Reproductive Biology Section

DHEA pre-treated patients, poor responders to a first IVF (ICSI) cycle: clinical results

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Summary

Purpose: To evaluate the effect of the premedication with dehydroepiandrosterone (DHEA) on the results of the in vitro fertilization (IVF) treatments in a group of women with evidence of diminished ovarian reserve. *Materials and Methods:* This experimental, prospective, pre-post study enrolled 29 patients with evidence of diminished ovarian reserve and poor-responders to a previous treatment. They received 75 mg/die of DHEA for a minimum of eight weeks; from the 18th day of the cycle before the stimulation with follicle stimulating hormone (FSH), they took trans-dermal estradiol (E2) (50 mcg every other day). The protocol of the stimulation consisted of a short cycle with follicle stimulating hormone receptor-human menopausal gonadrotropin (FSHr-HMG) and low doses of gonadotropin releasing hormone agonist (GnRH-a) (0.05 mg/die). The study was carried out comparing the results obtained respectively with the pre-DHEA and the post-DHEA treatments. *Results:* The comparative analysis of the results showed a significant increase in the number of the retrieved oocytes ($p < 0.01$), of the oocyte quality ($p = 0.02$) and a reduction of cancelled cycles ($p = 0.03$). Moreover, after the treatment with DHEA, there was an increase, though non-significant, in the number of embryos, in the fertilization rate, and in the number of pregnancies. *Conclusions:* This study confirms the beneficial effects of DHEA in patients who resulted *poor responders* to IVF treatments. Therefore, DHEA appears to be an effective treatment for age related sub-fertility.

Key words: Dehydroepiandrosterone (DHEA); Diminished ovarian reserve (DOR); FIVET/ICSI cycle; Non responders (OFF)<; Poor ovarian response (POR).

Introduction

With the trend in increased rates of infertility, connected to the aging of the Western population, the problem of low response to infertility treatment is becoming more and more frequent. With the advancing of the women's age, in fact, there is a reduction in the quantity and quality of the oocytes with increased rates of embryo aneuploidy and, consequently, reduced pregnancy rate and increased miscarriage rate [1].

In general, the majority of women reach menopause around 50 years. In the Venetian region, after the Resolution n. 822, 06/14/2011, the access to treatment (1st/2nd level) for infertility in women it was extended up to the age of 50 years at the expense of the NIH. The biological infertility, however, dramatically increases already 10-15 years before menopause [2].

The majority of these patients, in fact, due to the scarcity of the ovarian level of residual follicular pool, tend to be *poor-responders* to fertility treatment. It is estimated that the 5% - 18% of the treatments with in vitro fertilization (IVF) fail because of a low ovarian response to pharmacological stimulation [3]. The definition of *poor ovarian response* (POR), which was subjective and variable according to different researchers, was internationally standardized by European Society of Human Reproduction and Embryology (ESHRE) in 2011, according to the Bologna criteria [4]. As a result of this consensus conference, the definition of *low ovarian response* to treatments of IVF requires the presence of at least two of the following characteristics: 1) advanced maternal age (> 40 years) or the presence of other risk factors for low ovarian response; 2) a previous finding of poor-response (< three oocytes following a conventional stimulation protocol); 3) a test of the abnormal ovarian reserve (antral follicle count [AFC] < five to seven follicles in the two ovaries or anti-Müllerian hormone [AMH] < 0.5 to 1.1 ng /ml).

Various stimulation protocols were proposed, in the literature, for the treatment of *poor-responders* patients [5-8].

Casson *et al.* [9] were the first to highlight the beneficial effects of DHEA on the ovarian function. Barad and Gleicher in 2005 [10], demonstrated an increased production of follicles after a treatment with DHEA, and in the following year the same group underlined the beneficial effect of the hormone on various parameters of IVF (the peak of estradiol [E2], the number and quality of oocytes and embryos) in women with evidence of diminished ovarian reserve [11]. In 2007 [12], the same authors reported other positive effects such as increased pregnancy rate and a reduction in its "waiting time", related to DHEA intaking for at least six weeks before the ovarian stimulation. Wiser *et al.* in 2010

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[3], in the only prospective randomized study on this subject, affirmed that there is a direct proportional relationship between embryo's quality and the duration of treatment, showing also an increasing rate of live births as a result of a pre-treatment with DHEA. Gleicher, in 2010 [13], noted, finally, that the supplementation with DHEA induces a reduction in the number and percentage of aneuploid embryos. The studies in literature, therefore, seem to consider DHEA as a good candidate in the treatment of *poor-responder* patients with a low age-related ovarian reserve.

The purpose of this study was to evaluate prospectively the effects of supplementation with DHEA in the same patients, who were poor responders to a first conventional treatment of ovarian stimulation.

Materials and Methods

Selected patients

The study enrolled 29 patients with clinical evidence of diminished ovarian reserve, which were *poor responders*, according to the Bologna criteria, to the previous treatment with IVF.

The criteria for the exclusion from the study were: older than 45 years, FSH> 30 mIU/ml, AMH < 0.5 ng/ml, body mass index (BMI) > 30.

After an adeguate explanation and the signing of the informed consent, the recruited patients were subjected to the following therapeutic treatment:

a) Pre-treatment, of at least eight weeks, with DHEA with a dosage of 25 mg x3/die per os.
b) Application of E2, using 50 mcg trans-dermal patches every other day, from the 18th day of the cycle before the menstruation of the cycle of stimulation.
   Both A and B were interrupted on the day of menstruation, day 0.
c) Short cycle of stimulation with low doses of gonadotropin releasing hormone agonist (GnRH-a) (0.05 mg/die s. and follicle stimulating hormone receptor (FSHr) gonadotropin (300 IU/ die s. c.) and human menopausal gonadrotropin (hMG) (150 IU/ die s. c).

The criteria used for the definition of patients not responding to stimulation (OFF) were: an ultrasound checked number of ovarian growing follicles < two and/or E2 < 500 pg/ml.

Once obtained two or more follicles of dimensions ranging between 17 and 19 mm, ovulation was induced through the injection of 250 mg of human chorionic gonadotropin receptor (HCGr) s. c.

Thirty-six hours after induction, an oocyte pick-up (OPU) was carried out in sedation.

The oocytes collected were classified according to the degree of maturity in: germinal vesicles, metaphase I, and metaphase II. From the pool of the collected oocytes, those ones in metaphase II were isolated and used for fertilization with fertilization with intracytoplasmic sperm injection (ICSI).

Forty-eight hours after OPU, the embryo-transfer (ET) was executed.

The luteal support consisted in the use of micronized progesterone, per os or vaginally, (200 mg twice/die) and progesterone i. m. (50 mg/die) until at least the 14th day from the ET (beta-HCG dosage).

Study plan and statistical analysis

The study plan was *experimental pre-post*, it was performed enrolling patients initially subjected to a conventional treatment, and in succession to a short cycle with low doses of GnRH-a associated with pre-treatment with DHEA, lasting at least eight weeks. The authors then proceeded to the comparison of the results obtained with the two protocols, taking into account the following variables: number of retrieved oocytes, number of oocytes placed in fertilization (oocytes in metaphase II), number of fertilized oocytes, fertilization rate, percentage of patients not responding to treatment (OFF), beta-HCG positivity, and evolution of pregnancy.

The McNemar test was used for the statistical elaboration of the qualitative dichotomous variables (OFF and positivity of the beta-HCG). In case of qualitative multivaried variables and in the evolution of pregnancy, the Bowker test of symmetry was applied.

Quantitative variables, such as the number of retrieved oocytes, the number of oocytes placed in fertilization, the number of fertilized oocytes, and fertilization rate were analysed with the Wilcoxon signed ranks test, bringing the median (the value/modality assumed by the combined statistics that are in the middle of the distribution of a quantitative or qualitative sortable character) and maximum and minimum extremes.

All the tests used were performed at two code, considering as statistically significant a value of $p < 0.05$.

Results

The study was conducted from January 2011 to September 2012. In this period 29 patients were recruited. They were poor-responders to previous conventional treatment (called pre-DHEA) and then subjected to the protocol proposed by us using pre-medication with DHEA (defined post-DHEA).

The results, obtained using comparative analysis, are summarized in Table 1.

The proportion of patients not responding to treatment, defined as OFF, was found to be 9/29 (31%) in case of stimulation with conventional protocols (pre-DHEA), while only 2/29 (7%) with DHEA.

The differences proved to be statistically significant ($p = 0.03$), with evidence of increased response to hormonal stimulation cycle thanks to the pre-treatment with DHEA. During data processing it was showed that in a total of 27 women responding to the DHEA protocol, 19/27 completed treatment with both regimens, while 8/27 were *non-responders* with the classic protocols. Finally, only an enrolled patient was unresponsive to the medication with DHEA but responding to the previous cycle, and in one case there was no response (Table 2).

In the total number of treated patients (29 = 100%), therefore, 65.5% resulted responding to both treatments, 3.5% responded to conventional treatment but not to the DHEA protocol. Another 3.5% resulted non-responder in both protocols, 27% responded to the DHEA protocol, while it was cancelled from the previous cycle of stimulation.

A total of 27 patients, therefore, had access to the stage of oocyte sampling and to the possible IVF, thanks to the treatment with the new stimulation protocol with DHEA. By analysing the amount of retrieved oocytes, the median with conventional treatments resulted one oocyte, with a maximum of four and a minimum of zero and a total of

Table 1. — *Comparison of the results obtained with pre- and post-DHEA treatment.*

	Pre-DHEA	Post-DHEA	*p*
Patients	29	29	-
Mean age (years)	39.03 ± 1.2	39.7 ± 1.25	-
OFF	9/29 (31%)	2/29 (7%)	0.03
Median Retrieved oocytes (max - min)	1 (4-0) Tot = 35	2 (5-0) Tot = 66	< 0.01
Median oocytes in fertilization (max - min)	1 (4-0) Tot = 32	2 (5-0) Tot = 53	0.02
Median fertilized oocytes	0 (3-0) Tot = 20	1 (4-0) Tot = 33	0.05 (n.s.)
Median fertilization rate (max - min)	0 (1-0)	0.5 (1-0)	0.10 (n.s.)
Beta-hCG positivity (%)	0/29 (0%)	4/29 (13.8%)	0.12 (n.s.)
Evolutive pregnancies	0/29 (0%)	2/29 (6.9%)	0.26 (n.s.)

Table 2. — *McNemar Test: distribution of responding patients with conventional treatment (Pre-DHEA) and DHEA protocol (post-DHEA)*

		Pre-DHEA		
		ON	OFF	TOTAL
Post-DHEA	ON	19 (65.5%)	8 (27.5%)	27 (93%)
	OFF	1 (3.5%)	1 (3.5%)	2 (7%)
	TOTAL	20 (69.0%)	9 (31.0%)	29 (100%)

35, while it was two oocytes resulted with the protocol DHEA, with a maximum of five and minimum of zero and a total of 66. Applying the test function, the difference between the oocytes found in the two treatments was statistically significant ($p < 0.01$), with an evident greater number obtained using the DHEA protocol. Pre-treatment with DHEA, therefore, increased the amount of oocytes recruited, while increasing the chances of a successful fertilization.

This protocol demonstrated to increase not only the number of oocytes, but also the mature oocytes suitable for insemination, reducing the proportion of immature (germ vesicle, metaphase I) and degenerate cells. With the conventional treatment, in fact, the median of the oocytes placed in fertilization was found to be one, with a maximum of four and a minimum of zero and a total of 32, while DHEA resulted in a median of two, with maximum of five and a minimum of zero oocytes and the total of 53. The difference of oocytes "in fertilization " resulted statistical significant ($p = 0.02$), and the greater number of cells was obtained with the DHEA protocol.

To evaluate the success of insemination, the number of fertilized oocytes and fertilization rate (comparison between the fertilized oocytes and those put into insemination) were analysed.

The median of fertilized oocytes was found to be zero, with a maximum of three and a minimum of zero and a total of 20, with the pre-DHEA protocols, while it was one, with a maximum of four and a minimum of zero and a total of 33, with the post-DHEA protocol. The differences, however, were nearly but not statistically significant ($p = 0.05$). With regards to the fertilization rate, a median of zero was obtained by the first treatment, with a maximum of one and a minimum of zero, while the second one obtained a median of 0.5, with a maximum of one and a minimum of zero. Even in this case, however, the difference was not found to be statistically significant ($p = 0.10$). The lack of statistical significance was most likely caused by the low number of recruited patients and the small scale of the total embryos obtained, respectively 16 with conventional protocols and 31 with DHEA. However, the results indicate that DHEA has a tendency to increase the chance of fertilization and, therefore, the indication is to use it to enhance the chances of success of the IVF techniques. Thanks to the regimen of pre-medication with DHEA, a total of four pregnancies (13.8%) were obtained. Only two of them evolved: an early abortion occurred in the other two cases. However the difference between the two treatments, with regards to the number of pregnancies and their progression, did not reach statistical significance ($p = 0.12$ and $p = 0.26$, respectively), because of the low number of patients enrolled in the study and the few obtained pregnancies.

Discussion

This study confirms that pre-medication with DHEA, associated with a short cycle with low doses of GnRH-a, increases the overall probability of success in poor-responder patients with clinical evidence of diminished ovarian reserve (DOR). Therefore this clinical trial showed that the protocol with DHEA increases the response rate to stimulation treatments (in situations of poor response to ovarian stimulation), reducing the percentage of cancelled cycles. Besides increasing statistically the number of patients who reach the end of the treatment, DHEA significantly increases the number of retrieved oocytes and the pool of mature cells suitable for insemination, reducing the percentages of immaturity and degeneration. Therefore benefits of DHEA in improving the ovarian function in *poor responders* are confirmed, as reported by several studies in the literature [11-13].

Thanks to this regimen, a propensity to an increased success rates of stimulation treatments is also shown, as judged by the number of embryos and pregnancies obtained, though without reaching statistical significance, as highlighted in other trials [12].

The mechanism by which DHEA induces all the effects reported in the literature and partially demonstrated in this study, still remains unknown. One hypothesis is that DHEA has a synergistic effect with gonadotropins and exerts, therefore, a selective control in the early stages of follicular maturation [14]. Besides the synergy with the gonadotropins

(chiefly FSH), a direct role of DHEA on ovarian function is hypothesized. The notion derives from the observation of the evolution of the concentration levels of endogenous DHEA: the peak is reached when a woman is between 20 and 30 years of age, followed by a progressive decline of about two percent annually [15].

DHEA is not only the precursor of androstenedione, testosterone, and E2 but it can also influence the follicular growth acting as a ligand for the androgen receptors and/or through alternative non-receptorial ways [16]. Another possible mechanism was described by Casson *et al.* [17]. They experienced a transient increase of IGF-1 in patients undergoing induction with exogenous gonadotropins after pretreatment with DHEA, and they related it to the parallel increase in androgens.

Barad and Gleicher [11] postulated that the effects of DHEA can be due to the creation of a poycystic ovarian syndrome (PCOS) -like situation in elderly ovaries. In fact polycystic ovaries were described as a deposit of primordial follicles in transition to primary ones, by the action of GF, LH, and ovarian androgens, whose levels are increased in this pathology. The ovarian theca cells of the pre-antral follicles produce normally androstenedione, testosterone and DHEA: women with PCOS have higher levels of testosterone, androstenedione and DHEA in serum and in ovarian veins [18]. Therefore the prolonged exogenous androgens administration could induce the changes (histologically and sonographically seen) in the ovaries similar to those ones of women with PCOS (18). Therefore the creation of a PCOS-like environment, could explain the cumulative effect of DHEA on antral follicles, observed by Barad and Gleicher [11].

DHEA is a weak androgen that is administered in the U.S.A. as a dietary supplement, without the need for a prescription. The potential side-effects include: acne, hirsutism, alopecia, and oily skin. However, these androgenic effects are minimal at the therapeutic doses of 75 mg/day [19] and they did not occur in any patient in this study. Moreover, considering that different physiological situations of women, such as pregnancy, are themselves characterized by a high level of DHEA, low doses of DHEA are safe, without causing any health injury [20].

Doubts were raised regarding the safety of the treatment because DHEA, as a precursor of androgens and estrogens, could increase the risk of androgen or estrogen-dependent cancers [21]. However because of the low dose and short duration of administration of DHEA in the proposed protocol treatment, these risks cannot exist.

The most obvious limitation of this study is the lack of randomization that, in some way, might have influenced the results because of a possible effect of variables that were not considered in the study and not randomly distributed.

Moreover, strength of the study is the evaluation of the same patients who underwent both types of treatments, since this procedure eliminates the inter-personal variability from an ethnic, social, and pathologic point of view that could affect observations.

The fact that the same women were subjected to successive cycles of stimulation might suggest that the good results obtained with the proposed protocol are influenced by cumulatively previous stimulation. However, since the stimulations performed in the present centre are spaced by at least four months, to restore a condition of ovarian "rest", each treatment should be free from influences of the previous cycle.

The few samples (29 patients) are certainly a limitation of the study. However, the present work is one of the few perspective studies carried out on this topic. The only randomized perspective study on the effects of DHEA, for example, includes only 17 cases and 16 controls [3]. Moreover the sample quantity makes it difficult to achieve statistical significance regarding the analysis of probability in which the percentage of positivity is further reduced. In fact, given the limited probability of success related to the low ovarian reserve condition to *ovarian aging,* the number of pregnancies and their development, are quantitatively small and are therefore inadequate to extend the sample assessments to population.

Conclusions

The limitations of this study are mainly two: *a few samples* (moreso, the same women were perspectively evaluated in two successive cycles), *the diversity of the protocols and the doses of gonadotropins used in the two cycles* (furthermore the short protocol with high doses of FSH in the first cycle were also predominantly used). After these clarifications it is not an exaggeration to state that pre-medication with DHEA appears to be a valid approach in the treatment of subfertility caused by age related reduced ovarian reserve because of its efficacy and the lack of side-effects.

Further studies with larger, prospective, and randomized trials are necessary to confirm the evidence discovered until now.

References

[1] te Velde E.R., Pearson P.L.: "The variability of female reproductive ageing". *Hum. Reprod. Update*, 2002, *8*, 141.

[2] Hansen K.R., Knowlton N.S., Thyer A.C., Charleston J.S., Soules M.R., Klein N.A.: "A new model of reproductive aging: the decline in ovarian non- growing follicle number from birth to menopause". *Hum. Reprod*., 2008, *23*, 699.

[3] Wiser A., Gonen O., Ghetier Y., Shavit T., Berkovitz A., Shulman A.: "Addition of dehydroepiandrosterone (DHEA) for poor responder patients before and during IVF treatment improves the pregnancy rate: a randomized prospective study". *Hum. Reprod*., 2010, *25*, 2496.

[4] Ferraretti A.P., La Marca A., Fauser B.C.J.M., Tarlatzis B., Nargund G., Gianaroli L., ESHRE working group on Poor Ovarian Response Definition.: "ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria". *Hum. Reprod*. 2011, *7*, 1616.

[5] Dragisic K.G., Davis O.K., Fasouliotis S.J., Rosenwa ks S.J.: "Use of a luteal estradiol patch and a gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation for in vitro fertilization in poor responders". *Fertil. Steril.* 2005, *84*, 1023.

[6] Kim C.H., Jeon G.H., Cheon Y.P., Jeon I., Kim S.H., Chae H.D., Kang B.M.: "Comparison of GnRH antagonist protocol with or without oral contraceptive pill pretreatment and GnRH agonist low-dose long protocol in low responders undergoing IVF/intracytopasmic sperm injection". *Fertil. Steril*., 2009, *92*, 1758.

[7] Berin L., Stein D.E., Keltz M.D.: "A comparison of gonadotropin-releasing hormone (GnRH) antagonist and GnRH agonist flare protocols for poor responers undergoing in vitro fertilization". *Fertil. Steril*., 2010, *93*, 360.

[8] Shastri S.M., Barbieri E., Kligman I., Schoyer K.O., Davis O.K., Rosenwaks Z.: "Stimulation of young poor responder: comparison of the luteal estradioil gonadotropin-releasing hormone antagonist priming protocol versus oral contraceptive microdose leuprolide". *Fertil. Steril*., 2011, *95*, 592.

[9] Casson P.R., Lindsay M.S., Pisarska M.D., Carson S.A., Buster J.E.: "Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: a case series". *Hum. Reprod*., 2000, *15*, 2129.

[10] Barad D., Gieicher N.: "Increased oocyte production after treatment with dehydroepiandrosterone". *Fertil. Steril*. 2005, *84*, 756.

[11] Barad D., Gleicher N.: "Effect of dehydroepinadrosterone on oocytes and embryo yields, embryo grade and cell number in IVF". *Hum. Reprod*., 2006, *21*, 2845.

[12] Barad D., Brill H., Gleicher N.: "Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function". *J. Assist. Reprod., Genet*., 2007, *24*, 629.

[13] Gleicher N., Weghofer A., Barad O.H.: "Dehydroepiandrosterone (DHEA) reduces embryo aneuploidy: direct evidence from preimplantation genetic screening (PGS)". *Reprod. Biol. Endocrinol*., 2010, *8*, 140.

[14] Gleicher N., Weghofer A., Barad D.: "The role of androgens in follicle maturation and ovulation induction: friend or foe of infertility treatment?" *Reprod. Biol. Endocr*., 2011, *9*, 116.

[15] Jacob M.H., da R Janner D., Jahn M.P., Kucharski L.C., Belló-Klein A., Ribeiro MF.: "Age-related effects of DHEA on peripheral makers of oxidative stress". *Cell Biochem. Funct*., 2010, *28*, 52.

[16] Hiller S.G., Whitelaw P.E., Smyth C.D.: "Follicular estrogen synthesis: the "2-cell-two-gonadotrophin" model revisited". *Mol. Cell Endocrinol*., 1994, *100*, 51.

[17] Casson P.R., Santoro N., Elkind-Hirsch K., Carson S.A., Hornsby P.J,. Abraham G., Buster J.E.: "Post-menopausal dehydroepiandrosterone administration increases free insulin-like grow factor-1 and decreases high-density lipoprotein: a six-month trial". *Feritil. Steril*., 1998, *70*, 107.

[18] Amirikia H., Savoy-Moore R.T., Sundareson A.S., Moghissi K.S.: "The effects of long-term androgen treatment on the ovary". *Fertil. Steril*., 1986, *45*, 202.

[19] Kroboth P.D., Salek F.S., Pittenger A.L., Fabian T.J., Frye R.F.: "DHEA and DHEA-S: a review". *J. Clin. Pharmacol*., 1999, *39*, 327.

[20] MacCiamrock H.O. Adashi E.Y.: "Gestational hyperandrogenism". *Fertil. Steril*., 1992, *57*, 257.

[21] Kaaks R., Berrino F., Key T., Rinaldi S., Dossus L., Biessy C., *et al*.: "Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC)". *J. Natl. Cancer lnst*., 2005, *97*, 755.

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