomes than normal responders, with successful pregnancy rates as low as 2 % to 4 % 3.

Many treatment modalities have been suggested to improve ART outcomes in poor responders. These modalities include : [1] Variations in the type,dose,and timing of gonadotropins,or GnRH analogues(agonists and antagonists), [2] The use of oral contraceptive (OC) pills, clomiphene citrate (CC), aromatase inhibitors, growth hormone/growth hormone releasing hormone (GHRH), corticosteroids, estradiol (E2), testosterone (T), nitric oxide donors, or aspirin as adjuvant therapies. A part from these regimens, an alternative approach suggested for these patients is natural cycle ART 1, 4, 5. Previous studies have found that dehydroepiandrosterone (DHEA) supplementation increases oocyte production and augments ovarian stimulation in individuals who do not respond well to gonadotropin administration 6,7.
DHEA is an endogenous steroid that originates from the zona reicularis of the adrenal cortex and the ovarian theca cells in women. It is produced by the conversion of cholesterol and it is very important in the formation first of T and then E2 in peripheral tissues. Therefore, DHEA is an essential prohormone in ovarian follicular steroidogenesis8, 9.
The concentration of DHEA in women remains high during the reproductive years and progressively decreases with age. Numerous hypotheses have been made on how DHEA

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MATERIALS AND METHODS

This was a prospective cohort study involving poor responder patients undergoing IVF treatment at The Toronto Institute for Reproductive Medicine. Twenty-five women with significantly diminished ovarian reserve had IVF cycle before and after DHEA treatment, with otherwise identical hormonal stimulation from March2008 and March2009.Written informed consent was obtained from all patients and the study was reviewed and approved by our institutional review board.

As it is the policy of our centre to use GnRH antagonist protocol for poor responder patients, poor responders were defined when one or more of the following criteria is present in at least one previous failed ART cycle (using Gn RH antagonist protocol): [1] number of oocytes retrieved less than four, [2] level of E2 <1800 pmol/L on the day of hCG administration, or [3] a prior cancelled stimulation cycle due to poor ovarian response.

All patients had normal liver, kidney and thyroid function and had baseline ultrasound scans on cycle day 3, and blood was drown for serum FSH, LH, E2, progesterone, prolactin , testosterone and DHEA-S. Patients who agreed to participate in the study, began taking micronized DHEA by prescription compounded by a single pharmacy, 50 mg/day (two 25 micronized DHEA) for 2 months. All patients experienced both their pre- and post DHEA treatment IVF cycles at our centre. Monthly repeat baseline ultrasound scan on day 3, and liver, kidney, thyroid and hormonal assay were done. After 2 months of DHEA pretreatment, and while still remaining on this hormone, the subjects had a repeat IVF cycle using the same GnRH antagonist protocol.

Multiple dose GnRH antagonist protocol was given to all patients. Oral contraceptive pills were not used in this protocol. A combination of recombinant FSH, follitropin beta(Puregon, Organon)and hMG (Menopure, Ferring) was started on cycle day 2 and later 0.25 mg of Cetrorelix (Cetrotide, Serono) was administered daily when the leading follicle reached 14 mm in diameter or serum estradiol > 1000pmol/L until the day of hCG injection. An initial gonadotropin dose of 300IU of Puregon and 150 IU of Menopure for the first 5 days, followed by individual adjustments in gonadotropin dose, according to ovarian response was done.
When the average diameter of the leading follicles reached ≥ 18 mm and serum E2 levels were ≥ 2000 pmol/L, 10000 IU of hCG (Preganyl, Organon) was administered, followed 35-36 hours later by an ultrasound-guided transvaginal oocyte aspiration. The same multiple dose GnRH antagonist protocol was used both before and after DHEA treatment.Intracytoplasmic sperm injection (ICSI) procedure was performed 4-6 h after oocyte aspiration for all of the mature oocytes. Oocytes were examined 16-18h after ICSI for pronuclei (PN). Normal fertilization was defined as existence of (2 PN).

The embryos obtained were categorized on day 2 or 3 into 4 categories depending on their morphologic appearance, zonal thickness, cytoplasmic fragmentation and blastomere size .Grade 1[high quality]:embryos with equal blastomeres and no observed cytoplasmic fragmentation; grade II [good quality]: embryos with equal blastomeres and < 20 % fragmentation of the cytoplasm; grade III [fair quality]:embryos with unequal blastomeres and 20-50 % fragmentation of the cytoplasm; grade IV [poor quality]:embryos with unequal blastomeres and > 50 % fragmentation of the cytoplasm.

Cumulative embryo scores were calculated by multiplying the cell number and grade of each embryo, on its day 3 of development, and summing the scores for embryos produced by each patient in each cycle of treatment (Steer et al., 1992). Average cumulative embryo scores were calculated by dividing the cumulative embryo score by the total number of oocytes retrieved.

Embryo transfer was done on day3; one to four embryos were transferred depending on patient's age, embryo quality and the number of embryos available. The luteal phase was supported with 200mg three times per day of natural progesterone vaginally and daily until a pregnancy test was performed. Pregnancy was determined as positive by ß HCG levels after 14days from embryo transfer and confirmed by repeat the test after 48hours.Progesterone treatment was continued up to 12 weeks gestation. Clinical pregnancy was determined by the presence of intrauterine gestational sac 2weeks after pregnancy test. Criteria for cycle cancellation due to poor ovarian response included the presence of fewer than 3 growing follicles on ULS, with E2 level < 750pmol/ L on day7 of stimulation. All IVF cycle parameters including peak E2 level, total number of oocytes retrieved, embryo numbers and average cumulative embryo scores, rate of cycle cancellation, positive pregnancy tests and ongoing clinical pregnancies were analyzed.

STATISTICAL ANALYSIS

SPSS for windows; standard version 10.0.7(SPSS Co., Chicago, IL, USA) was used for the statistical analysis. The student's t-test or Mann-Whitney U test was used to compare the mean values between the endocrine variables, cycle characteristics, embryological data and clinical outcomes between pre and post DHEA IVF cycles. Differences in outcome rates were analyzed using an x^2 or Fisher's exact test. In all statistical analyses, P<0 .05 was considered statistically significant.

RESULTS

Twenty-five women with significantly diminished ovarian reserve had IVF cycle before DHEA treatment, six patients get clinical pregnancy. Five out of them continued their pregnancy and one patient aborted during first trimester. So, only twenty patients had their IVF cycle after DHEA treatment.

There were no significant differences in patient's age, BMI, day 3 LH, progesterone and prolactin. However after treatment with DHEA, patients demonstrated a significant improvement in day 3

FSH (p< 0.05), day 3 E2 (p< 0.05), day 3 testesterone (p< 0.05) and day 3 DHEA-S (p < 0.05) (Table 1).

Cycle characteristics before and after treatments with dehydroepiandrosterone (DHEA) were demonstrated in Table (II).There were no significant differences in duration of gonadotropin stimulation (days), total dose of rFSH(IU), total dose of hMG (IU)or endometrial thickness on the day of hCG administration. However, after treatment with DHEA, there were significant differences in number of follicles demonstrated by ULS on the day of hCG administration [Total (p = 0.01), ≥ 14 mm (p = 0.01) and ≥ 17 mm (p = 0.01)], Serum E2 level on the day of hCG administration (p = 0.001), number of oocytes retrieved (p = 0.01) and number of mature oocytes (Metaphase II) (p = 0.01).

Table (III) demonstrated embryological data and clinical outcomes before and after treatment with dehydroepiandrosterone (DHEA). There were no significant differences in number of embryos transferred or implantation rate. Although there were significant differences in fertilization rate (p<0.005), percentage of grade I/II embryos (p= 0.005), cumulative embryo score per oocyte retrieved (p = 0.001) and cancellation rate (p = 0.001).These were not reflected on biochemical pregnancy, clinical pregnancy rate/cycle or clinical pregnancy rate/embryo transfer.

Table 1: Demographic data and endocrine variables before and after treatment with dehydroepiandrosterone (DHEA)

	Pre - DHEA	Post- DHEA	P value
Number of patients	25	20	_
Number of cycles	25	20	_
Age (y)	39.6 ± 0.9	40.2 ± 0.9	NS
BMI (kg/m²)	24.73 ± 2.36	24.56 ± 2.43	NS
Day 3 FSH(mIU/ml)	9.9 ± 1.5	7.1 ± 0.5	< 0.05
Day 3 LH(mIU/ml)	5.6 ± 1.7	5.2 ± 1.4	NS
Day 3 E2(pmol/L)	227.37 ± 16.95	325.46 ± 9.7	< 0.05
Day 3 progesterone(nmol/L)	4.65 ± 1.3	4.57± 1.2	NS
Day 3 prolactin (mIU/L)	74±17.5	73±14.8	NS
Day 3 testesterone(μg/dl)	54. 64±10.31	79.84±10.93	< 0.05
Day 3 DHEA-S (μg/dl)	62 .54± 16.3	99.94 ± 15.4	< 0.05

Data presented as mean ± SD.P value determined by ANOVA analysis. P<0 .05 was considered statistically significant. NS = not significant.

Table II: Cycle characteristics before and after treatment with dehydroepiandrosterone (DHEA)

	Pre - DHEA	Post- DHEA	P value
Duration of gonadotropin stimulation (days)	11.0±1.0	10±1.0	NS
Total dose of rFSH(IU)	2650±1045	2575±1025	NS
Total dose of hMG(IU)	1500±150	1525±100	NS
Number of follicles on the day of hCG administration			
Total	6 ± 1.5	9 ±1.5	0.01
≥ 14 mm	3.5±1.5	4.5±1.5	0.01
≥ 17 mm	2.5±1.5	4.5±1.5	0.01
Endometrial thickness on the day of hCG administration(mm)	9.3 ± 1.9	9.2 ± 1.7	NS
Serum E2 level on the day of hCG administration(pmol/L)	3800±1684	4760±1524	0.001
Number of oocytes retrieved	5.5 ± 0.5	7.5 ± 0.5	0.01
Number of mature oocytes (Metaphase II)	3.5± 0.5	5.5± 0.5	0.01

Data presented as mean ± SD.P value determined by ANOVA analysis. P<0 .05 was considered statistically significant. NS = not significant.

Table III: Embryological data and clinical outcomes before and after treatment with dehydroepiandrosterone (DHEA)

	Pre - DHEA	Post- DHEA	P value
Fertilization rate (%)	45.8	72.6	<0.005
Number of embryos transferred	2.53±1.2	2.48±1.2	NS
Grade I/II embryos (%)	50.4	73.6	0.005
Implantation rate (%)	7.4	11.8	NS
Cancellation rate (%)	10/25(40%)	3/20 (15 %)	0.001
Cumulative embryo score per oocyte retrieved	10.5±1.2	18.5±1.2	0.001
Biochemical pregnancy (%)	6/25(24%)	6/20(30%)	NS
Clinical pregnancy rate/cycle (%)	4/25(16%)	4/20(20%)	NS
Clinical pregnancy rate/embryo transfer (%)	4/25(16%)	4/20(20%)	NS

Data presented as mean ± SD.P value determined by ANOVA analysis. P<0 .05 was considered statistically significant. NS = not significant.

DISCUSSION

To the best of our knowledge, this is the first trial employing DHEA supplementation for poor responder patients undergoing ovarian stimulation using GnRH antagonist protocol. Lack of a large number of adequately designed prospective randomized trials and inconsistency in inclusion criteria makes it extremely difficult to conclusively demonstrate an advantage of a single protocol for all poor responder patients. However, it is the policy of our centre to use Gn RH antagonist protocol for poor responder patients. This is in policy with several prospective trials which had evaluated GnRH antagonist in comparison with alternative regimens designed for poor responders.

Cheung et al., compared a fixed start (day 6) of multidose GnRH antagonist to a long GnRHa protocol in a prospective randomized trial of 66 poor responders. There were no significant differences in response, although a trend towards higher pregnancy rates/transfer in those receiving Gn RH antagonist (26.38 vs. 17.6%) did not achieve statistical significance. However, extremely high cancellation rates of over 30% were noted in both groups 12.

In the present study, DHEA supplementation for poor responder patients was associated with improvement in hormonal profile in the form of decrease in basal FSH and increase in basal estradiol, testosterone and DHEA-S. These observations were in agreement with Casson et al., 9 and Mamas and Mammas 14 and confirm numerous hypotheses which had been previously suggested on how DHEA promotes fertility.
DHEA and DHEA-S are ubiquitous steroids of primarily adrenocortical reticularis zonal origin. These hormones circulate in high amounts in female reproductive life; however concentrations fall progressively with age 13, leading to speculation that replacement of DHEA and DHEA-S in the elderly may have age-retardant effects 10.

Casson et al., (9) 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 gonadotropin ovulation induction after pretreatment for only 8 weeks of DHEA. 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 15.

In the present study, we observed also a significant increase in serum E2 level on the day of hCG administration and increase in the number of mature follicles demonstrated by ULS. Furthermore, this was associated with increase in the number of both retrieved and mature oocytes. Our results were in agreement with those of Barad and Gleicher15, although in their study, women received 75mg of DHEA daily (25 mg three times daily) for an average of 17.6≥ 2.13weeks and microdose GnRH agonist protocol was used for ovarian stimulation.

It seems that DHEA supplementation for poor responder patients, through its effect on increasing serum androgen level could produce its effect on ovarian follicular growth. Several theories had been previously suggested to explain the possible mechanism of association between increasing.

androgen level and improvement of follicular growth. Androgens may act as a metabolic precursor 16 or promote steroidogenesis 17, or act as a ligand for androgen receptors 18 or increase insulin-like growth factor (IGF-1) 19 or by other unknown mechanism yet. Haning et al., 20 demonstrated that DHEA is the prehormone for up to 48 % of follicular fluid testosterone, which is the prehormone for E2 during ovulation induction with exogenous gonadotropins.

Moreover, in the present study, DHEA supplementation for 2 months was associated with a significant increase in percentage of grade I/II embryos, cumulative embryo score per oocytes retrieved and decrease in cancellation rate. However these effects could not be reflected on implantation rate, biochemical and clinical pregnancy rates. This was in agreement with Barad et al., 21 regarding embryo quality and score however in contradiction regarding clinical pregnancy rate.

Barad et al., 21 in a case control study, assessed the role of DHEA supplementation on pregnancy rates in 190 women with diminished ovarian reserve. The study group included 89 patients who used supplementation with 75 mg daily of oral micronized DHEA for up to 4 months prior to entry into IVF. The control group composed of 101 couples who received infertility treatment, but did not use DHEA. They demonstrated that cumulative pregnancy rates were significantly higher in the study group (28.4% vs. 11.9 %).
As a result of the possible side effects which could be associated with DHEA including androgenic effects like acne, deepening of voice , facial hair growth, moreover, the long term effects of DHEA supplementation which remain unknown. Furthermore, since DHEA is a precursor of sex steroids. There may be concerns of a possibility of increased risk of estrogen and androgen dependant malignancy. So that is why we decided to use DHEA supplementation for only 2 months and in the minimal dose of 50 mg micronized DHEA per day. As a result, all patients in our study did well with DHEA, with no reported side effects and no impairment in kidney, liver or thyroid functions.

In summary, this study confirms the preliminary studies which reported beneficial effects of DHEA on ovarian follicular function, improvement in hormonal profile and embryological data after DHEA supplementation for poor responder patients. However, large prospective randomized placebo controlled trials are waited to demonstrate the improvement in clinical pregnancy rate after DHEA supplementation. Furthermore, the optimal dosage supplementation of DHEA should be demonstrated to outweigh the benefits of the drug for poor responder patients against the potential side effects.

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