F &S 17526 Decline and resubmit highlighted

Running title: RCT – Effect of DHEA in poor responders

Title Page

Full Title: A randomized controlled pilot trial on the effect of Dehydroepiandrosterone (DHEA) on ovarian response markers, ovarian response and IVF outcomes in poor responders

Authors:

Tracy Wing Yee YEUNG[†] (MBBS, MRCOG, FHKCOG, FHKAM (OG))

Joyce CHAI (MBChB, MRCOG, FHKCOG, FHKAM (OG))

Raymond Hang Wun LI (MBBS, MRCOG, FHKCOG, FHKAM (OG))

Vivian Chi Yan LEE (MBBS, MRCOG, FHKCOG, FHKAM (OG))

Pak Chung HO (MD, FRCOG, FHKAM (OG))

Ernest Hung Yu NG (MD, FRCOG, FHKAM (OG))

Department of Obstetrics & Gynecology, The University of Hong Kong, Hong Kong Special Administrative Region

Correspondence:

[†]To whom correspondence and reprint requests should be addressed

Department of Obstetrics and Gynecology,

The University of Hong Kong, Queen Mary Hospital,

Pokfulam Road, Hong Kong

Telephone: 852-22553374 **Fax**: 852-28175374

Email: tracyyeungwy@gmail.com

Any grant or fellowship supporting the writing of the paper: The Committee on Research and Conference Grants, University of Hong Kong

Disclosure summary: The authors have nothing to disclose.

Capsule: No significant improvement in ovarian response markers, ovarian response to standard dose gonadotrophin stimulation and IVF outcomes were detected in poor responders receiving pretreatment DHEA compared to placebo.

Structured Abstract and Key Words

Objective: To evaluate whether pre-treatment DHEA supplementation would improve ovarian response markers, ovarian response to standard low dose gonadotrophin stimulation and IVF outcomes in poor responders

Design: Randomized double-blinded placebo-controlled study

Setting: Tertiary reproductive medicine unit

Patients: 32 women with anticipated poor ovarian response

Interventions: Eligible subjects were randomized into the DHEA group (n=16) who received DHEA (GNC, 25mg three times a day) or the placebo group (n=16) who received placebo starting from at least 12 weeks before the scheduled IVF treatment according to a computer-generated randomization list. Monthly ovarian response markers including antral follicle count (AFC), serum anti-Mullerian hormone (AMH) and follicle stimulating hormone (FSH) levels, ovarian response to a standard dose of gonadotrophin stimulation at week 8 and IVF outcomes were compared.

Main outcome measures: Primary outcome was AFC after 12 weeks of intervention

Results: DHEA supplementation resulted in significantly higher serum DHEA-S, free androgen index and follicular DHEA-S levels. No significant differences in ovarian

response markers (AFC, AMH and FSH), ovarian response to standard dose gonadotrophin stimulation and IVF outcomes were found between the two groups.

Conclusions: No significant improvement in ovarian response markers, ovarian response to standard dose gonadotrophin stimulation and IVF outcomes can be found in poor responders receiving pre-treatment DHEA.

Clinical Trial Registration Number: HKCTR-1149 (www.hkclinicaltrials.com) and NCT01915186 (www.clincialtrials.org)

Keywords: DHEA, in-vitro fertilization, ovarian response markers, poor responders

INTRODUCTION

Dehydroepiandrosterone (DHEA) is an endogenous steroid produced mainly in the zona reticularis of adrenal cortex and ovarian theca cells in women. Androgens have been implicated in ovarian follicular steroidogenesis and is believed to increase follicular insulin-like growth factor-1 (IGF-1) that promotes folliculogenesis (1), potentiates the effects of gonadotropin (2) and reduces follicular arrest (3).

Previous observational studies have reported preliminary success in using DHEA in poor responders leading to improved ovarian response, increased oocyte yield, improved embryo quality, reduced miscarriage rates, as well as higher pregnancy rates following assisted reproductive treatments (2, 4-7). A recent meta-analysis including three randomized controlled trials (RCTs) (8-10) using transdermal testosterone and one RCT using DHEA (11) has shown an increased ongoing pregnancy /live-birth rates [RR 2.08; 95% confidence interval (1.10,3.93); p=0.002] when adjuvant androgen (DHEA or testosterone) pretreatments were given to poor responders (12). A worldwide survey conducted in 2010 revealed that over a quarter (26%) of IVF clinicians added DHEA as an adjuvant to IVF treatment protocols in poor responders (13). Even in women with primary ovarian insufficiency (POI), our group has previously demonstrated improvements in antral follicle count (AFC), ovarian volume and follicular activity after DHEA supplementation in an RCT (14).

Despite the wider use of DHEA in poor responders, there are still considerably diverse views among many clinicians. Most of the published studies were based on retrospective and/or observational data, and the results were not free from bias. The aim of this study is

to assess the effect of DHEA on ovarian response markers, ovarian response to standard gonadotrophin stimulation and the number of oocytes obtained in poor responders in an RCT setting.

MATERIALS AND METHODS

Study design and protocol

Consecutive women attending the Subfertility Clinic at the Department of Obstetrics and Gynaecology, University of Hong Kong who were indicated for IVF treatment were screened and recruited.

Inclusion criteria included: (a) age =<40 years; (b) subfertility > 1 year; (c) expected poor ovarian response defined as AFC < 5. Patients were excluded if they had (a) history of ovarian cystectomy or oophorectomy; (b) received cytotoxic chemotherapy; (c) received pelvic irradiation or (d) history of taking testosterone or DHEA supplement.

The study had been approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster and was registered under Hong Kong Clinical Trial Center (HKCTR-1149) and Clinicaltrials.gov (NCT01915186). All women were fully counseled and written consents were obtained.

Baseline assessments were performed on the second day of the menstrual cycle 12 weeks prior to the scheduled IVF treatment. Ovarian response markers including AFC, serum anti-Mullerian hormone (AMH) and follicle stimulating hormone (FSH) levels were measured. Serum estradiol (E2), testosterone, DHEA-S, sex hormone binding globulin (SHBG),

insulin-like growth factor-1 (IGF-1), complete blood picture and liver enzymes were also checked.

Assignment and masking

Women were randomized in 1:1 ratio according to a computer-generated randomization list generated by a research nurse not involved in the subjects' clinical management and were allocated in sealed, opaque, sequentially number envelopes. The hospital pharmacy packaged the DHEA and identical placebo capsules according to the randomization list and labeled the drug packs with subject numbers only. Physicians, research nurses involved and study subjects were all blinded to the assignment.

Treatment and Monitoring

Pretreatment and monitoring

Either DHEA (GNC LiveWell™) capsule at 25mg three times a day (i.e. 75 mg per day) or matching placebo capsules were started after baseline investigations. Subjects were followed up at 4-weekly intervals at week 0, week 4, week 8, and week 12. Transvaginal scans were performed by gynaecologists experienced in pelvic scanning using a 7 MHz vaginal probe (Voluson 730®, GE Healthcare, Wisconsin, USA) to determine AFC (2-9 mm) in both ovaries. The intra-observer coefficient of variation (CV) for AFC was 7%. Blood was collected for serum AMH, FSH, E2, testosterone, DHEA-S, SHBG, IGF-1, complete blood picture and liver function.

Standard low dose ovarian stimulation

At week 8, low dose gonadotrophin stimulation using 75 IU human menopausal gonadotrophin (HMG, Menogon®, Ferring Pharmaceuticals) was given on the 2^{nd} - 8^{th}

day as a standardized test for ovarian response. Ovarian response was assessed on the 10^{th} day by the number of follicle(s) >10mm and serum E2 levels (15).

IVF treatment

At week 12, subjects were treated with ovarian stimulation under the fixed antagonist protocol. HMG injections were started at 450 IU for 2 days followed by 300 IU daily. Ovarian response was monitored by serial transvaginal scanning with or without hormonal monitoring. Further dosage adjustments were based on the ovarian response. When the leading follicle was >/=18mm, human chorionic gonadotrophin (hCG, Pregnyl [Organon, Oss, the Netherlands]) 10,000 IU was given intramuscularly to trigger final maturation of oocytes. Cycles were cancelled if the follicles remained <10mm after 14 days of stimulation. Transvaginal ultrasound guided oocyte retrievals (TUGOR) were scheduled 36 hours later. A maximum of two embryos were transferred two days after TUGOR. Excess good quality embryos were frozen for subsequent transfer.

Serum samples were stored at -20^0C until assayed as a whole batch. Follicular fluid was collected from dominant follicles during oocyte retrievals. Samples were assayed for AMH, FSH, E2, progesterone, DHEA-S, testosterone and IGF-1. Serum and follicular AMH levels were measured using AMH Gen II ELISA (Beckman Coulter); IGF-1 levels were measured using Quantikine ELISA human IGF-1 (R&D System), whereas E2, progesterone, testosterone, DHEA-S and SHBG were measured using Beckman Coulter Access 2 Immunoassay system.

The intra-assay CVs were 3.4-5.4% for AMH, 3.5-4.3% for IGF-1, 12-21% for E2, 7.51-9.57% for progesterone, 1.67-3.93% for testosterone, 1.6-8.3% for DHEA-S and 4.5-4.8% for SHBG. The inter-assay CV were 4.0-5.6% for AMH, 7.5-8.1% for IGF-1, 12 – 21% for

estradiol, 6.11 – 11.19% for progesterone, 4.22-7.08% for testosterone, 3.7-11.3% for DHEA-S and 5.2-5.5% for SHBG. Detection limits were 0.08-22.5 ng/ml for AMH, 0.007-6 ng/ml for IGF-1, 73–17621pmol/L for E2, 0.25–127.2nmol/L for progesterone, 0.1-16 ng/ml for testosterone, 2-1000 μg/dL for DHEA-S and 0.33-200 nmol/L for SHBG.

Statistical analysis

AFC at week 12 was used as the primary outcome measure. We aimed at assessing any improvement in functional ovarian reserve as the first step. AFC was chosen since its predictive performance for functional ovarian reserve and ovarian response has been shown to be significantly better than that of basal FSH(16) and comparable to the use AMH(17, 18) or multivariate models(18, 19) in meta-analysis.

Secondary outcome measures included changes in FSH and AMH; serum and follicular hormonal profiles (E2, testosterone, DHEA-S, SHBG and IGF-1); post stimulation E2 and number of follicles > 10mm; and the number of oocytes obtained.

Based on our own database for anticipated poor responders (AFC <5) undergoing IVF treatment, the mean AFC was 3.10 with a standard deviation of 1.05 (unpublished data). Assuming an increase of AFC by 2.0 being clinically significant (i.e. with the resultant AFC of > 5 and beyond our current definition of anticipated poor responders), 6 subjects in each arm would be required for a test significance of 0.05 and power of 0.8. Considering possible dropouts, we aim at recruiting 8 patients in each arm with a total of 16 patients. 16 patients undergoing their first IVF treatment cycle and 16 patients undergoing their

subsequent treatment cycle were recruited. Continuous variables are expressed as median (25^{th} to 75^{th} centiles). Statistical comparisons were carried out with the intention to treat by Mann-Whitney-*U* test, Chi-square test and Fisher's exact test where appropriate using the Statistical Program for Social Sciences (SPSS Inc., Version 21.0, Chicago, U.S.A.). A two-sided $P < 0.05$ was taken as statistically significant.

RESULTS

Participant flow

Between August 2010 and August 2012, a total of 32 subjects were recruited with eighteen women undergoing their first IVF cycles and fourteen undergoing their subsequent treatment cycles (Supplemental Figure 1 – Consort 2010 Flow Diagram).

Baseline characteristics

Baseline characteristics of the DHEA and placebo groups including age of women, body mass index, duration, type and causes of subfertility and ovarian response markers are represented in Table 1.

Primary outcomes

No significant difference in median AFC had been detected between the DHEA and placebo groups throughout the study period (Figure 1). There was no significant improvement in AFC in DHEA group after 12 weeks of supplementation compared to its baseline [3.5 (1.75 – 4.25) vs 4 (3-4), p=0.436].

Secondary outcomes

Serum hormonal profiles

Similar to AFC, serum FSH and AMH levels were comparable between the two groups throughout the study period (Fig 1).

Serum testosterone and DHEA-S levels were significantly higher in DHEA group after DHEA supplementation in Week 4, 8 and 12 compared to the placebo group. Serum SHBG levels were significantly lower, leading to significantly higher free androgen indexes (FAI) in the DHEA group (Fig 1).

No significant difference in serum IGF-1 after 12 weeks was detected between DHEA group [88.0 (67.6 – 126.2) ng/ml] and placebo group [78.0 (47.5 – 113.2) ng/ml].

Response to a standard low dose gonadotrophin stimulation

Higher post-stimulation E2 level was observed after the standard dose ovarian stimulation with HMG at 75 IU daily for 7 days in the DHEA group, although it did not reach statistical significance [1076 (888–1232) vs 501 (216 - 1116) pmol/L, p=0.252]. Number of follicle(s) larger than 10mm was similar [1 (1-2) vs 1 (0-2), p=0.290].

IVF cycle characteristics

Shorter duration [median 10 (9 - 12.2) vs 12 (8 - 15) days, p=0.114] and lower dose [2475 (2475 - 3206) vs 3150 (2925 - 4425) IU, p=0.069] of gonadotrophin use were detected in the DHEA group, although they did not reach statistical significance. The number of follicles at various sizes and number of oocytes obtained were similar but there were higher numbers of fertilized, cleaved, transferred and top quality embryos – TQE (defined as 4-celled grade 1 or 2 on day 2 - i.e. blastomeres of equal size with no or minor fragmentation (20)) in the DHEA group, although again, they failed to reach statistical significance (Table

2). Three patients (18.6%) in DHEA group had cycle cancellation due to premature ovulation; while two patients (12.5%) in the placebo group had cycle cancelled, one due to premature ovulation and one due to absence of ovarian response despite prolonged ovarian stimulation.

Follicular fluid hormonal profiles

Median follicular DHEA-S level was significantly higher in the DHEA group. Follicular AMH was also higher in DHEA group, although it did not reach statistical significance. Follicular estradiol, progesterone and IGF-1 levels were similar for the two groups (Fig 2).

Pregnancy Outcomes

No significant difference in the clinical pregnancy (18.8% vs 25.0%, p = 0.380), ongoing pregnancy (18.8% vs 12.5%, p = 0.326), live birth (12.5% vs 12.5%, p = 1.000) and miscarriage (0 vs 12.5%, p=0.326) rates had been observed between DHEA and placebo groups. There was no multiple pregnancy in either group.

Subgroup analyses

Subgroup analyses were performed after stratifying subjects into those undergoing their first IVF cycles (n=18) or subsequent IVF cycles (n=14). There were no significant differences in AFC, AMH and FSH, gonadotrophin requirements and pregnancy rates throughout the study period (data not shown).

Subgroup analyses were also performed by dividing the subjects into halves according to their serum and follicular DHEA-S levels. Women in the subgroup with higher serum DHEA-S (cut-off at 220μg/dL) had significantly higher serum E2 level on the day of HCG

trigger [5272 (2902 - 7658) vs 3020 (989 - 4132) pmol/L, p=0.033]. Women having higher follicular DHEA-S (cut-off at 180μg/dL) had a significantly higher number of good quality embryos [1 (0-2) vs 0 (0-0.25), p = 0.013]. No significant differences in all parameters could be found in regards to follicular testosterone (cut-off at 5.5 ng/ml) and serum testosterone (cut-off at 1.0 ng/ml) levels or FAI (cut-off at 11).

Side effects

No major adverse effects were reported during the study period. One patient from DHEA group discontinued the intervention before week 4 complaining of increased acne. Monthly monitoring of liver function and complete blood picture did not reveal any derangement.

DISCUSSION

In our present study, we did not find any significant improvement in the functional ovarian reserve after 12 weeks of DHEA supplementation, using AFC as the surrogate marker.

Management of poor responders remains one of the biggest challenges in fertility treatment. Numerous studies have been performed to assess different stimulation protocols and adjuvant therapies to improve ovarian response, but the latest Cochrane review concluded that there is insufficient evidence to support the routine use of any particular intervention (21).

Accumulation of androgens in the micro-milieu of primate ovaries had been shown to play a critical role in early follicular development and granulosa cell proliferation (22, 23). Androgens promote recruitment and initiation of primordial follicle growth and induce significant increase in the number of primary, preantral and antral follicles through up-regulation of IGF-1 (1, 23); up-regulate FSH receptors expression in granulosa cells to potentiate the effect of FSH (1, 22, 24, 25); and exert paracrine regulation on follicular maturation and reduce follicular atresia (1, 24). At the same time, lack of androgen has been shown to reduce the number of antral follicles and ovulated oocytes (26) as well as accelerated follicular atresia (27).

Many observational studies have suggested improved ovarian response and pregnancy outcomes after DHEA supplementation in poor responders (2, 4, 5, 7, 28-33). Wiser et al conducted a RCT and concluded that DHEA could lead to significantly improved live birth rate among poor responders undergoing IVF treatment (11). However, there was no priori

sample size calculation, and concluding a significant improvement in live-birth rate by pooling the results from two treatment cycles with a p-value of 0.05 had been criticized. Our present study aimed at addressing the uncertain benefit(s) of DHEA in poor responders undergoing IVF treatment.

To the best of our knowledge, this is the first RCT that included the comprehensive serum and follicular fluid hormonal profiles and changes in ovarian response markers in poor responders throughout the DHEA pretreatment. Serum DHEA-S and total testosterone levels were significantly higher in the DHEA group starting from week 4. Together with the significantly lower SHBG, women were exposed to a much higher concentration of bioavailable free testosterone. After 12 weeks of DHEA, significantly higher follicular DHEA-S level was achieved. It confirmed the hypothesis that oral DHEA supplementation for 12 weeks leads to significantly higher intra-ovarian DHEA-S.

It has been reported that testosterone levels decline with advancing female age and is lower in women with premature ovarian aging (34). Previous non-randomized study suggested that lower functional ovarian reserve is an androgen deficient condition and androgen supplementation should be given to improve functional ovarian reserve(35). In our study, oral supplementation with DHEA for 12 weeks did manage to significantly increase the systemic DHEA-S and testosterone levels, as well as the local follicular DHEA-S. However, significant improvement of various ovarian response markers including AFC, AMH and FSH reported in previous uncontrolled studies (33, 36) cannot be replicated here.

Androgen treatment during follicular recruitment has been shown to increase the number of healthy follicles on morphological assessment in an animal study, despite similar total

number (37). Clinically, significant reduction in the number of aneuploid embryos after DHEA supplementation has been reported (38). In our present study, significantly higher number of TQE was found in subgroup of women having higher follicular DHEA-S but not in the group randomized to receive DHEA. It suggested that women with higher intra-ovarian DHEA-S, either naturally or achieved through DHEA supplementation, may have better embryos quality. It is possible that DHEA supplementation may improve the ovarian environment where follicular maturation takes place leading to reduced aneuploidy (38), although the underlying mechanism is not known. No significant differences were detected in terms of the gonadotrophin use or pregnancy outcomes between the two groups. However, it should be aware that our study was not powered to detect such a difference and a much larger sample size would be required to confirm or refute such observation.

It has been proposed that DHEA helps in regulating follicular development through increased IGF-1 in primate (2, 23). It increases the number of primary, preantral and antral follicles by increasing the follicular recruitment and initiation together with reduced follicular atresia, resulting in an increase in the FSH-sensitive growing pool. However, we did not detect any difference in either serum or follicular IGF-1 levels between the two groups. It is unlikely that DHEA exerts major effects on follicular development through IGF-1 in humans.

One of the major strengths of our study is the double-blinded randomized study design that minimized potential bias. We have also provided comprehensive data on monthly ultrasonographic and serum hormonal profiles to detect any changes in ovarian response markers; subjected all women to a low dose ovarian stimulation as a standardized test of ovarian response; followed by a IVF treatment cycle under a standard protocol to provide

clinical outcomes and allowed comparison of the follicular hormonal milieu. These created a complete picture on the possible effects of DHEA in poor responders.

Our study is not without limitations. Live-birth rate should be the ideal outcome measure in clinical trials assessing fertility outcomes. However, current belief in the potential benefit of DHEA in poor responders was based on the assumption that DHEA increases intra-ovarian androgen concentrations, which in turn improves the functional ovarian reserve, and ultimately the pregnancy rates. The primary aim of our study was to assess whether DHEA supplementation would indeed improve the functional ovarian reserve as the logical first step. AFC was chosen to be the primary outcome measure since it has been widely accepted as a marker for functional ovarian reserve and is a good predictor of ovarian response. Compared to other single ovarian response markers, the predictive performance of AFC towards poor response has been shown to be significantly better than that of basal FSH (16) and is comparable to AMH (17, 18) or multivariate models (18, 19) in metaanalyses. If significant improvement can be detected, further RCT could be performed using the live-birth rate as the primary outcome. Another limitation of our study is the relatively small sample size. Priori sample size calculation had been performed to ensure adequate power in assessing the primary outcome. However, the lack of significant differences especially in the secondary outcomes and/or in subgroup analyses may be limited by the sample size. Interpretation of these results has to be dealt with cautions.

Use of pre-treatment DHEA in poor responders had drawn much attention and increasing number of studies has been performed to assess its efficacy. Majority of the published studies used DHEA at 25mg 3 times per day for 5 to 16 weeks (11, 36). So far there is no good data to indicate the optimal duration of DHEA pre-treatment. In the present study, we

prescribed a 12-week pretreatment based on our published data on the use of DHEA in women with primary ovarian insufficiency who started to show some improvements in AFC and/or have growing follicles after 12 weeks of DHEA(14). Currently there has not been any dose-finding study to confirm the optimal dose and duration for DHEA supplementation and there is no available data to show the effective serum and/follicular DHEA-s or testosterone levels achieved from different DHEA regimens. It is possible that DHEA pre-treatment given at the present dosage and duration may not be adequate to achieve optimal intra-ovarian androgen levels in all women in order to improve the ovarian response and outcomes. Further studies may focus on the dose and duration of DHEA used prior to IVF treatment.

At the time when our study was started, there was no uniform definition of "poor responders". We used AFC <5 as a surrogate marker to predict poor ovarian response since it has been the widely accepted and adopted criteria (39-41). To compare our population against the Bologna criteria, all subjects fulfilled the criteria for abnormal ORT. 14 out of 36 of our subjects had previous IVF treatment. The median number of oocyte retrieved was 4 with a mean of 3.79 +/- SD 1.311. Although it does not strictly fit in the ESHRE consensus Bologna criteria (2011) of </=3 oocyte retrieved, it is compatible with most published studies which used </=4-5 oocytes as the definition of poor response (42-45) We exclude women over 40 and those who had previous oophorectomy or ovarian cystectomy, cytotoxic chemotherapy or pelvic irradiation from our present study to achieve a more homogenous population for comparison.

Currently a number of trials are underway to investigate the potential effects of DHEA on the ovarian response, embryo quality and pregnancy rates (http://clinicaltrials.gov). Further

studies should employ the definition of poor responders based on the Bologna criteria (46) to allow meaningful evaluation and meta-analysis of smaller studies.

Conclusion

No significant improvement in ovarian response markers, ovarian response to a standard low dose of gonadotrophin stimulation and number of oocytes obtained were detected in anticipated poor responders receiving 12 weeks of DHEA supplementation prior to the start IVF treatment compared to placebo. Further RCTs on the use of DHEA in poor responders should employ the Bologna criteria in defining poor responders and include the delineation of the optimal regimen.

Authors' role

T.Y. was involved in study design, execution, analysis, manuscript drafting, critical discussion and final approval of the manuscript. E.N. was involved in study design, execution, critical discussion and final approval of the manuscript. J.C., V. L., R.L, P.C.H. were involved in execution, critical discussion and final approval of the manuscript.

Reference

1. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. Journal of Clinical Investigation. 1998;101(12):2622.
2. Casson P, Lindsay M, Pisarska M, Carson S, Buster J. Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: a case series. Human Reproduction. 2000;15(10):2129-32.

3. Sen A, Hammes SR. Granulosa cell-specific androgen receptors are critical regulators of ovarian development and function. Molecular Endocrinology. 2010;24(7):1393-403.
4. Barad DH, Gleicher N. Increased oocyte production after treatment with dehydroepiandrosterone. Fertility and sterility. 2005;84(3):756. e1-. e3.
5. Barad D, Gleicher N. Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. Human Reproduction. 2006;21(11):2845-9.
6. Barad D, Brill H, Gleicher N. Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function. Journal of assisted reproduction and genetics. 2007;24(12):629-34.
7. Gleicher N, Ryan E, Weghofer A, Blanco-Mejia S, Barad DH. Miscarriage rates after dehydroepiandrosterone (DHEA) supplementation in women with diminished ovarian reserve: a case control study. Reprod Biol Endocrinol. 2009;7:108.
8. Massin N, Cedrin-Durnerin I, Coussieu C, Galey-Fontaine J, Wolf J, Hugues J-N. Effects of transdermal testosterone application on the ovarian response to FSH in poor responders undergoing assisted reproduction technique—a prospective, randomized, double-blind study. Human Reproduction. 2006;21(5):1204-11.
9. Fábregues F, Peñarrubia J, Creus M, Manau D, Casals G, Carmona F, et al. Transdermal testosterone may improve ovarian response to gonadotrophins in low-responder IVF patients: a randomized, clinical trial. Human Reproduction. 2009;24(2):349-59.
10. Kim C-H, Howles CM, Lee H-A. The effect of transdermal testosterone gel pretreatment on controlled ovarian stimulation and IVF outcome in low responders. Fertility and sterility. 2011;95(2):679-83.
11. Wiser A, Gonen O, Ghetler 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. Human Reproduction. 2010;25(10):2496-500.
12. Sunkara SK, Coomarasamy A. Androgen pretreatment in poor responders undergoing controlled ovarian stimulation and in vitro fertilization treatment. Fertility and sterility. 2011;95(8):e73-e4.
13. Leong M PP. Poor responders: how to define, diagnose and treat? http://www,ivf-worldwide.com/survey/poor-responders/results-poor-responders.html. 2010.
14. Yeung TWY, Li RHW, Lee VCY, Ho PC, Ng EHY. A randomized double-blinded placebo-controlled trial on the effect of dehydroepiandrosterone for 16 weeks on ovarian response markers in women with primary ovarian insufficiency. Journal of Clinical Endocrinology & Metabolism. 2013;98(1):380-8.
15. Fábregues F, Balasch J, Creus M, Carmona F, Puerto B, Quinto L, et al. Ovarian reserve test with human menopausal gonadotropin as a predictor of in vitro fertilization outcome. Journal of assisted reproduction and genetics. 2000;17(1):13-9.
16. Hendriks DJ, Mol B-WJ, Bancsi LF, te Velde ER, Broekmans FJ. Antral follicle count in the prediction of poor ovarian response and pregnancy after in vitro fertilization: a meta-analysis and comparison with basal follicle-stimulating hormone level. Fertility and sterility. 2005;83(2):291-301.
17. Li HWR, Lee VCY, Lau EYL, Yeung WSB, Ho PC, Ng EHY. Role of Baseline Antral Follicle Count and Anti-Mullerian Hormone in Prediction of Cumulative Live Birth in

the First In Vitro Fertilisation Cycle: A Retrospective Cohort Analysis. PloS one. 2013;8(4):e61095.
18. Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Human Reproduction Update. 2013;19(1):26-36.
19. Verhagen T, Hendriks D, Bancsi L, Mol B, Broekmans F. The accuracy of multivariate models predicting ovarian reserve and pregnancy after in vitro fertilization: a meta-analysis. Human Reproduction Update. 2008;14(2):95-100.
20. Veeck L. An atlas of human gametes and conception. 1999. London: Pathenon.
21. Pandian Z, McTavish AR, Aucott L, Hamilton M, Bhattacharya S. Interventions for poor 'responders' to controlled ovarian hyper stimulation (COH) in in-vitro fertilisation (IVF). Cochrane Database Syst Rev. 2010;1.
22. Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. Journal of Clinical Endocrinology & Metabolism. 1999;84(8):2951-6.
23. Vendola K, Zhou J, Wang J, Bondy CA. Androgens promote insulin-like growth factor-I and insulin-like growth factor-I receptor gene expression in the primate ovary. Human Reproduction. 1999;14(9):2328-32.
24. Hillier SG, Tetsuka M. Role of androgens in follicle maturation and atresia. Baillière's clinical obstetrics and gynaecology. 1997;11(2):249-60.
25. Nielsen M, Rasmussen I, Kristensen S, Christensen S, Møllgård K, Andersen EW, et al. In human granulosa cells from small antral follicles, androgen receptor mRNA and androgen levels in follicular fluid correlate with FSH receptor mRNA. Molecular human reproduction. 2011;17(1):63-70.
26. Wang H, Andoh K, Hagiwara H, Xiaowei L, Kikuchi N, Abe Y, et al. Effect of adrenal and ovarian androgens on type 4 follicles unresponsive to FSH in immature mice. Endocrinology. 2001;142(11):4930-6.
27. Hu YC, Wang PH, Yeh S, Wang RS, Xie C, Xu Q, et al. Subfertility and defective folliculogenesis in female mice lacking androgen receptor. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(31):11209.
28. Gleicher N, Goyal A, Weghofer A, Barad D. Supplementation with dehydroepiandrosterone (DHEA) improves ovarian reserve, as reflected by anti-müllerian hormone levels. Fertility and sterility. 2009;92(3):S54-S5.
29. Gleicher N, Weghofer A, Barad D. Increased euploid embryos after supplementation with dehydroepiandrosterone (DHEA) in women with premature ovarian aging. Fertility and sterility. 2007;88:S232-S.
30. Mamas L, Mamas E. Dehydroepiandrosterone supplementation in assisted reproduction: rationale and results. Current Opinion in Obstetrics and Gynecology. 2009;21(4):306.
31. Norbert G, Andrea W, David B. Dehydroepiandrosterone (DHEA) reduces embryo aneuploidy: direct evidence from preimplantation genetic screening (PGS). Reproductive Biology and Endocrinology.8.
32. Sönmezer M, Özmen B, Įil A, Özkavukįu S, TasįI T, Olmus H, et al. Dehydroepiandrosterone supplementation improves ovarian response and cycle outcome in poor responders. Reproductive biomedicine online. 2009;19(4):508-13.
33. Yilmaz N, Uygur D, Inal H, Gorkem U, Cicek N, Mollamahmutoglu L. Dehydroepiandrosterone supplementation improves predictive markers for

598 diminished ovarian reserve: serum AMH, inhibin B and antral follicle count. European
599 Journal of Obstetrics & Gynecology and Reproductive Biology. 2013.
600 34. Gleicher N, Kim A, Weghofer A, Shohat-Tal A, Lazzaroni E, Lee H-J, et al. Starting
601 and resulting testosterone levels after androgen supplementation determine at all
602 ages in vitro fertilization (IVF) pregnancy rates in women with diminished ovarian
603 reserve (DOR). Journal of assisted reproduction and genetics. 2013;30(1):49-62.
604 35. Gleicher N, Kim A, Weghofer A, Kushnir VA, Shohat-Tal A, Lazzaroni E, et al.
605 Hypoandrogenism in association with diminished functional ovarian reserve. Human
606 Reproduction. 2013;28(4):1084-91.
607 36. Gleicher N, Weghofer A, Barad DH. Improvement in diminished ovarian reserve
608 after dehydroepiandrosterone supplementation. Reproductive biomedicine online.
609 2010;21(3):360-5.
610 37. Cárdenas H, Jiménez E, Pope W. Dihydrotestosterone influenced numbers of
611 healthy follicles and follicular amounts of LH receptor mRNA during the follicular
612 phase of the estrous cycle in gilts. Reproduction. 2008;135(3):343-50.
613 38. Gleicher N, Weghofer A, Barad DH. Dehydroepiandrosterone (DHEA) reduces
614 embryo aneuploidy: direct evidence from preimplantation genetic screening (PGS).
615 Reprod Biol Endocrinol. 2010;8:140.
616 39. Klinkert E, Broekmans F, Looman C, Habbema J, Te Velde E. Expected poor
617 responders on the basis of an antral follicle count do not benefit from a higher starting
618 dose of gonadotrophins in IVF treatment: a randomized controlled trial. Human
619 Reproduction. 2005;20(3):611-5.
620 40. Mutlu MF, Erdem M, Erdem A, Yildiz S, Mutlu I, Arisoy O, et al. Antral follicle
621 count determines poor ovarian response better than anti-müllerian hormone but age
622 is the only predictor for live birth in in vitro fertilization cycles. Journal of assisted
623 reproduction and genetics. 2013:1-9.
624 41. Bancsi LF, Broekmans FJ, Looman CW, Habbema JDF, te Velde ER. Impact of
625 repeated antral follicle counts on the prediction of poor ovarian response in women
626 undergoing in vitro fertilization. Fertility and sterility. 2004;81(1):35-41.
627 42. Hendriks DJ, te Velde ER, Looman CW, Bancsi LF, Broekmans FJ. Expected poor
628 ovarian response in predicting cumulative pregnancy rates: a powerful tool.
629 Reproductive biomedicine online. 2008;17(5):727-36.
630 43. Saldeen P, Källen K, Sundström P. The probability of successful IVF outcome
631 after poor ovarian response*. Acta obstetricia et gynecologica Scandinavica.
632 2007;86(4):457-61.
633 44. Timeva T, Milachich T, Antonova I, Arabaji T, Shterev A, Omar HA. Correlation
634 between number of retrieved oocytes and pregnancy rate after in vitro
635 fertilization/intracytoplasmic sperm infection. The Scientific World Journal.
636 2006;6:686-90.
637 45. De Sutter P, Dhont M. Poor response after hormonal stimulation for in vitro
638 fertilization is not related to ovarian aging. Fertility and sterility. 2003;79(6):1294-8.
639 46. Ferraretti A, La Marca A, Fauser B, Tarlatzis B, Nargund G, Gianaroli L. ESHRE
640 consensus on the definition of 'poor response'to ovarian stimulation for in vitro
641 fertilization: the Bologna criteria. Human Reproduction. 2011;26(7):1616-24.
642
643

644

Figure legends

Figure 1a. Box-and-whisker plots of ovarian response markers (AFC, AMH, FSH), serum estradiol (E2), DHEA-S, testosterone, SHBG and FAI for women randomized into DHEA (shaded box) and placebo (open box) groups.
Boxes indicate 25th and 75th percentiles, with the horizontal line representing the median values. Whiskers span the range between the 5th and the 95th percentiles of the data. The x-axis represents the time of the blood taking after DHEA/placebo use. Statistically significant differences are defined as $P < 0.05$ and are indicated by an *asterisk*
FAI – free androgen index, defined as total testosterone /SHBG (both in nmol/L) x 100

Fig 2. Box-and-whisker plots of follicular fluid hormone concentrations for women randomized into DHEA (shaded box) and placebo (open box) groups. Boxes indicate 25th and 75th percentiles, with the horizontal line representing the median values. Whiskers span the range between the 5th and the 95th percentiles of the data. Statistically significant differences are defined as $P < 0.05$ and is indicated by an *asterisk*.

Supplemental Figure 1. CONSORT 2010 Flow Diagram

Supplemental Figure 1