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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

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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
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.clinicaltrials.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 2nd - 8th
day as a standardized test for ovarian response. Ovarian response was assessed on the 10th
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°C 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\textsuperscript{th} to 75\textsuperscript{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
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-
ovidian 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**


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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

Enrollment

Assessed for eligibility (n=576)

Excluded (n=544)
- Not meeting inclusion criteria (n=521)
- Declined to participate (n=23)

Randomized (n=32)

Allocation

Allocated to DHEA group (n=16)
- Received allocated intervention (n=16)
  - 8 first cycle
  - 8 subsequent cycle

Allocated to placebo group (n=16)
- Received allocated intervention (n=16)
  - 10 first cycle
  - 6 subsequent cycle

Follow-Up

Lost to follow up (n=0)

Discontinued intervention (n=2)
- 1 patient had spontaneous pregnancy after 8 weeks of DHEA
- 1 patient withdrew consent due to side effects of increase acne and hair loss

Embryo transfer

No embryo transfer (n=7)
- 1 spontaneous pregnancy
- 1 withdrew consent due to side effects
- 3 had cycle cancellation
  - 3 premature ovulation
- 2 had no fertilisation

Analysis (Intention to treat)

Analysed (ITT) (n=16)
- Excluded from analysis (n=0)

No embryo transfer (n=8)
- 1 spontaneous pregnancy
- 1 postponed her IVF treatment
- 2 had cycle cancellation
- 1 had no ovarian response
- 1 had premature ovulation
- 2 had no oocyte obtained
- 2 had no fertilisation

Lost to follow up (n=0)

Discontinued intervention (n=2)
- 1 patient had spontaneous pregnancy
- 1 patient postponed her IVF treatment due to family issue after 4 weeks of placebo