ORIGINAL ARTICLE

The effect of testosterone gel on fertility outcomes in women with a poor response in *in vitro* fertilization cycles: A pilot randomized clinical trial

Nasrin Saharkhiz, Shahrzad Zademodares, Saghar Salehpour, Sedighe Hosseini, Leyla Nazari, Hatav Ghasemi Tehrani[1]
Department of Obstetrics, Shahid Beheshti University, Tehran, [1]Department of Obstetrics, Isfahan University of Medical Sciences, Isfahan, Iran

Background: In this study, the effect of testosterone gel administration during ovulation induction on the fertility rate was examined in women with a poor ovarian response in *in vitro* fertilization (IVF) cycles. **Materials and Methods:** The current study is a single-blinded, randomized clinical trial. Patients who met inclusion (Bologna) criteria were placed in the antagonist cycle group. The patients were randomly divided into two groups each included 25 participants treated with a placebo (lubricant gel, the controls) and testosterone gel (intervention). Fertility outcomes were compared between two study groups. **Results:** The mean ± standard deviation (SD) age of intervention (41.04 ± 3.77) versus control group (39.69 ± 3.29) was not statistically different. The two studied groups were not statistically different in terms of follicle-stimulating hormone; antral follicle count, IVF, anti-Mullerian hormone, and the duration of infertility. The mean ± SD of oocyte 2.48 ± 1.64 versus 1.17 ± 1.27 and embryo 1.60 ± 1.58 versus 0.39 ± 0.58 in intervention group was significantly higher than control group ($P < 0.01$). The rate of pregnancy 16% versus 0% and embryo of quality A–B was significantly higher in intervention group than control (60% versus 17.4%, $P < 0.05$). **Conclusion:** The results of the current study showed that the testosterone gel has a significant impact on the fertility rate in women with a poor response in the IVF cycles. Further, randomized clinical trials with larger sample sized are recommended.

Key words: Embryo, *in vitro* fertilization cycles, infertility, oocyte, testosterone gel

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INTRODUCTION

Poor ovarian response to external gonadotropin drugs is one of the problems with assisted reproductive technology in 9%–26% of the *In vitro* fertilization (IVF) cycles.[1] It can lead to cycle stop, access to fewer oocytes and embryos, and finally reduced pregnancy rates.[2] IVF success rate per embryo transfer and live birth rate per treatment cycle are 25% and 18%, respectively. The ovarian response rate in the IVF cycle depends on various factors such as age, duration of infertility, cause of infertility, ovarian reserve, and cause of infertility, also previous surgery of the ovaries, pelvic adhesions, and high body mass index are associated with poor ovarian response, but poor response in some cases is also observed in young women.[1-6]

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Poor response to ovarian stimulation in IVF is a major challenge in IVF cycles and infertility treatment methods. No effective approach has been found yet to treat poor response to ovarian stimulation. However, there are several reports on possible methods affecting the performance of gonadotropins in the ovaries such as the use of high-dose gonadotropins,[7,8] treatment with growth hormone,[9,10] glucocorticoids,[10-12] and low-dose aspirin.[13] Another treatment is the use of low-dose androgens to improve ovarian response to gonadotropins.[14] The basis of this theory in literature is this fact that an increase in intrafollicular androgen increases the number of follicle-stimulating hormone (FSH) receptors on granulosa cells. As a result, the growth of follicles is improved leading to a better response to the gonadotropins.[15] During folliculogenesis, low-dose androgen plays an important

Address for correspondence: Dr. Hatav Ghasemi Tehrani, Department of Obstetrics, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: hatav.tehrani2014@gmail.com
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role as a substrate in the production of estradiol in the granulosa cells. They also play a trophic role in the growth of follicles, especially during the early growing stage of follicles when the dominant follicles are not yet selected. Studies on *Macaca mulatta* in the past decade show that administration of low doses of androgens stimulates the growth of follicles.[16] According to Vendola *et al.*, granulosa cells experienced a dramatic growth after administration of testosterone or dihydrotestosterone.[16]

Testosterone gel has been used as priming before the IVF cycle majority with the microdose flare protocol in previous studies. In the current study, the effect of testosterone gel during ovulation on the fertility rate in women with a poor ovarian response to IVF cycle was examined using antagonist protocol, and the results were compared with the lubricant gel as a control intervention.

MATERIALS AND METHODS

Study design and participants

This is a single-blinded, randomized clinical trial. Patients met the inclusion criteria were randomly divided into two groups each included 25 participants treated with a placebo (lubricant gel, the control group) and testosterone gel (intervention group).

Inclusion criteria were as follows: candidates for IVF cycles, patients older than 40 years, a cycle with early poor response, i.e., to obtain 3 or <3 oocytes of the cycles by normal stimulating, an antral follicle count (AFC) <5–7, anti-Mullerian hormone (AMH) <0.5–1.1 ng/ml, and male factor normal-FSH <15, and individual's consent to participate in the research project. Exclusion criteria in the current study were as the presence of endocrine disorders (thyroid, prolactin, etc.), endometrioma, and any history of surgery on the ovaries, reluctance to participate in the project, new clinical conditions or a change in a treatment procedure, sensitivity to testosterone gel and its complications. The conditions and possible side effects of testosterone gel were explained, and informed consent was obtained from the patients. The study protocol was registered in Iran center for clinical trials with clinical study registration number: IR.SBMU.RETECH.REC.1395.1007. Furthermore, the study protocol was approved by Ethic Committee of Shahid Beheshti University of Medical Sciences.

Procedures and assessment variables

The definition used in this study for poor responders to gonadotropins in IVF cycles is the Bologna criteria introduced by European Society of Human Reproduction and Embryology in 2011. A questionnaire was designed for data collection. The questionnaire consisted of three sections including age and patients history including the number of previous IVF, AFC, FSH, AMH, and duration of infertility (year); also the results after the intervention including oocyte, embryo, Grade A. B and grade C. D and the pregnancy was recorded in the designed questionnaire. The patients were randomly divided into two groups and were single blindly treated with placebo (lubricant gel, control group) and testosterone gel (intervention). Thus, the testosterone and placebo gel (lubricant) were purchased with the same shape, size, and color with similar package and labels. The patients and analyzer were not aware of the actual content of the gel until the end of analysis. Each batch contains 25 mg testosterone or placebo gel (lubricant).

First, estradiol, FSH, luteinizing hormone (LH), total testosterone, prolactin, and high thyroid hormones were measured by radioimmunoassay in the 3rd day of menstruation in a reputable laboratory. To ensure the normality, the patients received mercksern FSH gonadotropins (gonal-f) at a dose of 300 units from the 2nd day of the menstrual cycle. At the same time, the patients in the experimental group received 25 mg BayerShering Pharma testosterone gel daily for external use on the arm. The patients in the control group received lubricant gel and FSH. The patients were examined by ultrasonography every 48 h. When the follicle diameter reached 13–14 mm, antagonist (cetrotide) was administered to the patients, and the use of gels continued until human chorionic gonadotropin (HCG) administration.

When at least three follicles reached a size of >18 mm, the patients received 1000 IU HCG hormone intramuscularly in the form of 5000 IU ampules (Organon, Holand). Oocytes were transferred 36–38 h after injection of HCG under general anesthesia guided by transvaginal ultrasonography. Fertilized embryos were transferred into the uterus 48–72 h after the transfer of oocytes. If the endometrial thickness was <7 mm, the embryos were frozen and not transferred in that IVF cycle. The number of oocytes obtained, the number of embryos, the number of transferred embryos, the number of FSH ampules consumed, and the pregnancy rate were compared in the two groups.

Statistical analyses

Normality of continuous data was explored using Q-Q plot and Kolmogorov–Smirnov test and nonnormal positive skewed data were subjected to logarithmic transformation, and these data were expressed as mean ± standard deviation (SD) as well as median (minimum–maximum). Categorical data were expressed as frequency (percentage). Independent *t*-test and Chi-square (or Fisher's exact test) was used for comparing data between study groups. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to evaluate the impact of intervention

on categorical fertility outcomes (i.e., pregnancy and embryo of quality A-B and C-D). All statistical analyses were conducted using SPSS software version 20 (IBM, Inc., Chicago, IL, USA).

RESULTS

Twenty-three patients in the control group and 25 patients in the experimental group were completed the study protocol. Two patients in control group did not adhere to the study protocol then they were excluded from the study [Figure 1].

Table 1 presents the mean age and mean ± SD (median [minimum-maximum]) of basic clinical preintervention fertility-related variables of study participants in both intervention and control groups. As can be seen, no statistically significant differences were found between groups; indicating the comparable conditions in both groups before starting the study.

Table 2 presents the results of interventions on main outcomes of the study. The mean number of oocytes and embryo significantly higher in testosterone gel group than lubricant gel control group (both $P < 0.01$). The frequency of embryo with quality of A-B significantly higher in intervention group than control group, in which the testosterone gel increases the odds of embryo of quality A-B about 2 times (OR = 2.06, 95% CI: 1.23–3.46) while no significant difference was found between two groups in terms of embryo with quality C-D. The frequency of experienced pregnancies (16%) statistically significantly higher in intervention group than control group (0); the testosterone gel increases the odds of pregnancy 20% (OR = 1.20; 95% CI: 1.01–1.43; $P < 0.05$).

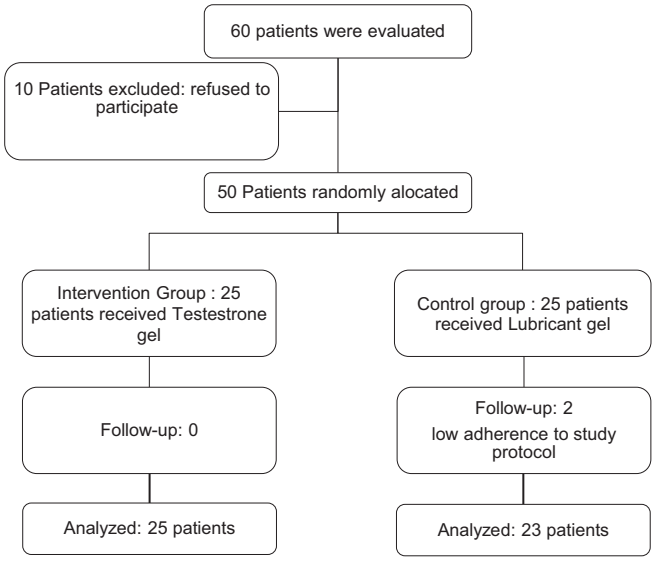

Figure 1: Flow diagram of patients recruitment

DISCUSSION

The results of the current study showed that the mean number of oocytes in patients used testosterone gel was significantly more than lubricant gel (control group). However, the results of Keay *et al.* and Sipe *et al.* showed no significant difference between two groups in terms of the number of oocytes. This discrepancy may be due to differences in the sample size in different studies.[1,17]

Our results showed that mean number of embryos in patients used testosterone gel was significantly more than that in the control group. In this regard, the results of a study by Haydardedeoglu and Isik on 25 patients showed that administration of 75 mg/day dehydroepiandrosterone for a 3-month period before the start of the IVF cycle lead to a significant increase in oocyte and embryo quality.[18]

The rate of pregnancy in our patients that used testosterone gel was significantly higher than control group. Hence, it can be concluded that the use of testosterone gel was effective treatment approach for infertility. Kyrou *et al.* found that a slight increase in serum androgen enhanced the success of

Table I: Demographic and basic clinical characteristics of participants in studied groups at the beginning of the study

Characteristics	Groups		*P*
	Intervention (*n*=25)	Control (*n*=23)	
Age (year)	41.04±3.77	39.70±3.29	0.20*
IVF	1.56±0.92 1 (1-4)	1.74±1.05 1 (1-4)	0.53*
AFC	2.24±0.59 2 (1-3)	3.14±2.64 2.5 (1-10)	0.11†
FSH	7.14±5.12 7 (1-25)	6.49±4.33 5.75 (0.6-20)	0.64†
AMH	051±0.57 0.4 (0.1-3)	0.57±0.51 0.3 (0.1-1.9)	0.67†
Duration of infertility (year)	5.42±6.39 3 (1-30)	4.94±3.19 3 (2-14)	0.79†

The data are presented as mean±SD or median (minimum-maximum), *P* values were calculated using *Independent sample *t*-test, †Mann-Whitney U-test. FSH = Follicle stimulating hormone; AFC = Antral follicle count; IVF = *In vitro* fertilization; AMH = Anti-Mullerian hormone; SD = Standard deviation

Table 2: Results of interventions on fertility outcomes

Characteristics	Groups		*P*
	Intervention (*n*=25)	Control (*n*=23)	
Oocyte (number)	2.48±1.64 2 (0-7)	1.17±1.27 1 (0-4)	0.004*
Embryo (number)	1.60±1.58 2 (0-7)	0.32±0.58 0 (0-2)	0.001†
Embryo with quality A-B (%)	15 (60)	4 (17.4)	0.003†
Embryo with quality C-D (%)	3 (12)	6 (26.1)	0.2†
Pregnancy outcome (%)	0	4 (16)	0.04†

The data are presented as mean±SD or median (minimum-maximum) for quantitative data and frequency (percentage) for categorical data, *P* values calculated using *Independent sample *t*-test, †Chi-square or Fisher's exact test. SD = Standard deviation

IVF cycles with a poor response to gonadotropins, leading a decrease in FSH dose needed to affect the ovary.[19]

According to Fábregues *et al.*, the use of 12.5 mg (the half of the dose in our study) transdermal testosterone for 2–4 weeks before the start of the IVF cycle decreased unsuccessful IVF cycles.[20]

Sipe *et al.* reviewed the impact of transdermal testosterone gel in patients with low ovarian reserve. According to this study, the required dose of FSH to stimulate the ovaries in patients used transdermal testosterone was much lower.[17]

González-Comadran *et al.* conducted a meta-analysis to investigate the impact of transdermal testosterone in pretreatment month in patients with poor response to gonadotropin. Their results showed an increase in the number of live births with less gonadotropin intake.[21]

In another study by Mitri *et al.* in Toronto, Canada, transdermal testosterone gel with microdose flare protocol was used for 26 women 34–47 years for 7 days. The use of this gel improved oocyte quality and follicle growth and estradiol while lowering FSH dose compared to the control group.[22]

All above-mentioned studies except Mitri *et al.*'s study[22] had used androgen as priming before starting the IVF cycle. In a pilot study by Mitri *et al.*, androgen was used with the microdose flare protocol at the start of IVF cycle. Seven days after the start of the cycle, gonadotropin discontinued, and the use of testosterone continued to HCG injections.

Study strengths and limitations

The current study is the only one with antagonist cycle, in which testosterone gel and gonadotropin were used from the beginning of cycle and continued until HCG injection. In fact, androgen alternatively plays the ultimate role of LH to enhance the follicular response to FSH. This in turn improves the quality of oocytes and the number and quality of embryos and consequently the fertility rate.

One limitation of our study is the low number of participants. Thus, a study on larger populations with more participants is recommended to be conducted. In addition, ultrasonography was performed for follow-up of participants until the diagnosis of clinical pregnancy; however, more long-term follow-up is needed.

CONCLUSION

According to the results of our study, the testosterone gel has a positive impact on fertility rate in women with poor response to IVF cycles. The use of testosterone gel improved the quality and number of oocytes as well as the number and quality of embryos and consequently the fertility rate. Further studies on larger sample with longer intervention period are recommended to evaluate the impact of testosterone gel on fertility and pregnancy.

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Conflicts of interest

There are no conflicts of interest.

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