ORIGINAL ARTICLE
https://doi.org/10.5653/cerm.2023.05946
pISSN 2233-8233 · eISSN 2233-8241
Clin Exp Reprod Med 2024;51(2):158-162

CERM

The effect of methyltestosterone on *in vitro* fertilization outcomes: A randomized clinical trial on patients with low ovarian response

Venus Haj Aliakbar[1], Fatemeh Davari Tanha[2], Firouzeh Akbari Asbagh[2], Mahbod Ebrahimi[2], Zahra Shahraki[3]

[1]Department of Obstetrics and Gynecology, Arash Women's Hospital, Tehran University of Medical Sciences, Tehran; [2]Department of Obstetrics and Gynecology, Yas Hospital, Tehran University of Medical Sciences, Tehran; [3]Department of Obstetrics and Gynecology, Amir-Al-Momenin Hospital, Zabol University of Medical Sciences, Zabol, Iran

Objective: The aim of this study was to compare the outcomes of *in vitro* fertilization (IVF) in patients with a poor ovarian response who used methyltestosterone, versus those using a placebo, in an infertility clinic setting.
Methods: This clinical trial included 120 women who had undergone IVF with intracytoplasmic sperm injection due to poor ovarian reserve and infertility. The study took place at the Yas Infertility Center in Tehran, Iran, between January 1, 2018 and January 1, 2019. In the intervention group, 25 mg of methyltestosterone was administered daily for 2 months prior to the initiation of assisted reproductive treatment. The control group was given placebo tablets for the same duration before starting their cycle. Each group was randomly assigned 60 patients. All analyses were performed using SPSS ver. 23 (IBM Corp.).
Results: The endometrial thickness in the intervention group was 7.57±1.22 mm, whereas in the control group, it was 7.11±1.02 (p=0.028). The gonadotropin number was significantly higher in the control group (64.7±13.48 vs. 57.9±9.25, p=0.001). However, there was no significant difference between the two groups in the antral follicular count. The chemical and clinical pregnancy rates in the intervention group were 18.33% and 15% respectively, compared to 8.33% and 6.67% in the control group. The rate of definitive pregnancy was marginally higher in the intervention group (13.3% vs. 3.3%, p=0.05).
Conclusion: The findings of this study suggest that pretreatment with methyltestosterone significantly increases endometrium thickness and is associated with an increase in the definitive pregnancy rate.

Keywords: Fertilization in vitro; Methyltestosterone; Ovarian stimulation; Suboptimal response

Introduction

Assisted reproductive technologies (ART) have become a global cornerstone in the management of infertility [1,2]. However, despite significant scientific advancements in the field of ART, a persistent challenge remains: the suboptimal ovarian response observed in a substantial number of women undergoing *in vitro* fertilization (IVF). Poor ovarian response, which is estimated to occur in 9% to 26% of cases [1], is characterized by inadequate increases in estradiol (EST) levels, a reduced yield of retrievable oocytes, and a limited number of maturing follicles [2].

In the field of therapeutic interventions aimed at addressing this issue, endocrine modulation has shown significant promise. Although the usefulness of dehydroepiandrosterone (DHEA) and its derivatives has been the subject of numerous studies, recent research has shifted focus towards methyltestosterone [3,4]. As an androgen, methyltestosterone has been proposed as a potential therapeutic agent that could improve pregnancy outcomes in women undergoing ART [5,6].

Received: February 7, 2023 Revised November 1, 2023 Accepted November 27, 2023
Corresponding author: **Zahra Shahraki**
Department of Obstetrics and Gynecology, Amir-Al-Momenin Hospital, Zabol University of Medical Sciences, Zabol, Iran
Tel: +98-918-1523144 E-mail: shahrakizahra86@gmail.com

*This study was supported by Tehran University of Medical Sciences (TUMS).

Previous studies have explored the intricate relationship between androgens and reproductive outcomes. For example, Vendola et al. [7] noted a direct stimulatory effect of testosterone on follicular maturation, which resulted in an increase in both follicular size and number. In contrast, the study conducted by Kara et al. [8] on DHEA did not identify any significant improvement in IVF-intracytoplasmic sperm injection (ICSI) outcomes for subjects with diminished ovarian reserve. In contrast, research by Barad and Gleicher [9] highlighted the potential of DHEA supplementation to increase both the number of oocytes and embryos.

Despite the extensive research conducted on various androgens, there remains a noticeable gap in our understanding of the definitive role of methyltestosterone. While androgens as a group have been thoroughly examined, the specific impact of methyltestosterone, particularly in the context of poor ovarian responders, has not been fully explored. The inconsistent results reported across different studies, coupled with the wide range of treatment protocols and dosages, underscore the need for a detailed understanding of the mechanistic and clinical implications of methyltestosterone.

With this backdrop, our study aimed to clarify this issue. We conducted a comparative analysis of IVF outcomes in women with documented poor ovarian response, comparing those treated with methyltestosterone to a placebo group. Our goal is to provide empirical evidence that could guide therapeutic strategies and inform clinical decisions.

Methods

1. Study design

This clinical trial study involved 120 women who had undergone IVF-ICSI due to poor ovarian reserve and infertility. The study took place at the Yas Infertility Center in Tehran, Iran, between January 1st, 2018 and January 1st, 2019. The study was conducted in strict accordance with the ethical guidelines set forth in the Declaration of Helsinki. The study protocol was thoroughly reviewed and subsequently approved by the Institutional Review Board of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1397.035). Additionally, this clinical trial was registered under the IRCT code "IRCT20091012002576N16."

In this clinical trial, we utilized a single-blind design. Patients were not aware of the specific treatment they were receiving—either methyltestosterone or a placebo—but the attending physicians and medical staff had access to this information. The reasoning behind this approach was to ensure the delivery of optimal patient care. In situations where individualized medical decisions, adjustments, or interventions were required, it was paramount for the attending physicians to be aware of the exact treatment each patient was receiving.

We utilized a convenience sampling method for participant recruitment. Each patient referred to the Yas Infertility Center underwent evaluation based on the study's inclusion criteria. The center accepts patient referrals from all regions of Iran, providing us with a broad catchment area. This allowed us to secure a diverse patient population, thereby enhancing the generalizability of our findings.

We utilized OpenEpi software to calculate the sample size [10]. We employed balanced block randomization (with a block size of 4) to assign subjects to groups, resulting in 60 patients per group. The patients were then randomly divided into two groups. In the intervention group, prior to the commencement of fertility-aid treatment, a daily dose of a 25-mg methyltestosterone tablet (25-mg tablet; Abu-Riyhan Pharmacy) was prescribed for a duration of 2 months. Following this, the patients immediately began the fertility-aid cycle. Conversely, patients in the control group were given placebo tablets for 2 months before initiating the cycle.

2. Eligibility criteria

We included patients who met the following criteria: women aged 35 to 42 years with a poor ovarian response, an antral follicle count of less than 5, and a serum anti-Müllerian hormone (AMH) level of less than 1.2, as per the Poseidon criteria for poor ovarian reserve [11]. Patients with a history of ovarian surgery, systemic disease, thyroid disorders, renal or hepatic dysfunction, as well as ovum donors were excluded from the study.

All participants provided written consent and agreed to participate in the study. They were informed about the potential side effects of methyltestosterone, which may include acne, oily skin, weight gain, and excessive sweating.

3. Study procedure

In both groups, we measured follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and thyroid hormones. For this study, we administered the standard protocol, which involved a daily dose of 300 U of gonadotropin FSH (Gonal-F; Merck Serono) starting on the second or third day of menstruation. Once the first 12-mm follicle was observed, we prescribed gonadotropin as a combination of FSH and LH (Merional; IBSA Slovakia). Starting from day 5 or upon observing a 14 mm follicle, we initiated a gonadotropin-releasing hormone antagonist at a dosage of 0.25 mg (CetrotideVR; Merck Serono). Each patient underwent sonography every 48 hours. Once the follicle measured between 18 and 20 mm, a prescription of 5,000 units of human chorionic gonadotropin was administered (Ovidrel1; Merck Serono Australia). Approximately 36 hours later, the patient's follicle was aspirated to retrieve the oocyte. Finally, we compared the number of oocytes obtained, the number of embryos, the number of embryos transferred, the number of FSH injections, the quality of eggs and embryos, and the pregnancy rates between the two groups.

Table 1. Age, duration of infertility, BMI, and basal hormone concentrations in the intervention and control groups

Variable	Intervention group	Control group	*p*-value
Age (yr)	33.53±4.77	33.4±6.13	0.89
Husband's age (yr)	34.83±4.35	35.95±6.1	0.25
Duration of infertility (yr)	4.57±2.02	5.29±3.43	0.16
BMI (kg/m^2)	23.45±3.03	21.09±3.4	0.038
Cycle day 3 FSH (IU/L)	9.48±2.13	8.15±2.2	0.01
Cycle day 3 AMH (IU/L)	0.63±0.28	0.83±0.27	0.03
Cycle day 3 EST (IU/L)	61.22±10.50	55.11±14.19	0.04
hCG (mIU/L)	856.85±307.58	957.53±370.2	0.11

Values are presented as mean±standard deviation.
BMI, body mass index; FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; EST, estradiol; hCG, human chorionic gonadotropin.

Table 2. Previous IVF, gravidity, parity, and childbearing history in the intervention and control groups

Variable	Intervention group	Control group	*p*-value
IVF times			0.18
1	51 (85.0)	45 (75.0)	
≥2	9 (15.0)	15 (25)	
Gravidity			0.20
0	46 (76.67)	38 (63.33)	
1	12 (20.0)	11 (18.33)	
≥2	2 (3.33)	11 (18.33)	
Parity			0.47
0	54 (90)	51 (85)	
≥1	6 (10)	9 (15)	
No. of children			0.50
0	55 (91.67)	52 (86.67)	
≥1	5 (8.34)	8 (13.34)	

Values are presented as number (%).
IVF, *in vitro* fertilization

4. Data analysis

Qualitative data are presented as frequency and percentage and quantitative variables are presented as mean±standard deviation. Categorical variables were compared using the chi-square test and continuous variables were compared using the Student t-test. All analyses were done using SPSS ver. 23 (IBM Corp.). A *p*-value less than 0.05 was considered to indicate statistical significance.

Results

Table 1 presents a comparison of patient age, duration of infertility, body mass index (BMI), and basal hormone serum concentrations between two groups. It is evident from the data that there was no significant difference between the two groups in terms of age, duration of infertility, and human chorionic gonadotropin levels. However, the mean BMI was significantly higher in the intervention group (23.45±3.03 kg/m^2 vs. 21.09±3.4 kg/m^2, $p<0.001$). Additionally, the cycle day 3 FSH and cycle day 3 EST levels were significantly higher in the intervention group. Conversely, patients in the control group exhibited higher cycle day 3 AMH levels ($p<0.05$).

Table 3. Quality of embryos in the intervention and control groups

Variable	Intervention group	Control group	*p*-value
Embryo A quality			0.72
0	36 (60.0)	34 (56.67)	
1	11 (18.33)	10 (16.67)	
≥2	13 (21.67)	16 (26.67)	
Embryo B quality			0.15
0	27 (45)	20 (33.33)	
1	24 (40)	33 (55.0)	
≥2	9 (15)	7 (11.67)	
Embryo C quality			0.88
0	24 (40)	30 (50)	
≥1	36 (60)	30 (50)	

Values are presented as number (%).

Table 2 presents the previous history of IVF, gravidity, parity, and childbearing in the groups studied. While the control group had higher gravidity, the difference was not statistically significant. Specifically, 76.67% of patients in the intervention group had no history of gravidity, compared to 63.33% in the control group. However, this difference was not significant ($p=0.20$). Likewise, no significant differences were observed between the two groups regarding the number of previous IVF cycles, parity, and childbearing. Regarding embryo quality, as detailed in Table 3, there was no significant difference between the intervention and control groups.

After treatment, the endometrial thickness in the intervention group was 7.57±1.22 mm, with a minor increase of 0.40%, whereas in the control group it was 7.11±1.02 mm, showing a minimal change of 0.14% ($p=0.028$). The gonadotropin number in the control group was significantly higher posttreatment, at 64.7±13.48, compared to the intervention group's value of 57.9±9.25, with respective percentage changes of 3.52% and 3.98% ($p=0.001$). There was no significant difference between the two groups regarding the posttreatment antral follicular count (Table 4).

As indicated in Table 5, the intervention group exhibited more favorable pregnancy outcomes. The rates of chemical pregnancy (18.33% vs. 8.33%, $p=0.22$) and clinical pregnancy (15% vs. 6.67%, $p=0.25$) were higher in the intervention group than in the control group, but these differences did not reach statistical significance. The rate of definitive pregnancy was also higher in the intervention group than in the control group (13.33% vs. 3.33%), a difference that

https://doi.org/10.5653/cerm.2023.05946

Table 4. Comparison of study predictors (ET, AFC, and GN) between the intervention and control groups

Variable	Intervention	Control	*p*-value
Pretreatment	7.54 ± 1.20	7.10 ± 1.13	0.82
Posttreatment	7.57 ± 1.22	7.11 ± 1.02	0.028
Percentage change (%)	0.4	0.14	-
Antral follicular count			
Pretreatment	5.98 ± 3.18	5.90 ± 2.66	0.90
Posttreatment	6.0 ± 3.19	5.91 ± 2.75	0.88
Percentage change (%)	0.33	0.17	-
Gonadotropin number			
Pretreatment	60.3 ± 11.4	62.5 ± 13.80	0.78
Posttreatment	57.9 ± 9.25	64.7 ± 13.48	0.001
Percentage change (%)	3.98	3.52	-

Values are presented as mean±standard deviation.
ET, endometrial thickness; AFC, antral follicular count; GN, gonadotropin number.

Table 5. Pregnancy outcomes in the intervention and control groups

Variable	Intervention group	Control group	*p*-value
Chemical pregnancy			0.22
Yes	11 (18.33)	5 (8.33)	
No	49 (81.67)	55 (91.67)	
Clinical pregnancy			0.25
Yes	9 (15.0)	4 (6.67)	
No	51 (85.0)	56 (93.33)	
Definitive pregnancy			0.05
Yes	8 (13.33)	2 (3.33)	
No	52 (86.67)	58 (96.67)	

Values are presented as number (%).

is borderline statistically significant with a *p*-value of 0.05. Abortions were reported in three patients in each of the study groups.

Discussion

The findings of our study suggest that pretreatment with methyltestosterone significantly enhances endometrial thickness, which is associated with an increased definitive pregnancy rate.

Our study aligns with that of Nagels et al. [12], who demonstrated in a review study that pretreatment with testosterone may enhance live birth rates in women identified as poor responders undergoing ART. In recent years, the role of testosterone in ovarian function has garnered considerable interest. Historically, androgens were thought to have a negative impact on ovarian function. However, recent research has highlighted the importance of androgens, particularly in the early stages of follicle maturation [13,14]. The pattern of androgen receptor (AR) expression in the granulosa cells (GCs) of immature preantral and early antral follicles is particularly noteworthy. As the follicle matures, AR expression decreases, underscoring the importance of androgens during the initial stages of follicle development [13,14]. Many researchers now posit that androgens play a crucial role in follicular recruitment, promoting follicular growth, and reducing GC apoptosis, which in turn leads to an increase in the number of growing follicles [15]. A meta-analysis conducted by Noventa et al. [16] suggested an increase in the total number of oocytes, M2 oocytes, and total embryos following testosterone therapy.

In our study, the posttreatment endometrial thickness in the intervention group exhibited a slight yet significant increase compared to the control group. This finding aligns with previous studies suggesting a correlation between optimal endometrial thickness and improved IVF outcomes [17]. Furthermore, Andreeva et al. [15] reported in their study that extended testosterone pretreatment could potentially enhance IVF treatment in patients with low ovarian response. Their results demonstrated an increase in pregnancy rates, which is consistent with our findings. We observed a nearly significant difference between the patients who received testosterone pretreatment and the control group in terms of definitive pregnancy [15].

Patients with poor ovarian responses pose significant challenges in the field of reproductive medicine, as a universally successful treatment method has yet to be established. It is postulated that androgens are crucial in the early stages of follicle development, and a deficiency in these hormones may lead to a decrease in ovarian responsiveness to FSH [18]. Our research findings suggest that pretreatment with methyltestosterone can enhance fertility outcomes for those with diminished ovarian reserves. Identifying the specific group of poor responders who might benefit most from testosterone therapy would be advantageous in future research. We recommend comprehensive studies with larger sample sizes for further investigation.

While the sample size of 120 participants in our study may limit its broader generalizability, the diversity of our patient population, drawn from various regions of Iran, enhances its representativeness. Any potential biases arising from the single-blind design were mitigated through the standardization of procedures and rigorous data collection.

In conclusion, This study's findings suggest that pretreatment with methyltestosterone significantly increased endometrial thickness and was associated with a higher definitive pregnancy rate. However, it did not enhance chemical and clinical pregnancy outcomes compared to the control group. Consequently, we recommend conducting additional prospective randomized clinical trials with larger sample sizes to better understand the role of methyltestosterone in improving pregnancy outcomes.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

We gratefully acknowledge the kind support of the participants for their invaluable collaboration in this study, as well as the staff of Yas Hospital.

ORCID

Venus Haj Aliakbar https://orcid.org/0009-0002-2132-7515
Zahra Shahraki https://orcid.org/0000-0002-6118-4718

Author contributions

Conceptualization: VHA, FDT, ZS. Data curation: FAA, ME. Formal analysis: FDT, ME. Funding acquisition: ZS. Methodology: FDT, ZS. Project administration: VHA, FDT, ZS. Visualization: FDT. Writing-original draft: FAA, ME. Writing-review & editing: VHA, FDT, ZS.

References

1. Vollenhoven B, Osianlis T, Catt J. Is there an ideal stimulation regimen for IVF for poor responders and does it change with age? J Assist Reprod Genet 2008;25:523-9.
2. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update 2003;9:61-76.
3. Barad D, Brill H, Gleicher N. Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function. J Assist Reprod Genet 2007;24:629-34.
4. Burger HG. Androgen production in women. Fertil Steril 2002;77 Suppl 4:S3-5.
5. Bosdou JK, Venetis CA, Kolibianakis EM, Toulis KA, Goulis DG, Zepiridis L, et al. The use of androgens or androgen-modulating agents in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Hum Reprod Update 2012;18:127-45.
6. Gonzalez-Comadran M, Duran M, Sola I, Fabregues F, Carreras R, Checa MA. Effects of transdermal testosterone in poor responders undergoing IVF: systematic review and meta-analysis. Reprod Biomed Online 2012;25:450-9.
7. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest 1998;101:2622-9.
8. Kara M, Aydin T, Aran T, Turktekin N, Ozdemir B. Does dehydroepiandrosterone supplementation really affect IVF-ICSI outcome in women with poor ovarian reserve? Eur J Obstet Gynecol Reprod Biol 2014;173:63-5.
9. Barad D, Gleicher N. Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. Hum Reprod 2006;21:2845-9.
10. Dean AG, Sullivan KM, Soe MM. OpenEpi: open source epidemiologic statistics for public health [Internet]. OpenEpi; 2011 [cited 2023 Dec 8]. Available from: www.openepi.com.
11. Esteves SC, Roque M, Bedoschi GM, Conforti A, Humaidan P, Alviggi C. Defining low prognosis patients undergoing assisted reproductive technology: POSEIDON criteria: the why. Front Endocrinol (Lausanne) 2018;9:461.
12. Nagels HE, Rishworth JR, Siristatidis CS, Kroon B. Androgens (dehydroepiandrosterone or testosterone) for women undergoing assisted reproduction. Cochrane Database Syst Rev 2015;2015:CD009749.
13. Gleicher N, Weghofer A, Barad DH. The role of androgens in follicle maturation and ovulation induction: friend or foe of infertility treatment? Reprod Biol Endocrinol 2011;9:116.
14. Prizant H, Gleicher N, Sen A. Androgen actions in the ovary: balance is key. J Endocrinol 2014;222:R141-51.
15. Andreeva P, Oprova I, Valkova L, Chaveeva P, Dimova I, Shterev A. The benefits of testosterone therapy in poor ovarian responders undergoing in vitro fertilisation (IVF). EMJ 2020;5:71-9.
16. Noventa M, Vitagliano A, Andrisani A, Blaganje M, Vigano P, Papaelo E, et al. Testosterone therapy for women with poor ovarian response undergoing IVF: a meta-analysis of randomized controlled trials. J Assist Reprod Genet 2019;36:673-83.
17. Nielsen ME, Rasmussen IA, Kristensen SG, Christensen ST, Mollgard K, Wreford Andersen E, et al. In human granulosa cells from small antral follicles, androgen receptor mRNA and androgen levels in follicular fluid correlate with FSH receptor mRNA. Mol Hum Reprod 2011;17:63-70.
18. Jindal PC, Singh MJ. How testosterone therapy is advantageous in improving the art outcome in poor ovarian responders undergoing IVF-ICSI cycles. Fertil Steril 2021;116:e367-8.

https://doi.org/10.5653/cerm.2023.05946