ORIGINAL ARTICLE

The Effect of Transdermal Testosterone Gel Pretreatment on IVF Outcomes in Patients with Poor Ovarian Reserve

Nidhi Sharma[1], Kanad D Nayar[2]

Received on: 11 November 2022; *Accepted on:* 15 December 2022; *Published on:* 16 September 2023

Abstract

Aim: To investigate the effect of pretreatment with testosterone gel on IVF outcomes in patients with poor ovarian reserve.

Materials and methods: In this prospective, single-blinded, randomized controlled trial, 87 patients, young or old (< or ≥35 years) with diminished ovarian reserve (DOR) (AFC ≤5, AMH ≤1.2 ng/mL) undergoing IVF treatment were enrolled and randomly divided into two groups. In the treatment group, 41 patients received pretreatment with testosterone gel (12.5 mg/day; from the 6th day of the previous menstrual cycle to 2nd day of stimulated cycle), and in the control group, 46 patients received lubricant gel.

Results: Total number of oocytes retrieved (4.05 vs 2.72; $p < 0.05$) was significantly more in the treatment group than in the control group. Similarly, the number of grade A embryos (2.78 vs 1.96; $p < 0.05$) was significantly greater in the TTG group. The clinical pregnancy rate per patient was higher in the TTG group (37% vs 29.3%) but the difference was not statistically significant.

Conclusion: Testosterone gel application before stimulation helps in reducing the dose and duration of gonadotrophins and to increase the number of oocytes retrieved and the quality of embryos formed in DOR patients.

Clinical significance: Pretreatment with androgen before ovarian stimulation can be offered to patients with poor ovarian reserve.

Keywords: Poor ovarian reserve, POSEIDON classification, Testosterone gel.

Journal of South Asian Federation of Obstetrics and Gynaecology (2023): 10.5005/jp-journals-10006-2278

Introduction

Poor response to controlled ovarian stimulation during *in vitro* fertilization (IVF) is a major challenge in assisted reproductive technology (ART) cycles. There are many studies on methods for improving the ovarian stimulation protocols, such as the use of higher doses of gonadotropins, Day 2 embryo transfer, growth hormone, glucocorticoids, low-dose aspirin as well as use of androgen have been done.[1] Over the past years, multiple studies have assessed the role of testosterone supplementation in POR patients. The rationale is the fact that an increase in intrafollicular androgen improves the follicle-stimulating hormone (FSH) receptor number on granulosa cells and helps in the growth of a greater number of follicles and better response to the gonadotropins.[2] Additionally, it has been hypothesized that during the early phases of follicular maturation, testosterone accelerates the transition of follicles from the quiescent to the developing pool.[3] In 2011, Bologna criteria were established to identify "poor responders" on the basis of age, previous ovarian response, and abnormal ovarian reserve test. However, it did not address the issue of change in oocyte number and quality with age and included all patients with diverse characteristics and prognoses in one group. For this reason, a new classification, Patient-Oriented Strategies Encompassing Individualize DOocyte Number (POSEIDON), was introduced in 2016 for patients with poor response to standard stimulation or reduced ovarian reserve. This is based on both quantitative and qualitative parameters: age and expected oocyte aneuploidy rate, Antral follicle count (AFC), anti-Mullerian hormone (AMH), and response to ovarian stimulation.[4] Groups 1 and 2 are the patients who have good follicle reserve but had unanticipated poor response to stimulation whereas group 3 (age < 35, AFC <5, and/or AMH <1.2 ng/mL) and 4 (age >35, AFC <5, and/or AMH <1.2 ng/mL) are the patients who are expected to be poor responders due to diminished ovarian reserve. The use of adjuvant therapy in the form of oral or transdermal androgens have been found to be useful in such patients.[5–7] Therefore, we planned this study to examine the effect of transdermal testosterone when administered over 4 weeks before initiation of controlled ovarian hyperstimulation on IVF outcomes in patients with reduced ovarian reserve (POSEIDON Group 3 and 4) who are expected to have a low response to standard stimulation.

[1]Department of Fertility Solutions, Medicover Fertility, Chandigarh, India

[2]Department of Reproductive Medicine, Akanksha IVF Centre, Mata Chanan Devi Hospital, Janakpuri, New Delhi, India

Corresponding Author: Nidhi Sharma, Department of Fertility Solutions, Medicover Fertility, Chandigarh, India, Phone: +91 9780959512, e-mail: drnidhi.sharma16@gmail.com

How to cite this article: Sharma N, Nayar KD. The Effect of Transdermal Testosterone Gel Pretreatment on IVF Outcomes in Patients with Poor Ovarian Reserve. J South Asian Feder Obst Gynae 2023;15(4):419–423.

Source of support: Nil

Conflict of interest: None

Materials and Methods

It was a prospective, single-blinded, randomized trial conducted from August 1, 2019 to March 31, 2020. After clearance from the ethical committee of the institution and registration of the trial with the institution bearing registration no. MCDH/2019/27, 87 patients meeting the inclusion criteria were enrolled in the study.

Inclusion Criteria

Patients fulfilling the criteria of groups 3 and 4 of POSEIDON classification will be included in the study viz young or old (< or ≥ 35 years) women with diminished ovarian reserve (DOR) (AFC ≤5, AMH ≤1.2 ng/mL).

Exclusion Criteria

- Presence of endocrine disorders (thyroid, prolactin).
- Presence of endometrioma and any history of surgery on the ovaries.
- Sensitivity to testosterone gel.
- Patients with male factor infertility.
- Patients with deranged liver and renal function tests.
- Oocyte donation cycle.

After the complete history, physical examination, and baseline investigation, patients were randomly divided into two groups with the help of a computer-generated random number list. Both testosterone and lubricant gel were kept in numbered bottles with dispensing pumps for each patient according to the randomization schedule. Each woman was assigned an order number and received the corresponding bottle. Only treating physicians were aware of the allotment (single-blinded study). In the treatment group (TTG group), once daily application of 12.5 mg testosterone gel (TTG; Androtas gel, 1% w/v, Intas pharmaceuticals, Ahmedabad, India) per day was started from the day 6 of the previous menstrual cycle to day 2 of the stimulated cycle. The gel was applied onto the dry, clean area with intact skin, on the inner upper arm or shoulder, abdomen, or inner aspect of thigh before bedtime and covered with clothing and was asked to report any side effects of the gel, such as skin rashes, itching, and burning sensation. Patients in the control group received lubricant gel (placebo) in similar way. On day 2 or 3 of the next menstrual cycle, serum FSH, LH, oestradiol, and progesterone were done along with a transvaginal ultrasound to assess AFC. For controlled ovarian hyperstimulation, fixed GnRH antagonist protocol (Cetrorelix 0.25 mg; Torrent Pharmaceuticals Ltd., Ahmedabad, India) was followed and the starting dose of gonadotrophin (recombinant FSH) was individualized according to each patient's parameters, with minimum dose as 225 IU/d and maximum upto 300 IU/day. Follow-up ultrasound was done to assess the number and size of growing follicles and the findings were recorded in the patient's chart. Once most follicles (at least 3) reached ≥ 18 mm, serum oestradiol was measured and a decision for ovulation trigger was taken accordingly. Recombinant HCG trigger (250 µg; Ovitrelle, Serono S.A. Spain) was administered as an ovulation trigger agent and ovum pick up was performed after 34–36 hours of ovulation trigger. After oocyte pickup, the oocytes were inseminated with the husband's semen (washed sample) for *in vitro* fertilization. Vaginal progesterone was started from the day of ovum pickup. This was followed by a fresh Day 5 blastocyst transfer using Sydney IVF embryo transfer (Cook's Medical, Australia) set. For luteal support, vaginal progesterone pessaries (Uterogestan 400 mg twice daily; Abott India, Mumbai) were continued for the next 14 days. A urine pregnancy test was done after 14 days of embryo transfer. A transvaginal ultrasound was advised after 2 weeks of urine pregnancy tests to document the presence, location, and number of intrauterine gestation sacs. The patients were followed till 12 weeks of pregnancy and then referred to the obstetrics unit of the hospital for further management.

Outcome Measures

The primary outcome has been assessed in terms of the number of oocytes retrieved in patients receiving testosterone gel pretreatment.

Secondary outcomes included total duration and dose of gonadotrophins, number of grade A embryos formed, implantation rate, and clinical pregnancy rate.

Ethical Justification

This study was approved by the Ethical Committee of the Indian fertility society, New Delhi. A written informed consent was taken from all the participants and was given the right to opt out at any time during the study.

Statistical Analysis

The total sample size was set as 45 calculated in each group with a power of 80%, at α of 0.05 to detect the assumed mean difference of 1 in the mean total number of oocytes between any two groups. Statistical testing was done with the SPSS version 22 (IBM Corp, USA).

Results

Forty-six patients in the TTG group and 41 patients in the control group completed the study protocol. Three patients in the control group were excluded from the study due to poor response (Fig. 1).

As depicted in Table 1, baseline characteristics, such as age, BMI, type, causes, and duration of infertility, serum AMH, TSH, serum prolactin, and the AFC on Day 2/3 of the patients in the TTG group and control group were similar. In the control group, 3 out of 44 cycles initiated (6.8%) were canceled prior to ET (poor follicular growth), whereas there was none in the TTG group (Fig. 1). Retrieved oocyte number was significantly higher in the TTG group (4.05 vs 2.72; $p < 0.05$) along with mature (MII) oocyte number (3.25 vs 2.11; p-value < 0.05) and grade A embryos (2.78 vs 1.96; $p < 0.05$) as compared with the control group. On analyzing the cycle characteristics (Table 2), it was found that the dosage and number of days of gonadotrophin stimulation in the TTG group were lower as compared with the control group ($p < 0.05$). The frequency of clinical pregnancies in the TTG group (36.9%) was also higher than in the control group (22.89%) but the difference was statistically insignificant. Similarly, a greater ongoing pregnancy rate (27.5% vs 22.9%, $p = 0.45$) and a lower miscarriage rate were observed in the TTG group (11.8% vs 8.3%, $p = 0.87$; Table 3).

Discussion

As has been described previously based on the POSEIDON classification, the key factor in achieving a higher pregnancy rate is the oocyte number obtained leading to the formation of at least one euploid blastocyst.[8] This study demonstrated that TTG pretreatment can increase the number of oocytes retrieved, the number of MII oocytes, and grade A embryos. These findings are further supported by other trials as well.[9,10] Use of testosterone gel resulted in the lower amount of gonadotrophin usage and a lesser number of days to reach our target follicle growth, as documented in other studies [5,9,11,12] This finding further strengthens the concept that androgens increase the sensitivity of the growing follicle to FSH by increasing the FSH receptors on the follicles, thereby reducing the dose of gonadotrophins required to achieve the desired follicle growth.[3,11] Similarly, the implantation and clinical pregnancy rate

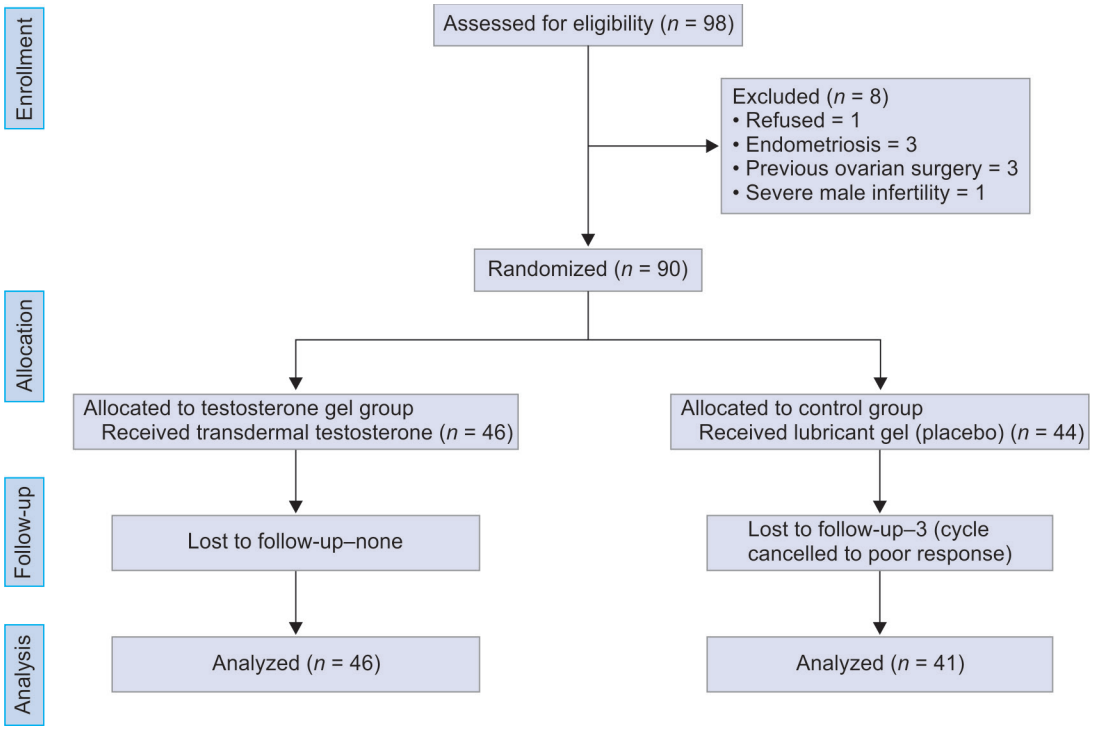

Fig. 1: CONSORT diagram of the progress through the phases of the randomized trial comparing transdermal testosterone gel with a lubricant gel in patients with diminished ovarian reserve

Table 1: Comparison of baseline variables in the two groups

Variables	*Testosterone gel (TTG) group (n = 46)*	*Control group (n = 41)*	*p-value*
Age (in years)	32.96 ± 3.76	33.78 ± 3.30	0.28
BMI (Kg/m^2)	23.5 ± 3.19	22.8 ± 2.9	0.29
Duration of infertility (years)	5.49 ± 2.8	6.56 ± 3.41	0.11
Type of infertility			
Primary (%)	30 (65.22%)	26 (63.41%)	0.86
Secondary (%)	16 (34.78%)	15 (36.59%)	0.86
Cause of infertility			
DOR	25 (55.56%)	25 (60.1%)	0.71
DOR + Tubal disease	20 (43.47%)	12 (29.2%)	
DOR + Others	1 (2.17%)	4 (9.76%)	
AFC (Day 2)	3.74 ± 0.83	4 ± 0.86	0.15
Serum AMH (ng/mL)	0.92 ± 0.30	0.86 ± 0.3	0.35
Serum TSH (mIU/L)	2.68 ± 0.95	3.25 ± 3.16	0.27
Serum Prolactin (ng/mL)			
Day 2 hormonal profile	14.78 ± 8.80	14.32 ± 9.32	0.81
Serum FSH (IU/L)	9.86 ± 3.29	9.97 ± 4.50	0.89
Serum LH (IU/L)	4.46 ± 2.04	4.87 ± 2.65	0.43
Serum oestradiol (pg/mL)	38.15 ± 12.40	37.64 ± 12.74	0.85
Serum progesterone (ng/mL)	0.48 ± 0.28	0.51 ± 0.26	0.57
Total serum testosterone (ng/dL)	115.2 ± 41.54	40.56 ± 18.12	0.001*

Data represented by Mean ± SD or by No. of patients (%), AFC, antral follicle count; AMH, anti-Mullerian hormone; BMI, body mass index; DOR, diminished ovarian reserve; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid stimulating hormone

was also higher in the TTG group than in controls. This has also been reported in various systematic reviews and meta-analysis.[3,9] However, one such meta-analysis by Jeve et al. involving three studies (total 225 patients) did not report any significant

Table 2: Comparison of cycle characteristics between the two groups

Parameters	*TTG group (n = 46)*	*Control group (n = 41)*	*p-value*
Total dose of gonadotrophins	3345.11 ± 1223.95	3826.22 ± 935.96	0.04*
Total duration of stimulation (days)	9.8 ± 1.42	10.56 ± 0.97	0.0045***
Serum E_2 on day of trigger	1041.37 ± 447.26	864.86 ± 310.79	<0.05*
No. of follicles on the day of trigger (total)	4.65 ± 1.84	3.21 ± 1.56	<0.05**
Total oocytes retrieved	4.05 ± 2.56	2.72 ± 2.03	<0.05*
Number of MII oocytes*	3.25 ± 1.32	2.11 ± 1.05	<0.05*
Number of Grade A blastocyst	2.78 ± 1.34	1.96 ± 1.51	<0.05*
No. of Grade B + C blastocyst	1.91 ± 1.28	1.54 ± 1.02	0.13
Endometrial thickness on day of trigger (mm)	10.1 ± 1.1	10.3 ± 0.7	0.32
No. of blastocysts transferred per patient	1.13 ± 0.48	1.04 ± 0.53	0.41

Data represented by Mean ± SD or by No. of patients (%), *Assessed in patients with ICSI only

Table 3: Comparison of clinical variables between the TTG group and control group

	TTG group (n = 46)	*Control group (n = 41)*	*p-value*
Implantation rate*	27.47% (24/91)	22.89% (19/83)	0.59
Clinical pregnancy rate†	36.95% (17/46)	29.26% (12/41)	0.45
Multiple pregnancy rate	11.76% (2/17)	8.33% (1/12)	0.75
Miscarriage rate	11.76% (2/17)	16.67% (2/12)	0.87
Ongoing pregnancy rate†	32.6% (15/46)	24.39% (10/41)	0.39

Data represented by % (number of patients), *No. of g sacs seen per embryo transferred, †Calculated per cycle initiated (%)

improvement in oocyte number while CPR and LBR demonstrated significant improvement (high evidence) in patients with poor ovarian response.[13] Another study by Bosdou et al. found that the difference between the number of oocytes retrieved after the use of transdermal testosterone was not 1.5 or more, as hypothesized in the study. However, the dose of testosterone used was 10 mg per day and for 21 days and GnRH agonist, long protocol was followed.[14] Patient selection is a limitation in most of the previous studies and meta-analysis as it has been done based on Bologna Criteria which is now being replaced by a better classification system, namely, the POSEIDON classification as it helps the clinician to plan the treatment, for every patient, individually with the help of an ART calculator.[8] Our present study is one of the few studies in which patients have been selected based on POSEIDON criteria. All the expected poor responders (< or ≥ 35 years) with DOR (AFC ≤5, AMH ≤1.2 ng/mL) that is, POSEIDON groups 3 and 4 were included. The dose and duration of testosterone application have been widely variable from 5 to 25 mg for 5–28 days in these studies. This drawback has also been addressed in the present study and patients received 12.5 mg/day of testosterone for 28 days which has been found to be effective in improving the clinical pregnancy rate.[15,16] Unlike oral therapy, transdermal testosterone has better bioavailability as it diffuses into circulation at a relatively constant rate during the 24-hour cycle, maintaining stable serum testosterone levels with minimum variation.[17]

Conclusion

Based on the results of our study, we can conclude that testosterone gel improves the number of oocytes as well as the quality of embryos formed in patients with DOR undergoing IVF stimulation. Further randomized studies with larger sample sizes need to be done to assess the effect of pretreatment with testosterone gel on IVF outcomes.

Acknowledgment

Ms Neha Sharma, MSc Statistics, a self-employed Bengaluru based statistician, helped in statistical analysis.

Orcid

Nidhi Sharma https://orcid.org/0000-0002-7116-3582

References

1. Gardner DK, Weissman A, Howles CM, et al. Textbook of Assisted Reproductive Techniques. 5th Edition. Florida, USA: CRC press, 2018.
2. Walters KA, Handelsman DJ. Role of androgens in the ovary. Mol Cell Endocrinol 2018;465:36–47. DOI: 10.1016/j.mce.2017.06.026.
3. Gervásio CG, Bernuci MP, Silva-de-Sá MF, et al. The role of androgen hormones in early follicular development. ISRN Obstet Gynecol 2014; 2014:818010. DOI: 10.1155/2014/818010.
4. Humaidan P, Alviggi C, Fischer R, et al. The novel POSEIDON stratification of 'Low prognosis patients in Assisted Reproductive Technology' and its proposed marker of successful outcome. F1000Res. 2016;5:2911. DOI: 10.12688/f1000research.10382.1.
5. Conforti A, Esteves SC, Picarelli S, et al. Novel approaches for diagnosis and management of low prognosis patients in assisted reproductive technology: The POSEIDON concept. Panminerva Med 2019;61:24–29. DOI: 10.23736/S0031-0808.18.03511-5.
6. Papathanasiou A, Searle BJ, King NM, et al. Trends in 'poor responder' research: lessons learned from RCTs in assisted conception. Hum Reprod Update 2016;22(3):306–319. DOI: 10.1093/humupd/dmw001.
7. Haahr T, Dosouto C, Alviggi C, et al. Management strategies for POSEIDON Groups 3 and 4. Front Endocrinol 2019;10:614. DOI: 10.3389/fendo.2019.00614.
8. Esteves SC, Carvalho JC, Bento FC, et al. A novel predictive model to estimate the number of mature oocytes required for obtaining at least one euploid blastocyst for transfer in couples undergoing *in vitro* fertilization/intracytoplasmic sperm injection: the ART Calculator. Front Endocrinol 2019;10:99. DOI: 10.3389/fendo.2019.00099.

9. Noventa M, Vitagliano A, Andrisani A, et al. Testosterone therapy for women with poor ovarian response undergoing IVF: a meta-analysis of randomized controlled trials. Journal of assisted reproduction and genetics 2019;36(4):673–683. DOI: 10.1007/s10815-018-1383-2.
10. Doan HT, Quan LH, Nguyen TT. The effectiveness of transdermal testosterone gel 1% (androgel) for poor responders undergoing in vitro fertilization. Gynecol Endocrinol 2017;33(12):977–979. DOI: 10.1080/09513590.2017.1332586.
11. Saharkhiz N, Zademodares S, Salehpour S, et al. The effect of testosterone gel on fertility outcomes in women with a poor response in in vitro fertilization cycles: A pilot randomized clinical trial. J Res Med Sci 2018;23:3. DOI: 10.4103/jrms.JRMS_864_17.
12. Kim CH, Howles CM, Lee HA. The effect of transdermal testosterone gel pretreatment on controlled ovarian stimulation and IVF outcome in low responders. Fertil Steril 2011;95(2):679–683. DOI: 10.1016/j.fertnstert.2010.07.1077.
13. Jeve YB, Bhandari HM. Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis. J Hum Reprod Sci 2016;9(2):70–81. DOI: 10.4103/0974-1208.183515.
14. Bosdou JK, Venetis CA, Dafopoulos K, et al. Transdermal testosterone pretreatment in poor responders undergoing ICSI: a randomized clinical trial. Hum Reprod 2016;31(5):977–985. DOI: 10.1093/humrep/dew028.
15. Vishwakarma P, Joy I, Varma TR. Role of testosterone pretreatment in poor ovarian responders undergoing in vitro fertilization/intracytoplasmic injection in comparison with growth hormone. IVF Lite 2016;3:90–97. DOI: 10.4103/2348-2907.204667.
16. Kim CH, Ahn JW, Moon JW, et al. Ovarian features after 2 weeks, 3 weeks and 4 weeks transdermal testosterone gel treatment and their associated effect on IVF outcomes in poor responders. Dev Reprod 2014;18(3):145–152. DOI: 10.12717/DR.2014.18.3.145.
17. Shoskes JJ, Wilson MK, Spinner ML. Pharmacology of testosterone replacement therapy preparations. Transl Androl Urol 2016;5(6):834–843. DOI: 10.21037/tau.2016.07.10.