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Original Research Article

Comparative analysis of dehydroepiandrosterone and transdermal testosterone pre-treatment in POSEIDON group 3 and 4 women undergoing *in vitro* fertilization: a retrospective cohort study

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ABSTRACT

Background: Patients classified under patient-oriented strategies encompassing individualised oocyte number (POSEIDON) groups 3 and 4 typically demonstrate suboptimal response to controlled ovarian stimulation (COS) in *in vitro* fertilization (IVF) cycles. Androgen-based pre-treatments like dehydroepiandrosterone (DHEA) and transdermal testosterone have been studied for their role in enhancing ovarian response, although direct comparative data are limited. Objective of the study was to evaluate and compare the effects of DHEA versus transdermal testosterone gel administered before stimulation on ovarian and embryological outcomes in women within POSEIDON groups 3 and 4 undergoing IVF.

Methods: A retrospective cohort study was conducted at a tertiary fertility centre in India between January 2018 and January 2020. Eligible women received either DHEA (75 mg/day for 12 weeks) or testosterone gel (12.5 mg/day for 21 days) before controlled ovarian stimulation (COS). All patients underwent antagonist protocol with dual trigger followed by IVF. Primary outcomes included number of oocytes retrieved, metaphase II (MII) oocytes, and follicular output rate (FORT). Secondary outcomes included fertilization rate, good-quality embryos, and stimulation burden.

Results: Of 237 women analysed, 144 received DHEA and 93 received testosterone gel. The testosterone group showed significantly higher mean oocyte yield (7.2 versus 5.4; $p<0.01$), MII oocytes (5.6 versus 4.0; $p<0.01$), and FORT (58.2% versus 49.3%; $p<0.01$). While fertilization rate (63.5% versus 61.2%; $p=0.37$) and embryo quality (59.1% versus 57.6%; $p=0.75$) were similar, testosterone-treated patients required fewer days of stimulation (9.7 versus 10.3; $p=0.04$) and lower gonadotropin doses (2291 IU versus 2576 IU; $p<0.01$). No OHSS cases occurred in either group.

Conclusions: This study supports the use of short-course transdermal testosterone as a more practical and efficient adjuvant strategy in poor prognosis IVF cycles. It may be especially valuable in resource-constrained settings, where cost-effectiveness and cycle efficiency are critical to success. Further prospective trials are needed to evaluate long-term reproductive outcomes.

Keywords: Transdermal testosterone, DHEA, Poseidon classification, IVF, Poor ovarian reserve, FORT

INTRODUCTION

The success of *in vitro* fertilisation (IVF) is closely associated with the number and quality of oocytes retrieved during controlled ovarian stimulation (COS). Women with a diminished ovarian reserve—especially those aged over 35 years or younger women with an unexpectedly poor ovarian response—represent a significant clinical challenge in assisted reproductive technology (ART). To address the heterogeneity among

poor responders, the patient-oriented strategies encompassing individualised oocyte number (POSEIDON) classification was introduced. This framework stratifies women on the basis of age, antral follicle count (AFC), and ovarian biomarkers such as anti-Müllerian hormone (AMH) levels.[1,2] POSEIDON group 3 included younger women (<35 years) with low AFCs or AMH levels, whereas group 4 included women (≥35 years) with similarly diminished ovarian reserves.[1,2] Androgens, such as dehydroepiandrosterone (DHEA) and testosterone, have been explored as potential adjuvants in COS because of their capacity to upregulate follicle-stimulating hormone (FSH) receptor expression and enhance granulosa cell responsiveness.[3-5] Preclinical studies suggest that androgens play a synergistic role in early follicular development, promoting antral follicle recruitment and improving ovarian sensitivity.[6] DHEA is a weak androgen that is typically administered orally over extended periods, whereas testosterone, when delivered via the transdermal route, provides more rapid systemic absorption and stable intraovarian bioavailability.[7] A recent Cochrane review reported that testosterone pre-treatment may improve clinical pregnancy and live birth rates in poor responders undergoing IVF, but the evidence for DHEA remains inconsistent and debated.[8] However, data comparing these two agents directly in POSEIDON-defined populations, particularly groups 3 and 4, are scarce.

We hypothesise that transdermal testosterone, owing to its greater bioavailability and shorter pre-treatment duration, may result in improved follicular output and oocyte yield compared with those of DHEA. This retrospective study aimed to compare the effects of these two androgen-based pre-treatments on ovarian response parameters specifically the AFC, follicular output rate (FORT), and oocyte retrieval in POSEIDON groups 3- and 4-women undergoing IVF. The findings may guide more personalised treatment approaches in this challenging patient population.

METHODS

Study design and setting

This retrospective, comparative cohort study was conducted at a tertiary fertility center in India between January 2018 and January 2020. The study received approval from the institutional ethics committee. All patients were counselled about the potential benefits and risks of DHEA and testosterone supplementation before initiating treatment, as per the centre's standard protocol.

Participants

Of the 284 patients, who met the POSEIDON group 3 or 4 criteria, eight cycles were cancelled due to poor response, and the other patients did not proceed with IVF. A total of 237 patients who underwent stimulation and oocyte retrieval were included in the final analysis.

Inclusion criteria

Women included in the study were between 21 and 42 years of age and met the criteria for either POSEIDON group 3 (younger than 35 years with an AFC <5 or AMH <1.2 ng/ml or POSEIDON group 4 (aged 35 years or older with similarly low AFC or AMH). Eligible participants had a body mass index (BMI) between 19 and 32 kg/m^2, were undergoing either their first or second IVF/ICSI cycle, and had received coenzyme Q10 (CoQ10) supplementation as part of their pretreatment regimen.

Exclusion criteria

Patients were excluded if they had severe endometriosis (stage III or IV), congenital uterine anomalies, or uncontrolled endocrine disorders such as thyroid dysfunction or hyperprolactinemia. Women with a history of androgen-secreting tumours, previous ovarian surgery, or those who had received other adjuvant therapies—such as growth hormone apart from CoQ10 were also excluded. Additionally, patients whose cycles were cancelled prior to oocyte retrieval, those who used donor oocytes or underwent preimplantation genetic testing (PGT), and individuals who received both DHEA and testosterone sequentially or in combination were not included in the study.

Patients were divided into two groups based on clinician recommendation and patient preference. Group A (DHEA group) received oral DHEA at a dose of 75 mg/day for 12 weeks prior to stimulation. Group B (testosterone group) applied transdermal testosterone gel (12.5 mg/day; 1.25 g of 1% formulation) to the inner arms for 21 consecutive days. Day 2-3 of the same cycle for all participants using standardized ultrasound protocols and laboratory assays.

Controlled ovarian stimulation protocol

All patients underwent controlled ovarian stimulation using an individualised GnRH antagonist protocol. Recombinant FSH or HMG (225–300 IU/day) was initiated on day 2 or 3 and antagonist 0.25 mg was started on day 5 or when the lead follicle reached ≥13 mm. Despite the individualisation of gonadotropin type and dose the consistent antagonist protocol ensured comparability between groups; final oocyte maturation was triggered by a dual trigger (recombinant hCG 250 µg + GnRH agonist 0.1 mg) when ≥3 follicles reached 17-18 mm. Oocyte retrieval was performed 35–36 hours after the trigger. All patients underwent ICSI, in accordance with institutional policy.

Outcome measures

Primary outcomes

The primary outcome measures included the AFC at baseline, the total number of oocytes retrieved, the number of MII oocytes, and FORT, which was calculated as the

number of pre-ovulatory follicles on the trigger day divided by the baseline AFC, multiplied by 100.

Secondary outcomes

The secondary outcome measures included the duration of ovarian stimulation (in days), total gonadotropin dose administered (in international units), fertilisation rate calculated per injected oocyte, and embryo quality assessed on day 3 and day 5 based on the Gardner and Schoolcraft scoring system. Additional outcomes included the number of embryos cryopreserved and the incidence of adverse events, particularly ovarian hyperstimulation syndrome (OHSS).

Owing to variability in embryo transfer timing (fresh versus frozen) and stage (cleavage versus blastocyst), clinical pregnancy and live birth outcomes were not assessed in this retrospective dataset.

Statistical analysis

Statistical analysis was performed using statistical package for the social sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY). Normality of continuous variables was assessed using the Shapiro–Wilk test. Between-group comparisons were made using Student's t-test or Mann–Whitney U test for continuous variables, and Chi-square or Fisher's exact test for categorical variables. A p value of <0.05 was considered statistically significant. No missing data imputation was required. Confounder adjustment was not applicable due to baseline comparability.

RESULTS

Baseline characteristics

Among the 237 women included, 144 received DHEA and 93 received testosterone. Age, BMI, AMH, AFC, and infertility duration were comparable between groups (Table 1). The distributions of POSEIDON group 3 and Group 4 classifications did not differ significantly between the groups (p=0.63).

Ovarian response and primary outcomes

Ovarian stimulation outcomes are summarised in (Table 2). Compared to DHEA, the testosterone group had significantly greater FORT (58.2±11.6 versus 49.3±12.4, p<0.01) and oocyte yield (7.2±2.8 versus 5.4±2.5, p=0.02). Post-treatment AFC was marginally greater in the testosterone group, but the difference was not statistically significant (4.4±1.3 versus 4.1±1.2, p=0.21) (Figures 1 and 2).

Table 1: Baseline demographic and ovarian reserve parameters in women undergoing IVF stratified by pre-treatment type (DHEA versus testosterone).

Parameter	DHEA group (n=144)	Testosterone group (n=93)	P value
Age (years), mean±SD	37.9±2.1	37.7±2.3	0.48
BMI (kg/m²), mean±SD	24.3±2.9	24.1±3.1	0.62
Infertility duration (years), mean±SD	5.8±2.2	5.6±2.1	0.51
AMH (ng/ml), median (IQR)	0.92 (0.7–1.1)	0.89 (0.7–1.2)	0.59
Baseline AFC, median (IQR)	3.9 (3–5)	4.0 (3–6)	0.66
POSEIDON group 3 (%)	41.7	43.0	0.83
POSEIDON group 4 (%)	58.3	57.0	0.83

*Values presented as mean±SD or median (IQR) as appropriate. Statistical significance calculated using student's t-test or Mann–Whitney U test for continuous variables, and Chi-square test for categorical variables. Multivariate analysis was not performed as baseline characteristics were comparable

Table 2: Controlled ovarian stimulation and embryological outcomes between DHEA and testosterone groups.

Outcome	DHEA group (n=144)	Testosterone group (n=93)	P value
Post-treatment AFC, mean±SD	4.1±1.2	4.4±1.3	0.21
FORT, mean±SD	49.3±12.4	58.2±11.6	<0.01
Oocytes retrieved, mean±SD	5.4±2.5	7.2±2.8	0.02
Stimulation days, mean±SD	10.3±1.9	9.7±1.6	0.04
Total gonadotropin dose (IU), mean±SD	2576±532	2291±484	<0.01
MII oocytes (%)	4.0	5.6	<0.01
Fertilization rate, mean±SD	61.2±10.9	63.5±11.2	0.37
Embryo quality (good grade) (%)	57.6	59.1	0.75
Adverse events (OHSS)	0	0	—

FORT=Follicular output rate; MII=metaphase II; AFC=antral follicle count; OHSS=ovarian hyperstimulation syndrome

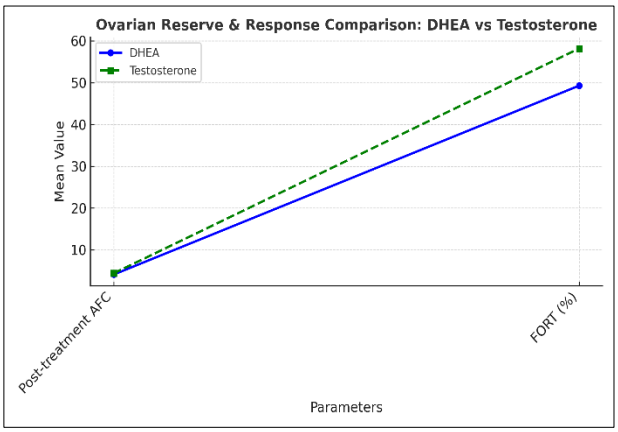

Figure 1: Ovarian reserve and follicular response in DHEA versus testosterone groups.

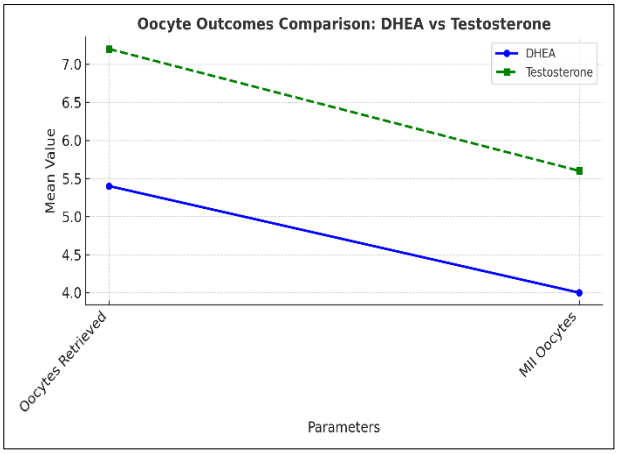

Figure 2: Oocyte yield and maturation in DHEA versus testosterone groups.

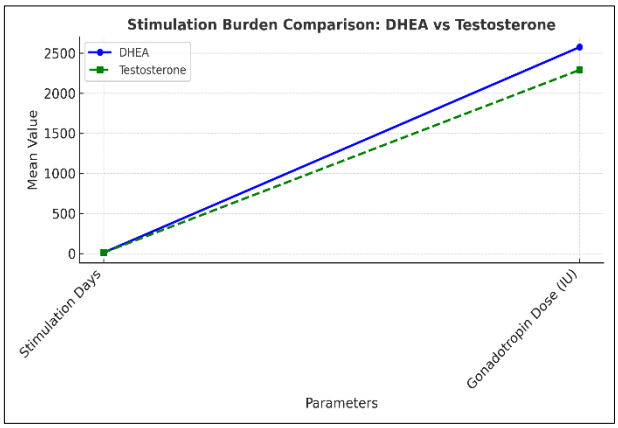

Figure 3: Stimulation burden comparison: days and gonadotropin dose.

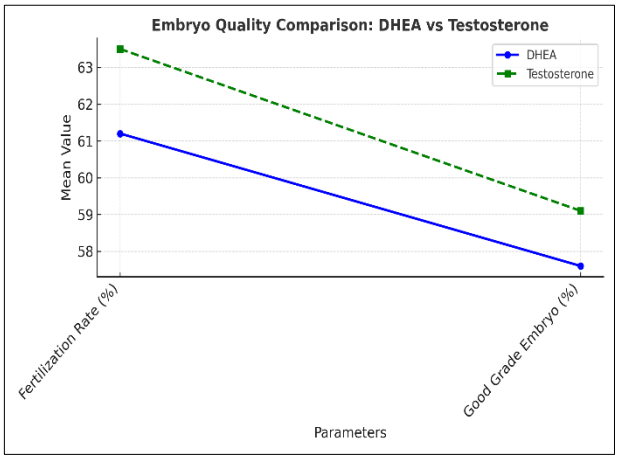

Figure 4: Fertilization rate and embryo quality outcomes.

Secondary outcomes

Testosterone-treated patients had shorter stimulation duration (9.7±1.6 versus 10.3±1.9 days, p=0.04) and lower gonadotropin usage (2291±484 IU versus 2576±532 IU, p<0.01). MII oocytes were also significantly greater in the testosterone group (5.6 versus 4.0, p<0.01) (Figure 3).

Additionally, a significantly greater number of MII oocytes were retrieved in the testosterone group (5.6 versus 4.0; p<0.01) (Figure 3).

While fertilisation rates (63.5% versus 61.2%; p=0.37) and the proportion of good-quality embryos (59.1% versus 57.6%; p=0.75) showed a favourable trend in the testosterone group, these differences did not reach statistical significance (Figure 4).

Safety and adverse events

No cases of OHSS were reported. No participants discontinued pre-treatment because of side effects.

DISCUSSION

This study compared the efficacy of pre-treatment with DHEA versus transdermal testosterone gel in women classified under POSEIDON groups 3 and 4 undergoing IVF. Testosterone directly addresses the low androgen milieu seen in POSEIDON patients. Our results demonstrate that short-term testosterone pre-treatment was associated with a significantly greater FORT, increased oocyte and MII oocyte yield, and lower gonadotropin requirements compared to DHEA.

These findings are supported by the growing body of literature indicating a critical role for androgens in promoting follicular recruitment and enhancing ovarian responsiveness through the upregulation of follicle-stimulating hormone (FSH) receptor expression and intraovarian insulin-like growth factor-1 (IGF-1) signalling.[9-11] A recent randomized controlled trial by Lu et al confirmed that testosterone pre-treatment improved oocyte yield, clinical pregnancy, and live birth rates in poor responders.[12]

In contrast, the benefits of DHEA have remained inconsistent. Although DHEA is believed to modulate AMH levels and androgen receptor activity within the follicular environment.[13,14] Multiple meta-analyses suggest only marginal improvements in oocyte yield and negligible effects on live birth rates.[15,16] Our findings are consistent with this evidence, showing the limited impact of DHEA compared to testosterone.

Notably, patients in the testosterone group also required significantly fewer days of stimulation and lower total gonadotropin doses. This supports earlier findings that testosterone may facilitate early follicular recruitment and synchronisation, thus reducing stimulation burden and treatment costs, an important consideration in resource-constrained settings.[17,18]

Interestingly, both POSEIDON group 3 (younger women with low reserve) and group 4 (older women with low reserve) appeared to benefit from testosterone supplementation. While several earlier studies suggested androgen responsiveness may be age-dependent, emerging data show potential benefits even in group 4 patients when adjuvant strategies are carefully individualised.[19-21]

Several mechanisms may underlie the superior outcomes observed with testosterone. Unlike DHEA, testosterone exerts a direct androgenic effect at the receptor level and bypasses enzymatic conversion, allowing for more predictable intra-ovarian exposure.[22] Transdermal formulations have been shown to achieve stable serum androgen levels and superior bioavailability compared to oral DHEA.[23,24]

Our findings are further reinforced by the 2024 Cochrane review, which reported moderate-quality evidence that testosterone improves live birth and clinical pregnancy rates in poor responders, while DHEA likely offers little to no benefit.[8] Fuentes et al observed that POSEIDON groups 3 and 4 are characterized by low serum levels of testosterone and androstenedione, perhaps testosterone supplementation is more effective because it directly addresses this deficiency.[3] Nevertheless, optimal dose, duration, and selection of patients remain unresolved, underscoring the need for standardized protocols.

Strengths

Strengths of the study include targeted POSEIDON stratification, homogeneity of stimulation protocols, consistent use of a dual trigger approach to optimise oocyte maturation in poor responders, and a direct head-to-head comparison, and a head-to-head comparison of commonly used androgen adjuvants.

Limitations

Limitations include the retrospective design, absence of randomisation, lack of serum androgen monitoring, and lack of live birth data. CoQ10 was included as part of the inclusion criteria to reflect the standard of care at our center for patients with poor ovarian reserve.

CONCLUSION

This study supports the use of short-term transdermal testosterone gel as a more effective pre-treatment strategy than DHEA in women with poor ovarian reserve, as defined by POSEIDON groups 3 and 4. Testosterone supplementation was associated with significantly higher FORT, improved oocyte yield, and reduced stimulation burden. However, prospective, randomised trials are warranted to validate these outcomes and define optimal protocols. Research should focus on identifying patient subgroups most likely to benefit from testosterone pre-treatment and on elucidating the underlying mechanisms of action.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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