

# Pregnancy Chances in Women over the Age of Forty

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## ABSTRACT—

**Objective:** To determine whether Dehydroepiandrosterone (DHEA) improve ovarian response and pregnancy potential in women whose age is  $\geq 40$  years, and whether such potential is age dependent.

**Methods:** Between January 2013 and October 2013, 39 infertile women  $\geq 40$  years were prospectively evaluated for ovarian response and pregnancy chances without and with DHEA treatment (A= 22 normal ovarian reserve & B= 17 poor ovarian reserve).

**Results:** Group B women have a significant lower baseline Anti Mullerian Hormone and Estradiol indicating a poor ovarian reserve. There were insignificant difference between the two groups in term of follicle numbers after controlled ovarian stimulation, and the pregnancy rate is significantly better in group A.

**Conclusion:** our study failed to demonstrate any beneficial effect of DHEA to improve OR in women above or = 40 with baseline poor OR in term of follicular recruitment and pregnancy rate after controlled ovarian stimulation. Efficacy of androgen supplementation with DHEA seems to vary depending on female age and baseline OR.

**Keywords—** Ovarian Reserve, Dehydroepiandrosterone (DHEA) supplementation, ovarian stimulation.

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## 1. INTRODUCTION

Ovarian reserve (OR) is defined by a woman's remaining follicular pool, which declines with advancing female age. A patient is considered to suffer from diminished (OR) if this pool, at any given age, is smaller than expected (1). Diminished (OR), whether due to physiological ageing of the ovaries (2) or premature ovarian ageing, represent one of the few unresolved problems of modern infertility care (1). Casson and his team work (2000) were the first to report that dehydroepiandrosterone (DHEA) supplementation of women with diminished (OR) may positively impact ovarian function by increasing oocyte yields after stimulation with gonadotrophins(3). Their study, however, received almost no attention until the observation was confirmed by Barad and Gleicher, 2005(4) and expanded by demonstrating that DHEA also improves egg and embryo quality(5), pregnancy rates and time to conception (6) and reduces miscarriage rates (7). One of the most challenging issues for clinicians is to identify predictive factors of response to androgen (8). The aim of the present study was to evaluate whether the administration of DHEA to women  $\geq 40$  years with poor OR (defined by AMH) would improve ovarian response, and pregnancy rate.

## 2. PATIENTS AND METHODS

Between January 2013 and October 2013, 39 women were recruited from Al- Amal clinic/Babylon –Iraq, for infertility diagnosis and management. All women were referred because of infertility and underwent work up for infertility treatment at our clinic .At all women serum levels of Anti Mullerian Hormone (AMH) concentrations were evaluated as a reflection of OR, they underwent hormonal assessment for Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) , Estradiol (E2), prolactin, testosterone and thyroid function test (TFT) to exclude hyperprolactinemia ,hyperandrogenism or thyroid dysfunction as a cause of their infertility. Tubal patency was assessed by hysterosalpingeography (HSG); seminal fluid assessment for the husband was done to exclude male contribution. Inclusion criteria: female age above or equal 40 years, normal seminal fluid analysis and HSG and normal serum prolactin, testosterone and TFT. All the subjects had baseline ultrasound scans of the ovaries to exclude Polycystic Ovaries( PCO) and liver function tests and kidney function and had an average Body Mass Index (BMI) ( $<28\text{kg/m}^2$ ). The included women were categorized into two groups, group A (n= 22, those women who are  $\geq 40$  years of age and they have reasonable OR defined by AMH concentration, and Group B (n=17, those women  $\geq 40$  years and have poor OR defined by low AMH concentration. We defined poor OR by universal AMH concentrations (obtained on a random day of the menstrual cycle) below 0.8 ng/ ml, which approximately correlates to an FSH of 11.0 mIU/ml (9). All women were asked about current use of hormones or drugs or any medical condition that might affect ovarian function, smoking,

pregnancy, lactation, previous ovarian surgery. All participating women underwent a comprehensive history and thorough physical examination, calculation of BMI. The Sera were withdrawn, separated and stored at  $-20^{\circ}\text{C}$  until assayed. Measurement of serum FSH was performed using the MiniVIDAS method (bioMérieux® France). Inter-assay Coefficient of Variance % ( CV %) 4.7; Intra-assay CV % 5.9, lower limit of detection  $\leq 0.1\text{mIU/ml}$  within 95% probability. For E2 the kit we used (bioMérieux® France) with Mini VIDAS technique Inter-assay CV % 4.6; Intra-assay CV% 3.2. Lower limit of detection 9 pg/ml Within 95% probability .Serum levels of AMH were determined by enzyme-linked immunosorbent assays (ELISA) using ( Beckman coulter INC,USA) kit , the assays were done according to the manufacturer’s instructions. The detection limits of this assay were 0.08 ng/ml within 95% probability, and its intra-assay and inter-assay coefficients of variation were 5.6% and 4.5% respectively.

Both groups underwent ovarian stimulation, but patients in group B have received for at least 2 months supplementation with pharmaceutical-grade, micronized and pharmacy-compounded DHEA (DHEA Chatsworth.CA 91311.USA, 25mg/tablet) at dosages of 25mg three times daily before progressing to ovarian stimulation (all women have been told about the benefit of DHEA supplementation and consent have been given to them in which they write down their agreement to use this drug). The stimulation protocol was started on day 2 consisted of two ampules of 75 IU recombinant FSH (Gonal-f, Follitrop Alfa 75 IU /ampule,serona .Switzerland). On day 7 repeat ultrasound and E2 concentrations measurements were performed and recombinant FSH dose was subsequently adjusted for amaxinal response.

Monitoring was continued till the leading follicle reached a mean diameter of 18mm, at which human Chorionic Gonadotropin(hCG) ( 5,000-10,000 IU) was administered (HCG, Pregnyl, Organon, 5000 IU/ ampule, Holland I.M.) to induce final oocyte maturation followed at 36 h by timed intercourse as the included couple refuse to undergo any type of Assisted Reproductive Technologies.

The primary outcome measure was the biochemical pregnancy rate defined as a rising  $\beta$ -hCG in 2 occasions. The secondary outcome measures were ovarian response to stimulation defined by number of follicles recruited seen on ultrasound.

Data are shown as mean  $\pm$  SD or as raw numbers and percentages. Data analysis was performed using the statistical package for social science for Windows version 20.0 (IBM, SPSS). Continuous data were analyzed with unpaired Student’s t-test while categorical variables by chi-square test. Values of  $P < 0.05$  were considered to indicate significant differences.

### 3. RESULTS

Table 1: Demographic data of the study groups. Values are mean  $\pm$ SD or percentages.

Parameter	Group A	Group B	P value
Age ( year)	41.40 $\pm$ 2.05	42.35 $\pm$ 1.99	P>0.05
BMI (Kg/m <sup>2</sup> )	26.5 $\pm$ 3.50	26.35 $\pm$ 1.65	P>0.05
FSH (mIU/ml)	13.61 $\pm$ 6.39	17.58 $\pm$ 8.73	P>0.05
E2 (pg/ml)	56.36 $\pm$ 21.84	41.99 $\pm$ 14.39	P<0.05
AMH (ng/ml)	1.26 $\pm$ 0.98	0.49 $\pm$ 0 .36	P<0.05
Type of infertility%			
Primary	40.9	47.1	p>0.05
secondary	59.1	52.9	
Duration of infertility (years)	3.72 $\pm$ 2.93	9.82 $\pm$ 16.58	P<0.05
Follicle number	2.14 $\pm$ 0.56	1.94 $\pm$ 0.24	p>0.05
Pregnancy rate%	36.4	5.9	P<0.05

Table 1 shows that the mean age of group A is  $41.40 \pm 2.05$  years which differ insignificantly from group B women ( $p > 0.05$ ). BMI in both groups is of no significant difference ( $26.5 \pm 3.50$  vs  $26.35 \pm 1.65$  Kg/m<sup>2</sup>). The basal mean serum level of FSH in both groups is of no significant difference. Basal serum level of E2 in group A is  $56.36 \pm 21.84$  pg/ml while it is  $41.99 \pm 14.39$  in group B, the difference is significant among them ( $p < 0.05$ ). Serum AMH in group A is significantly higher than group B ( $1.26 \pm 0.98$  VS  $0.49 \pm 0.36$ ) ng/ml.

Primary infertility is present in 40.9 % of women in group A and 47.1% in group B while secondary infertility is present in 59.1% in the former group and in 52.9 % in the latter group, the difference is insignificant. All women in group A suffered from infertility for  $3.72 \pm 2.93$  years while women in group B have infertility for  $9.82 \pm 16.58$  years the difference is significant. Ovarian stimulation in group A resulted in follicle number of  $2.14 \pm 0.56$  while in group B  $1.94 \pm 0.24$  the difference is insignificant. The pregnancy rate in group A was 36.4% while its 5.9% in group B the difference is of significant value.

#### 4. DISCUSSION

Our results regarding baseline characteristic of the study groups are comparable to many studies in the US (10, 11). As table 1 demonstrates that serum levels of AMH and FSH and E2 concentrations in the DHEA group (B) are highly confirmatory of a significant degree of diminished (OR). There were no significant difference between the two included groups from the age of women, BMI, and baseline FSH. While baseline E2 and AMH are significantly lower in group B, as well as the duration of their infertility is significantly longer due to their diminished (OR). According to the two cells /two gonadotrophin theory, androgens play an essential role in ensuring adequate follicular steroidogenesis in humans (12), therefore female fertility. Androgen receptors are abundant in the granulosa cells of healthy preantral and antral follicles of rhesus monkeys and their expression is up-regulated by androgen (13, 14). Androgens exert effects through transcriptional regulation but also in non-genomic ways, with ligand-activated androgen receptors modulating (FSH) activity in granulosa cells by synergistic effect (15, 16).

Dehydroepiandrosterone is of predominantly adrenal but also ovarian in origin, it is the major source of androgen synthesis in women and is converted to androgens (androstenedione and testosterone (17) or estrogens based on the expression of steroidogenic enzymes present in peripheral target tissues, including the ovarian follicle (18). (DHEA), appear effective in improving functionality OR in women with diminished OR best assessed by (AMH) and antral follicle count (AFC) (19). Previously published clinical observations suggested that approximately 2 months of DHEA supplementation were required before statistically significant differences in outcomes could be observed (3, 6). It seems that DHEA may enhance the follicular environment through: augmentation of the growth promoting and survival enhancing effect of Insulin Growth Factor-I (IGF-I); LH-stimulated follicular androgen and estrogen production (5); and the augmentation of granulosa cell FSH-receptor (FSH-r) expression and associated increase in the number of growing preantral and small antral follicles (20, 16).

DHEA could potentially improve oocyte quality as well via the GH axis through the promotion of DNA repair in oocytes (21) and mitochondrial activity in both follicular cells and oocytes (22).

This prospective comparative study investigated patients with diminished OR, supplemented for 2 months with DHEA (25 mg three times daily) prior to gonadotrophin stimulation, this short period in our opinion is to minimize the potential side effects of DHEA. We were not able to demonstrate any beneficial effect of DHEA administration on the OR in term of the number of follicles obtained and pregnancy rate in women undergoing controlled ovarian stimulation. These results matches well with those of a recent studies using a more physiological model, in monkeys that could not confirm that androgens improve the ovarian sensitivity to gonadotrophins(23), it also matches with Kelani co-workers 2010 (24) who didn't prove that DHEA supplementation in IVF patients to improve oocyte yield and pregnancy outcome. He had used it before controlled ovarian stimulation but for female with normal OR. Gleicher et al demonstrate improve in OR and pregnancy rate in a study conducted in 2010, he used DHEA for longer time and his study group was younger in age than ours (<38 years) (10) and also OR of our study group is lower than their study group.

Barad and Gleicher and their work teams (4, 5, 6) who was the most active team in investigating the effect of DHEA on improving OR demonstrated that follicular numbers and oocytes yield increase up to approximately 5 months of DHEA supplementation which equal to the approximate time period from primordial stages to gonadotrophin sensitivity. DHEA supplementation in their study may have increased the AFC which we haven't included it in our study.

Our results go well with the most recent review of Narkwichean et al. 2013 (25) who reviewed all the published controlled studies (including all the above mentioned studies) and they summarize the role of DHEA as an adjuvant to stimulation protocol in women with diminished OR or poor-responders and they concluded that there are insufficient data to support a beneficial role of DHEA as an adjuvant to controlled ovarian stimulation. So it is clear that the opinions

are clearly divided on this issue, and the prevailing uncertainty perhaps suggests that it is time to evaluate the clinical and cost effectiveness of DHEA in the context of a large well-designed multicenter randomized controlled trial.

## **5. CONCLUSION**

our study failed to demonstrate any beneficial effect of DHEA to improve OR in women above or = 40 with baseline poor OR in term of follicular recruitment and pregnancy rate after controlled ovarian stimulation. Efficacy of androgen supplementation with DHEA seems to vary depending on female age and baseline OR.

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