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Original Article

Efficiency of DHEA on diminished ovarian reserve patients undergoing IVF-ET based on ultrasonography

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Abstract: Objective: To evaluate the efficiency of DHEA in diminished ovarian reserve (DOR) patients treated with *in vitro* fertilization and embryo transfer (IVF-ET) using ultrasonography. Methods: A total of 193 DOR patients undergoing IVF-ET from Jan. 2012 to Dec. 2014 were enrolled. The included patients were classified into DHEA group (n = 98) receiving DHEA treatment and control group (n = 95) with placebo treatment. Endocrine indexes and ultrasonography parameters before and after DHEA treatment were compared. Number of oocytes retrieved after IVF-ET was counted. Based on the ultrasonography results, ROC curve was plotted to compare the DHEA efficiency. Results: The post-treatment follicle-stimulating hormone (FSH) levels, follicle-stimulating hormone/luteinizing hormone (FSH/LH) were remarkably decreased, while testosterone (T) levels were elevated in the DHEA group, compared with the pre-treatment and those in the control group (all $P < 0.05$). Increases of vascularization index (VI), vascularization-flow index (VFI) and antral follicle counts (AFC) in the DHEA group were observed compared with control group. The ROC curve of VI demonstrated that area under the curve (AUC) was 0.774 (95% CI = 0.708-0.840, $P < 0.001$) with cut-off value, sensitivity and specificity of 1.908, 73.5% and 68.4%, respectively. The ROC curve of VFI revealed that AUC and cut-off value were 0.676 (95% CI = 0.599-0.753, $P < 0.001$) and 0.837 with sensitivity and specificity of 70.4% and 57.9%, respectively. The ROC curve of AFC also showed that the AUC was 0.604 (95% CI = 0.524-0.684, $P = 0.013$) with cut-off value, sensitivity and specificity of 7.775, 64.3% and 51.6%, respectively. After IVF-ET, the number of retrieved oocytes, fertilization rate and pregnancy rate in the DHEA group were significantly improved compared with those in the control group (all $P < 0.05$). Conclusion: DHEA showed great benefits in the improvement of ovarian reserve function for DOR patients and the application of ultrasonography can effectively evaluate the efficiency of DHEA in IVF-ET, thus ultrasonography provides valuable information for DOR treatment.

Keywords: Diminished ovarian reserve, *in vitro* fertilization and embryo transfer, dehydroepiandrosterone, ultrasonography, ultrasonography parameters, endocrine indexes

Introduction

Diminished ovarian reserve (DOR), also known as poor ovarian reserve, is a condition of low fertility characterized by decreased quality and quantity of oocytes in the ovaries [1]. DOR, resulted from physiological ageing of the ovaries or premature ovarian ageing, is a major challenge in reproductive medicine, which is associated with miscarriage leading to disappointing live-birth rates [2, 3]. Controversy exists due to the undefined diagnostic criteria of DOR and the accuracy of the tests used to predict poor ovarian reserve. The poor ovarian response, a measurable variable of DOR, is stilling improving with modern female tend to defer their fertility [4, 5]. It is estimated that about 5~18% of assisted reproductive technology (ART) cycles are cancelled due to poor ovarian response [6]. Currently, people become more aware of the importance of aging on fertility, ovarian reserve assessment and interventions to prevent age-caused infertility [7]. Although several strategies are commonly applied, such as increasing gonadotroph in dosage, the oocyte yield is still unsatisfactory for low pregnancy rates [8]. Moreover, none of them have been proven to be the optimal [9].

Dehydroepiandrosterone (DHEA), an important endogenous steroid hormone, is primarily produced in the zonareticularis of the adrenal cortex and in the ovaries [10]. Despite its function *in vivo* remains inconclusive, DHEA acts as a

steroid prehormone for ovarian follicular sex steroidogenesis, which will be highly concentrated in reproductive stage and progressively decrease with aging [8]. In 2000, Casson *et al*. first suggested that a short-term exogenous DHEA administration was partially effective in improving ovarian function in elderly women with DOR [11]. Accumulating evidence shows positive effects of DHEA supplementation on improving ovarian response in DOR women undergoing *in vitro* fertilization and embryo transfer (IVF-ET) treatment, presenting better end points of peak estradiol level, more number of retrieved oocytes and embryos reserved, improved embryo quality and anti-Mullerian hormone expression levels [1, 12]. Ovarian reserve markers, antral follicle counts (AFC), vascularization index (VI) and vascularization-flow index (VFI) are critical in DOR treatment [13], and a previous study demonstrated that three-dimensional (3-D) ultrasonography would be reliable and valid in AFC assessment [14], however, the ultrasonography advantages in evaluating DHEA efficiency on DOR need further confirmation [15]. Therefore, in current study, ultrasonography was used to evaluate the efficiency of DHEA in DOR patients treated with IVF-ET.

Methods and materials

Ethics statement

All experiments were in strict accordance with protocol approved by the local Committee of the First Affiliated Hospital of Guangxi Medical University as well as Guangxi Nanning Center for Disease Control and Prevention. Study protocols followed the ethical principles for medical research involving human subjects of the Helsinki Declaration [16]. Each subject was aware of the potential side effects of DHEA and written informed consent was obtained.

Subjects

We included 193 women diagnosed as poor ovarian reserve in the First Affiliated Hospital of Guangxi Medical University from Jan 2012 to Dec 2014. The included subjects had a mean age of of 31.85 ± 2.64 years, ranging from 23 to 42 years. Subjects were classified into DHEA group (treated with DHEA, n = 98) and control group (treated with placebo, n = 95). The diagnostic criteria for DOR were [7]: (1) bilateral AFC < 10, basic follicle-stimulating hormone (FSH) > 101 U/L, FSH/luteinizing hormone (FSH/LH) > 3.6, basic serum E2 level > 80 pg/mL; (2) after HCG injection, mature follicle < 3-5 with diameter > 14 mm or number of oocytes retrieved < 3-5 and peak E2 serum level < 300-500 pg/mL, daily dosage for Gn > 300 IU or total dosage > 3000 IU (25-44 packs).

The inclusion criteria were: (1) serum hormone levels on the 3rd day of menstrual cycle meet diagnostic standards; (2) patients had following high risk factors for DHEA: i, age > 35 years; ii, ovarian diseases: endometriosis, ovarian tuberculosis, severed inflammations and adhesions around the pelvic cavity and ovary, chlamydia infection, ovarian inflammation caused by cytomegalovirus infection, ovarian dysplasia and unilateral ovary; iii, history of ovarian and fallopian tube surgery, including unilateral ovariectomy and ovarian cyst stripping; iv, immunological factors: antibodies to zonapellucida, anti-ovarian antibodies (AoAb) and anti Gn receptor antibody; v, environmental and lifestyle factors: unhealthy lifestyle, such as smoking and alcoholism; long-term exposure to physical radiation and hazardous substances, such as organic solvent, insecticide, heavy metal; (3) patients had no history of drug therapy within 2 months and patients were compliance with return visit to complete the clinical observation. Patients were excluded based on following criteria: (1) irregular menstruation or infertility caused by congenital dysplasia of female genital organs or acquired female genital organs lesions; (2) received hormonal drugs in past 3 months; (3) subjects failed to take the medicine properly, or subjects dropped out of experiment due to intolerance to DHEA, or subjects with incomplete clinical data.

Medication and detection of endocrine and baseline indexes

During the prophase of IVF-ET treatment, oral DHEA (General Nutrition Corporation, America) or placebo for continuous 3 months thrice a day (25 mg per 8:00 am, 14:00 pm and 20:00 pm) was delivered for the subjects in the corresponding groups. Age, infertility duration and basic antral follicle count were also obtained and collected. The serum levels of endocrine indexes before and after the treatment were examined and recorded. Specifically, on the 2nd or 3rd day of the menstrual cycle, 5 mL fasting blood were extracted from each subject. Then the FSH, LH, estradiol (E2) and testosterone (T)

levels were measured by applying the automated chemiluminescence immunoassay system (ADVIA Centaur XP, SIEMENS) with sensitivities for FSH of 0.3 mIU/mL, LH of 0.07 mIU/mL, E2 of 7 pg/mL and T of 10 ng/dL.

Ultrasonography

A 3-D ultrasonic device from Shenzhen Mindray Bio-Medical Electronics Co., Ltd was applied to scan the bilateral ovary of each subject both before and after three-month treatment to obtain complete and clear images of ovary. Then the multi-plane imaging model was used to describe the ovarian trails within the region of interest (ROI) and then the longitudinal section was rotated for 30°. Automatic volume calculation (SonoAVC) was used to calculate the AFC. Three-D power Doppler ultrasound with SonoAVC histogram was applied to calculate the volume of ovary and ovarian arterial blood flow indexes, including VI, FI and VFI.

Ultrasonography results were blinded assessed by two physicians who were blind to the research process or subjects information. Both of the two physicians had an experience of respectively 3 and 5 years.

IVF-ET treatment

After case group were received oral administration of DHEA for three month and control group were received placebo instead, both groups underwent IVF-ET, which was began with the injection of GnRH-a (Decapepthl, Germany) on the luteal phase before ART for pituitary down regulation, followed by ovarian stimulation with recombinant FSH (Gonal-F; Serono, Geneva, Switzerland) to observe the follicular development. Once the diameter of one follicle > 20 mm, or two follicles > 18 mm, the administration of GnRH-a was stopped and 250 μg of human chorionic gonadotropin (HCG, Merck, Serono, Switzerland) was injected on that night. Then transvaginal ultrasound-guided oocyte retrieval was performed 36 hours afterwards and embryo transfer was conducted in the 3rd day afterwards. The time of receiving Gn, Gn dosage and HCG dosage of each patient were recorded for further analysis.

Assessment of the embryo

Fertilization was assessed 20 hours after the embryo transfer via the appearance of pronucleus. Embryo quality was evaluated based on the criteria proposed by Modern Assisted Reproductive Technology [17]. The embryo was evaluated based on the shape and size of the blastomere as well as the percentage of fragmentation: grade I, equal-sized and well-shaped blastomeres, translucent ooplasm and 0 to 5% of fragmentation; grade II, more or less uniformly sized blastomeres, ooplasm with particles and 6% to 20% of fragmentation; grade III, non-uniformly sized blastomeres, ooplasm with large particles and 21% to 50% of fragmentation; grade IV, serous non-uniformly sized blastomeres, ooplasm with-coarse particles and > 50% of fragmentation. Number of retrieved oocytes, fertilization rate (number of prokaryotic embryo/number of retrieved oocytes), cleavage rate (number of cleavage cells/number of prokaryotic embryo), implantation rate (number of gestational sac/number of embryo transferred) and pregnancy rate (number of pregnancy/periodicity) were also calculated and recorded.

Statistical analysis

SPSS 19.0 software (SPSS, Chicago, IL) was used for data analysis. Continuous data were presented with mean ± standard deviation (SD) and compared by *t* test, while categorical data were expressed as percentage or rate and compared by χ^2 test. Receiver operating characteristic (ROC) curve was applied to evaluate the diagnostic values of ultrasonography in ovarian function. Two-tailed tests were conducted and $P < 0.05$ was considered statistically significant.

Results

Comparison on baseline characteristics

The baseline characteristics from each group were compared and found that age, infertility duration and basic antral follicle count between DHEA and control group had no statistical significance (all $P > 0.05$). Comparisons on the medication parameters after IVF-ET suggested that the time of receiving Gn, Gn dosage and HCG dosage were not statistical different between two groups (all $P > 0.05$) (**Table 1**).

Comparisons of endocrine indexes before and after DHEA

Our study found that the FSH level in DHEA group was significantly decreased after DHEA

Table 1. Comparisons on the baseline characteristics between case and control group

	Control (n = 95)	Case group (n = 98)	*P*
Age (year)	31.26 ± 3.15	31.85 ± 2.64	0.159
Infertility duration (year)	5.21 ± 4.56	5.36 ± 3.96	0.807
Basic antral follicle count	7.72 ± 2.67	7.69 ± 2.45	0.935
Gn received time (day)	11.69 ± 2.12	11.76 ± 2.56	0.837
Gn dosage (pack)	33.75 ± 9.89	34.01 ± 8.95	0.848
HCG dosage (pg/ml)	5425.18 ± 1256.31	5395.97 ± 1351.26	0.877

Note: HCG = human chorionic gonadotropin; Gn, gonadotropin releasing hormone analogues.

Table 2. Comparisons on endocrine indexes before and after DHEA treatment between case group and control group

Index	Before treatment (n = 193)		After treatment (n = 193)	
	Control group (n = 95)	Case group (n = 98)	Control group (n = 95)	Case group (n = 98)
FSH (IU/L)	11.11 ± 3.20	11.30 ± 3.23	10.82 ± 3.00	9.60 ± 2.96*,#
LH (IU/L)	3.39 ± 1.14	3.50 ± 1.11	3.60 ± 1.22	3.79 ± 1.23
FSH/LH	3.48 ± 1.07	3.36 ± 1.13	3.62 ± 1.52	2.67 ± 0.89*,#
E2 (pg/mL)	44.04 ± 10.74	44.23 ± 9.83	44.54 ± 11.72	42.46 ± 10.81
T (ng/dL)	32.11 ± 8.14	32.19 ± 8.32	32.59 ± 7.20	35.68 ± 7.01*,#

Note: DHEA = dehydroepiandrosterone; *, compared with before DHEA treatment, $P < 0.05$; #, compared with before DHEA treatmentin control group, $P < 0.05$; FSH = Follicle-Stimulating Hormone; LH = Luteinizing Hormone; FSH/LH = Follicle-Stimulating Hormone/Luteinizing Hormone; E2 = Estradiol; T = Testosterone.

treatment compared with that before DHEA treatment. Moreover, the post-treatment FSH level in DHEA group was lower than that in control group (DHEA group: after treatment vs. before treatment, 9.60 ± 2.96 *vs*. 11.30 ± 3.23; after treatment: DHEA group *vs.* control group, 9.60 ± 2.96 *vs*. 10.82 ± 3.00, both $P < 0.05$). Patients in the DHEA group had a decreased FSH/LH value after DHEA treatment (after treatment *vs.* before treatment, 2.67 ± 0.89 *vs*. 3.36 ± 1.13, $P < 0.05$), which was significantly reduced compared with that in the control group (after treatment: DHEA group *vs*. control group, 2.67 ± 0.89 *vs*. 3.62 ± 1.52, $P < 0.05$). In the DHEA group, T levels were increased (35.68 ± 7.01 *vs.* 32.19 ± 8.32, $P < 0.05$). Compared with the control group, T levels in DHEA group were also elevated (35.68 ± 7.01 *vs*. 32.59 ± 7.20, $P < 0.05$). No significant differences in post-treatment LH and E2 levels between two groups were found (all $P > 0.05$). In addition, no significant changes on LH and E2 levels were observed in DHEA group (all $P > 0.05$). The levels of endocrine indexes were not significantly different betweem before and after treatment in control group (all $P > 0.05$) (**Table 2**).

Comparisons on ultrasonography parameters after DHEA treatment

SonoAVC was utilized to calculate the AFC after ultrasonography was performed on each subject after treatment (**Figure 1A** and **1B**). The volume of ovary and ovarian arterial blood flow indexes were obtained from 3-D power Doppler histogram. The data in the current study showed that case group had increased VI, VFI and AFC after treatment compared with control group (VI, 2.012 ± 0.311 *vs*. 1.807 ± 0.213, $P < 0.05$; VFI, 0.894 ± 0.211 *vs*. 0.826 ± 0.125, $P < 0.05$; AFC, 8.46 ± 3.15 *vs*. 7.78 ± 2.32, $P < 0.05$, respectively). The FI and ovarian volume between case and control group were not significantly different (both $P > 0.05$) (**Table 3**).

ROC curve in evaluating ovarian function after DHEA

ROC area was applied to assess the DHEA efficiency in patients with poor ovarian reserve with larger area suggesting higher accuracy and diagnostic value. Ultrasonography parameters with statistical significances were chosen for ROC curve. The ROC curves of VI demonstrated that areas under the curve (AUC) was 0.774 (95% CI = 0.708-0.840, $P < 0.001$) with cut-off value, sensitivity and specificity of 1.908, 73.5% and 68.4%, respectively. The ROC curves of VFI revealed that AUC and cut-off value were 0.676 (95% CI = 0.599-0.753, $P < 0.001$) and 0.837 with sensitivity and specificity of 70.4% and 57.9%, respectively. The ROC curves of AFC also showed that the AUC was 0.604 (95% CI = 0.524-0.684, $P = 0.013$) with cut-off value, sensitivity and specificity of 7.775, 64.3% and 51.6%, respectively (**Figure 2**).

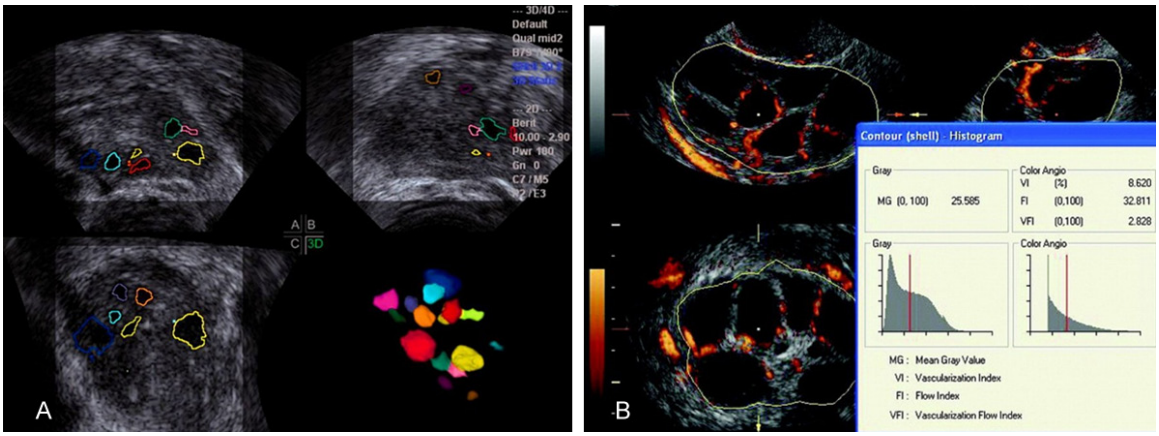

Figure 1. The ultrasonography results in patients with diminished ovarian reserve (DOR) (A. The antral follicle counts (AFC) calculated by three-dimensional (3-D) ultrasonic device; B. Ovarian volume and arterial blood flow indexes calculated by 3-D ultrasonic device).

Table 3. Comparisons on ultrasonography parameters after treatment between case group and control group

Parameters	Control group (n = 95)	Case group (n = 98)	*P*
VI (%)	1.807 ± 0.213	2.012 ± 0.311	< 0.001
FI (%)	25.423 ± 2.125	26.122 ± 4.029	0.301
VFI (%)	0.826 ± 0.125	0.894 ± 0.211	0.009
Ovarian volume (cm^3)	6.066 ± 0.453	6.182 ± 0.597	0.301
AFC	7.78 ± 2.32	8.46 ± 3.15	0.008

Note: DHEA = dehydroepiandrosterone; VI = vascularization index; FI = flow index; VFI = vascularization-flow index; AFC = antral follicle counts.

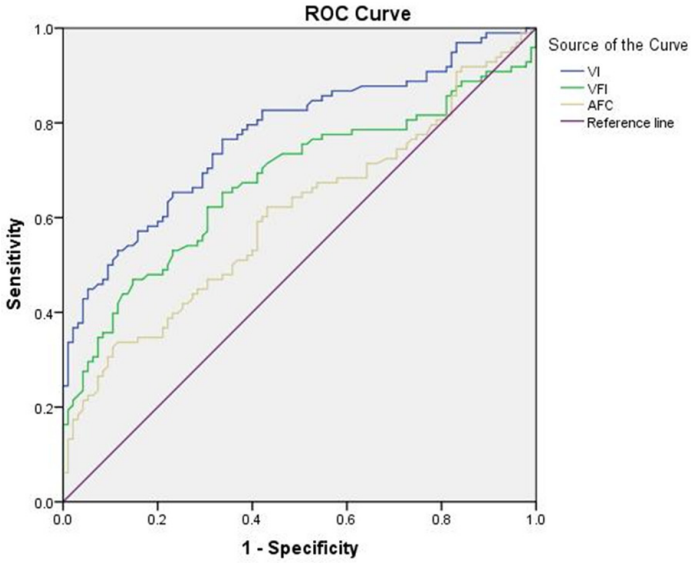

Figure 2. The receiver operating characteristic (ROC) curves of three-dimensional (3-D) ultrasonic device parameters, vascularization index (VI), vascularization-flow index (VFI) and antral folliclecounts (AFC).

Comparisons on IVF-ET treatment

Compared with control group, the DHEA group had an increased number of retrieved oocytes (10.81 ± 3.23 *vs.* 9.21 ± 3.15, $P < 0.05$), significantly higher implantation rate (16.7% *vs.* 9.29%, $P < 0.05$), and pregnancy rate (25.6% *vs.* 11.8%, $P < 0.05$). High-quality embryo formation rate, fertilization rate and cleavage rate in DHEA group were not statistically different from those in control group (all $P > 0.05$) (**Table 4**).

Discussion

In current study, we found that the DHEA supplementation would significantly increase the T level and reduce the FSH and FSH/LH level, which was consistent with the results confirmed in previous literatures, demonstrating the benefits of DHEA treatment in higher cumulative pregnancy rates [10, 12, 18]. More importantly, the application of 3-D ultrasonography in current study revealed that the administration of DHEA could increase VI, VFI and AFC in DOR patients, based on the more pronounced increase in VI, VFI and AFC in case group. The rise in FSH and decrease in AFC could indicate the presence of DOR [15]. The improvement in AFC implies changes in potential ovarian response and the

Table 4. The comparisons on IVF-ET indexes between case group and control group

Index	Control (n = 95)	Case group (n = 98)	*P*
Good-quality embryo formation rate (%)	67.1 (251/374)	69.3 (181/261)	0.796
Number of retrieved oocytes	9.21 ± 3.15	10.81 ± 3.23	0.001
Fertilization rate (%)	75.9 (662/872)	77.6 (743/957)	0.779
Cleavage rate (%)	85.1 (529/621)	85.4 (599/701)	0.998
Implantation rate (%)	9.29 (21/226)	16.7 (39/234)	0.040
Pregnancy rate (%)	11.8 (12/102)	25.6 (30/117)	0.041

Note: IVF-ET = in vitro fertilization and embryo transfer.

increasing availability of follicles for recruitment and maturation. Consistent with our result, Wiser implied that the supplementation of DHEA can have a beneficial effect on ovarian reserves for poor-responder patients on IVF treatment [12]. The IVF-ET improvement by DHEA treatment may be explained by a quantitative increase in recruitable follicles, or by a qualitative change, yielding high quality oocytes and embryos [19]. The efficiency for DHEA was reported in previous studies. For instance, previous study demonstrated that the miscarriage rates after DHEA were significantly lower at all ages in women with DOR, implying DHEA may be able to be used as an important drug in fertility practice [20]. In addition, DHEA has also been reported to improve oocyte/embryo yields and oocyte/embryo quality in women with DOR [2]. However, the exact mechanism of DHEA on the ovary function is not clear yet [21]. DHEA supplementation improved oocyte retrieval rates, number of mature oocytes and number of good-quality embryos, leading to increased pregnancy rates with acceptably low cancellation rates, and ovarian reserve markers may help to identify women at high risk of poor ovarian response [6].

In this study, VI, VFI and AFC were used to assess the diagnostic value on ovarian function. Our results demonstrated high accuracies of VI, VFI and AFC in ovarian evaluation, suggesting ultrasonography is of great value in ovarian assessment during IVF-ET and DHEA supplementation, and these findings are consistent with previous reports. A study by Deb S *et al.* showed that small antral follicles are significant predictors of pregnancy following IVF-ET treatment [22]. While there is discrepancy that reproductive response may also be predicted by AFC, it can be attributed to the major role of AFC in comprising small antral follicles [23]. The VI measurements are informative for the number and/or size of vessels within the volume assessed, and VFI is a feature of both vascularization and volume flow [24]. Partially consistent with our results, ovarian stromal VI and VFI were significantly different between women with polycystic ovary syndrome (PCOS) and normal fertile respectively, implying VI and VFI may be potential parameters for ovarian function [25].

Our results signified that the efficiency of DHEA on patients with DOR can be easily obtained during IVF-ET by using 3-D SonoAVC ultrasonography. The advantages of ultrasonography have been observed in a previous study, which suggested SonoAVC offers advantages for clinical practice with accuracy or reliability comparing with 2D manual measurements when examining follicular volumes in IVF-ET [26]. A recent study has shown 3-D ultrasonography can yield the objective assessment of ovarian volume and total blood flow to the ovary and, both of which can be important determinants of ovarian reserve [15].

In conclusion, our results suggest DHEA showed great benefits in the improvement of ovarian reserve function for DOR patients and the application of ultrasonography can effectively evaluate the efficiency of DHEA in IVF-ET, thus ultrasonography provides valuable information during DOR treatment. Although the baseline characteristics between the DHEA and the control group were not statistically significant, the administration of DHEA or placebo was not randomized distributed, which may had a slight influence on the credibility of our results. Further evaluations of ultrasonic device and the value of DHEA in DOR treatment with larger study population are required.

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Disclosure of conflict of interest

None.

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