RESEARCH ARTICLE

The Effect of Testosterone Gel for Patients with Poor Ovarian Response Prior to IVF Cycles: Is It really Effective?

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Received: 10th December, 2022; Revised: 15th January, 2023; Accepted: 26th February, 2023; Available Online: 25th March, 2023

ABSTRACT

Background: Classically, poor women respond with old maternal age and little ovarian standby. Though, some females unpredictably have little reply to measured ovarian stimulation.

Aim of the Study: To evaluate the effectiveness of transdermal testosterone gel (TTG) before controlled ovarian stimulation (COS) in poor responders undergoing intracytoplasmic sperm injection (ICSI).

Methods: An interventional prospective randomized control trial that included 60 females who were definite as unfortunate responders undergoing an intracytoplasmic sperm injection (ICSI) procedure. The population was divided into two groups. Approximately 30 patients received 10 mg TTG that was applied daily for 21 days in the cycle preceding COS for ICSI Transdermal testosterone gel (TTG) pretreatment group, while the other 30 patients are the control group. All patients were assessed for their AFC again and to begin *in-vitro* futilization (IVF) treatment with the beginning of the menstrual cycle. Serum testosterone was measured again in the study group. The primary outcome is the number of retrieved oocytes while the secondary outcome is the number and quality of embryos transferred, chemical gestation rate.

Results: No important differences in sociodemographic features between both groups compared. Also there was an increase in total number of oocytes in the study group but again, this difference was statically non-significant. Although the total dose of gonadotrophin (GT) and the duration of stimulation days were less in study group but this was statically non-significant. No differences experiential regarding number and grading of embryos, chemical pregnancy rate nor clinical pregnancy rate.

Conclusion: Pretreatment of transdermal testosterone at a dose of 10 mg/day for 21 days not upsurge the number of oocyte retrieved nor the number of embryos or pregnancy rate to a significant level in poor responders undergoing ICSI.

Keywords: Androgens, Intracytoplasmic sperm injection, Poor ovarian response, Transdermal testosterone pretreatment.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.1.46

How to cite this article: Muhammed EM, Al-khafajy ZH, Rasool HA. The Effect of Testosterone Gel for Patients with Poor Ovarian Response Prior to IVF Cycles: Is It really Effective?. International Journal of Drug Delivery Technology. 2023;13(1):285-289.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Poor response to ovarian stimulation represents around one-third of women undergoing assisted reproduction.[1] Till these last years, many debates have arisen concerning the meaning of deprived ovarian reply.[2,3] Women have very poor *in-vitro* futilization (IVF) outcomes regardless of age, meaningfully lesser when compared to females not achieving the Bologna principles.[4] In order to increase the recruitable pool of follicles, it would be necessary to enhance continued healthy follicle development. Unfortunately, gonadotropins (GT) only play a role during the last recruitment period and final follicular maturation. Therefore, some interventions would be required to overcome the age (or other pathological) related decline in follicle numbers.[5] Many strategies, adjuvant therapy and even procedures have been tested to improve the response to GT stimulation, though results from some studies have shown conflicting results.[6] Unfortunately, there is still insufficient data to support an effective role of rLH, DHEA, androgens or letrozole administration in the probability of pregnancy in poor responders undergoing ovarian stimulation for IVF.[7] Androgen is produced by the theca cells and acts as a substrate for the biosynthesis of estrogen and steroidogenesis.[8] Androgens also up-regulate their receptors and augment FSH receptors in granulosa cells.[9] Androgen plays a critical role in initial folliculogenesis and granulosa cell propagation and upsurge the number of prenatal and antral sacs.[10,11] Administration of exogenous testosterone or increased local ovarian androgen concentrations due to aromatase inhibition or exogenous LH

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and or hCG lead to an increase ovarian response to GT.[12] These possibilities point to treatment strategies that may increase circulating or local androgens in poor responders.

THE AIM OF THE STUDY

To evaluate the effectiveness of transdermal testosterone gel (TTG) before controlled ovarian stimulation (COS) in poor responders undergoing intracytoplasmic sperm injection (ICSI).

MATERIALS AND METHODS

This study is an interventional prospective randomized control trial conducted from September 2021 to August 2022 at infertility centers in Baghdad, Iraq. A total of 60 females were included in the study predicted poor responders. Poor responder was defined as such according to Bologna measures. The population was divided into two distinct groups of women. TTG pretreatment group (n=30) and control group (n=30), the randomization was done according to the randomization list order.

Inclusion Criteria

- Any predicted poor responder infertile female who fulfil two of three Bologna criteria at the start of enrolment, these criteria are:
- Advanced maternal age (or any other risk factor for poor ovarian reserve)
- Had previous poor response in an IVF cycle (cancelled cycle or number of retrieved oocytes are ≤ 3 with conventional GT stimulation dose)
- Poor ovarian reserve test (Antral follicle counts (AFC) < 7 or anti-mullerian hormone (AMH) < 1.1 ng/mL)
- Two previous episodes of POR after maximal stimulation alone even in the absence of other criteria are enough to consider the patient's poor responders.

Exclusion Criteria

Any woman was excluded from the study if she is one of the following:

- Menopause female
- Oocyte donation cycle
- Patients have liver or kidney diseases
- History of allergy to testosterone gel
- Uncontrolled DM, hypertension or heart disease
- Patients with an ovarian cyst or uterine pathology

All women in both groups were assessed their basal AFC by transvaginal ultrasound and hormonal status. After stimulation regimens and oocyte aspiration, vaginal ultrasonography 35 to 36 hours after hCG management, embryos graded 1 or 2 were reflected of high class. Both embryo transmission groups were done in the cleavage stage at day three.[13,14] The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2010. The descriptive statistics, including frequency, range, mean and standard deviation, were measured to describe the data. The groups were compared by applying an independent sample t-test (Unpaired t-test comparison between two groups), chi-square (Compare non-continuous data or percentage) and unpaired sample t-test (Comparison of continuous variables in 2 periods *i.e.* before and after). The results were considered statistically significant when *p-value* was ≤ 0.05.

RESULTS

Total 60 infertile female with poor ovarian reserve were enrolled in the present interventional case-control study. The first group, including 30 patients, used daily 10 mg TTG (1%) for 3 weeks as a pretreatment for poor ovarian reserve (Study group). These patients were compared with another 30 patients without pretreatment testosterone gel (Control group). The comparison of mean patient's age and body mass index between study and control groups were demonstrated in Table 1. Accordingly, there was an insignificantly higher age mean and body mass index in study group with p equal to 0.110 and 0.860, respectively. The comparison of the duration, type and causes of infertility was illustrated in Table 1. No significant differences between both groups regarding the above parameters ($p > 0.05$).

The comparison of hormonal levels between study and control groups was demonstrated in Table 2. According to the results there were no significant differences regarding FSH levels (p=0.119), LH levels (p=0.148), prolactin levels (p=0.272), E2 levels (p=0.967), TSH levels (p=0.675), AMH levels (p=0.870), testosterone levels before the treatment (p=0.898) and testosterone levels after the treatment (p=0.936).

Table 1: Comparison of demographic features between study and control groups

Parameters		*Study group n=30*	*Control group n=30*	*p-value*
Age (years) (Mean ± SD)		38.29 ± 6.58	35.21 ± 7.09	0.110 NS
BMI (Kg/m²) (Mean ± SD)		29.12 ± 4.67	28.87 ± 5.05	0.860 NS
Duration of infertility (years) (Mean ± SD)		7.92 ± 1.12	8.61 ± 3.64	0.588 NS
Type of infertility (%)	Primary	18 (60 %)	20 (66.7 %)	0.592 NS
	Secondary	12 (40 %)	10 (33.3 %)	
Cause of infertility (%)	Female causes	13 (43.3%)	8 (26.7 %)	0.393 NS
	Male factors	12 (40.0 %)	12 (40.0 %)	
	Unexplained	2 (6.7 %)	3 (10.0 %)	
	Mixed causes	3 (10.0 %)	7 (23.3 %)	

SD: Standard Deviation; S: Significant ($p \leq 0.05$); NS: Not significant ($p > 0.05$).

Table 2: Comparison of hormonal levels between study and control groups

Hormone	*Study group (Mean ± SD)*	*Control group (Mean ± SD)*	*p-value*
FSH (mIU/mL)	10.53 ± 2.47	6.05 ± 0.88	0.119 NS
LH (mIU/mL)	5.05 ± 0.79	4.49 ± 0.27	0.148 NS
Prolactin (ng/mL)	17.96 ± 1.13	20.18 ± 1.57	0.272 NS
E2 (pg/ mL)	37.78 ± 5.57	37.52 ± 3.22	0.967 NS
TSH (mIU/mL)	2.03 ± 0.19	1.95 ± 0.11	0.675 NS
AMH (ng/mL)	0.77 ± 0.23	0.74 ± 0.35	0.870 NS
Testosterone before the treatment (nmol/l)	0.49 ± 0.20	0.53 ± 0.18	0.898 NS
Testosterone before treatment (nmol/l)	0.56 ± 0.23	0.53 ± 0.31	0.936 NS

SD: Standard deviation; FSH: Follicle stimulating hormone; LH : Luteinizing hormone;E2: Estradiol; TSH: Thyroid stimulating hormone; AMH: Antimullerian hormone; NS: Non-significant (p > 0.05).

Table 3: Comparison of gonadotropin dose and duration of stimulation between study and control groups

Parameters	*Study group n=30*	*Control group n=30*	*p-value*
Gonadotropins dose (IU)	276.2 ± 28.6	330.6 ± 14.2	0.094 NS
Duration of gonadotropins (Days)	8.71 ± 0.54	9.86 ± 0.40	0.087 NS

NS: Non-significant ($p > 0.05$)

Table 4: Comparison of oocytes characteristics between study and control groups

Oocytes characteristics	*Study group (Mean ± SD)*	*Control group (Mean ± SD)*	*p value*
Antral follicles count before treatment	3.32 ± 1.91	4.21 ± 1.69	0.101 NS
Antral follicles count after treatment	4.15 ± 0.70	4.58 ± 0.34	0.562 NS
Total oocytes count	5.00 ± 1.21	4.68 ± 0.60	0.791 NS
Metaphase II (MII)	2.91 ± 0.62	3.41 ± 0.37	0.470 NS

SD: Standard deviation; NS: Non-significant ($p > 0.05$).

Table 5: Comparison of embryos characteristics between study and control groups

Parameter	*Study group (Mean ± SD)*	*Control group (Mean ± SD)*	*p-value*
Total embryos count	1.89 ± 0.41	2.36 ± 0.36	0.186 NS
Grade I embryo	1.22 ± 0.32	1.54 ± 0.32	0.572 NS
Grade II embryo	0.67 ± 0.24	0.79 ± 0.20	0.729 NS
Grade III embryo	0.00	0.13 ± 0.34	0.331 NS

NS: Non-significant ($p > 0.05$).

Table 6: Comparison of testosterone levels &AFC pre and post-testosterone therapy

Parameter	*Before treatment (Mean ± SD)*	*After treatment (Mean ± SD)*	*p-value*
Serum testosterone	0.55 ± 0.22	0.56 ± 0.23	0.264 NS
Antral follicle counts (AFC)	3.42 ± 0.45	4.16 ± 0.74	0.756 NS

NS: Non-significant ($p > 0.05$).

In Table 3, the results showed no significant differences regarding the dose of GT (p=0.094) and duration of GT treatment (p=0.087).

The comparison of oocytes characteristics between study and control groups was presented in Table 4. Accordingly, there were no significant differences regarding AFC before (p=0.101) and after (p=0.562) testosterone gel treatment, total oocytes count (p=0.791), and metaphase II oocytes count (p=0.470).

There were also no significant differences between study and control groups regarding the characteristics of the embryo, including total embryos, grade I embryos, grade II embryos and grade III embryo counts ($p > 0.05$) as demonstrated in Table 5.

Paired sample t-test had been applied for the comparison of serum testosterone levels and AFC before and after testosterone gel treatment, the results showed insignificantly higher serum testosterone and AFC after testosterone gel treatment with *p-value* equal to 0.264 and 0.756, respectively as presented in Table 6.

Total nine females out of 30 females treated with testosterone gel had a positive pregnancy test with a pregnancy rate equal to 30.0%. In the control group 7 patients (23.3%) had positive pregnancy tests (Table 7).

In the study group 6 patients (20.0%) had clinical pregnancy while in the control group only 5 (16.7%) patients had clinical pregnancy, so accordingly the miscarriage rates were 33.3 and 28.6%, respectively; however, there was no significant differences regarding clinical pregnancy and miscarriage rate between the two groups of patients ($p > 0.05$) as presented in Table 8.

Table 7: Chemical pregnancy rate of the study and control groups

Parameter	*Study group (%)*	*Control group (%)*	*p-value*
Positive pregnancy test	9 (30.0%)	7 (23.3%)	0.559 NS
Negative pregnancy test	21 (70.0%)	23 (76.7%)	

NS: Non-significant ($p > 0.05$).

Table 8: Comparison of clinical pregnancy and miscarriage rates of the study and control groups

Parameter	*Study group (%)*	*Control group (%)*	*p-value*
Clinical pregnancy rate	6/30 (20.0%)	5/30 (16.7%)	0.739 NS
Miscarriage rate	3/9 (33.3%)	2/7 (28.6%)	0.838 NS

NS: Non-significant ($p > 0.05$).

DISCUSSION

Increasing the number of developing follicles and oocytes by COS is the critical clinical milestone to improve the pregnancy of females who are arranged to experience IVF.[15] Reduced responders remain flop to reply sufficiently, although giving them the best dose of GT.[16] A variety of strategies to improve ovarian stimulation in poor responders have been proposed. Although unfortunately, most of these have not been significantly effective in improving ovarian response in this group of patients. However, some strategies that utilized pharmacological agents and manipulating endocrinology may augment follicular recruitment or cytoplasmic integrity, thus improving the prognosis for these women. Peterson and Fratarelli evaluated androgen levels in a number of females before IVF management and experiential that patients who had little levels of testosterone after down-regulation necessary an advanced dose of FSH or a lengthier period of ovarian stimulus and they were less likely to attain a pregnancy than patients with advanced baseline testosterone levels.[17] Barad and Gleicher presented that DHEA recovers the ovarian reply to COS in females with reduced ovarian backup.[18] In 2006 a study evaluated the effectiveness of treatment with a transdermal testosterone patch before stimulation in poor responders undergoing the IVF cycle.[19] In 2010 a study demonstrated that TTG pretreatment can increase the number of oocytes retrieved, number of good quality embryos, clinical pregnancy rate and reduction in the total FSH dose required in poor responders undergoing IVF/ICSI.[20] However, these promising primary studies explored the effect of testosterone management before ovarian stimulus in unfortunate responder females and showed a better live birth rate with testosterone pretreatment.[21] Moreover, a recent study conducted by Bosdou *et al*. which concluded in their publication in 2016 that pretreatment transdermal testosterone not upsurge the amount of "cumulus-oocyte complexes (COCs)" saved by ≥ 1.5 and they also described that there was no upsurge in LBR.[22] No significant differences neither in baseline characteristics nor in hormonal level between study and control groups. No important differences concerning the dose of GT (p=0.094) and duration of GT treatment (p=0.087). These results were similar to that found by Bosdou *et al.* where the duration of FSH stimulation was similar between the groups compared 12.5 *et al.* 12 days.[22] While other studies found a significant reduction in the duration of stimulation and the doses of FSH required among poor responders.[6,23] Although serum testosterone increased in our study group after the administration of testosterone gel, the difference did not reach a significant level. This finding differs from that seen in previous studies where they found significantly higher serum testosterone levels in study groups on the day of start of FSH stimulus.[7,20] Although we observed an increase in the AFC after using the gel in the study group, the number of oocytes was more than that in the control group. Unfortunately, there were no significant differences regarding AFC before and after testosterone gel treatment, total oocytes count in comparison to that in the control group. These findings differ from those seen in some previous studies when they found a significant increase in the number of oocytes retrieved.[23,24] In contrast, other studies found similar to our finding regarding the number and quality of oocytes.[6,7] No important differences between both groups regarding the embryo's characteristics and total numbers.

CONCLUSION

Pretreatment of transdermal testosterone at a dose of 10 mg/day for 21 days not upsurge the number of oocytes retrieved nor the number of embryos or pregnancy rate to a significant level in poor responders undergoing ICSI.

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