

# Therapeutic effect of prolonged testosterone pretreatment in women with poor ovarian response: A randomized control trial

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## Abstract

**Purpose:** This study compared the therapeutic effects of transdermal testosterone gel (TTG) application at 4 and 6 weeks before controlled ovarian hyperstimulation (COH) in women with poor ovarian response (POR).

**Methods:** In this randomized control trial, infertile women with POR who underwent in vitro fertilization (IVF) were recruited and randomly classified into 4 week (n = 42) and 6 week (n = 38) TTG treatment groups and control group (n = 42). The primary outcome was total number of retrieved mature oocytes. The secondary outcomes were the biochemical pregnancy rate, clinical pregnancy rate, and ongoing pregnancy rate.

**Results:** No significant differences were observed in the number of oocytes retrieved, mature oocytes and embryos between all groups. Human chorionic gonadotropin (hCG) positive, clinical, and ongoing pregnancy rates were significantly higher in the TTG pretreatment groups than in the control group but no differences were observed between the 4- and 6 week groups.

**Conclusions:** Applying TTG in infertile women with POR may ameliorate the outcomes of IVF. The extended application of TTG to 6 weeks did not improve the response to ovarian stimulation regarding the number of retrieved oocytes nor pregnancy outcomes compared to the 4 week pretreatment.

## KEYWORDS

controlled ovarian hyperstimulation, in vitro fertilization, infertility, poor ovarian response, testosterone

## 1 | INTRODUCTION

Poor ovarian response (POR), defined as the retrieval of a low number of oocytes despite adequate ovarian stimulation in an assisted reproduction cycle, occurs in 9%-24% of infertile women undergoing in vitro fertilization (IVF).<sup>1,2</sup> Despite recent advances in assisted

reproductive technology (ART), POR remains one of the most challenging tasks for reproductive clinicians during controlled ovarian stimulation (COS). This is mainly due to inadequate standard protocols and drugs for the management and treatment of POR.<sup>3</sup> Various interventions have been proposed to improve pregnancy outcomes for patients with POR. These include increased gonadotropin

**Clinical Trial Registry:** The trial was registered at ClinicalTrials.Gov as NCT04602143.

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dosages, implementation of a modified natural IVF cycle, and addition of adjuvant agents during ovarian stimulation.<sup>1</sup>

One of the promising interventions is the administration of additional testosterone before or during ovarian stimulation because the low response to ovarian stimulation frequently reflects an age-related decline in testosterone levels. There is a positive association between testosterone levels and the number of oocytes retrieved.<sup>4,5</sup> Moreover, patients with higher testosterone baseline levels require a lower follicle-stimulating hormone (FSH) dose, a shorter duration of ovarian stimulation, and are more likely to achieve pregnancy than females with lower testosterone levels.<sup>6</sup> Testosterone supplementation in POR treatment could facilitate the transition of follicles from the dormant to the growing pool, during the early and intermediate stages of follicular maturation.<sup>7</sup> Available data suggest that testosterone may increase the number of pre-antral and antral follicles and augment the expression of FSH receptors in granulosa cells, potentially enhancing ovarian sensitivity to FSH.<sup>1</sup>

Regarding transdermal testosterone gel (TTG) supplementation, evidence suggests the effect of time-effectiveness ratio is more than the dose-effectiveness ratio.<sup>8</sup> Androgen treatment duration might be critical in stimulating follicular growth. However, limited evidence exists on the optimal treatment duration. While several studies do not support the use of testosterone for less than 21 days due to negative results,<sup>9-11</sup> only one study evaluated the effects of testosterone pretreatment based on different application durations.<sup>12</sup> Compared with the control group, TTG pretreatment for 3 or 4 weeks increased antral follicle count (AFC) and ovarian stromal blood flow, therefore, augmenting the numbers of oocytes retrieved and matured oocytes. However, significantly better results in terms of clinical pregnancy rate (CPR) and live birth rate (LBR) were only achieved in patients treated with transdermal testosterone extensively (4 weeks).<sup>12</sup> These results seem to agree with the fact that the transition period from the pre-antral to the antral follicular stage lasts approximately 70 days in humans.<sup>13</sup> To evaluate the effect of a longer duration of androgen supplementation, we hypothesized that increasing the testosterone treatment duration to 6 weeks may potentially increase the number of recruited follicles. Therefore, the present study aimed to determine the comparative therapeutic effect of 6 week and 4 week TTG application in women with POR.

## 2 | METHODS

### 2.1 | Study design and participants

This randomized control trial (RCT) was conducted between January 2018 and September 2019 at the National Center for Reproductive Medicine, Hanoi, Vietnam. In total, 165 women with POR undergoing IVF treatment in the study was assessed. For eligibility, inclusion criteria based on Bologna criteria included patients who met at least two of the following criteria: (a) advanced maternal age (40 years) or any other POR risk factor; (b) A previous incident of POR (cycles canceled or 3 oocytes with a conventional ovarian stimulation

protocol); (c) A low ovarian reserve test (AFC  $\leq$  5-7 or AMH  $\leq$  0.5-1.1 ng/mL). In contrast, exclusion criteria included severe primary ovarian insufficiency (POI) (which was defined as basal FSH level over 40 mIU/mL, basal estradiol level under 5 pg/mL, and female age < 40 years), donated gamete IVF cycles, male factor infertility, severe endometriosis or unexplained infertility, thyroid disease, liver and kidney dysfunction, and abnormal genitalia. After screening, eligible patients were randomly allocated into two TTG intervention groups (4 week and 6 week groups) and one control group through a manual lottery. Allocation concealment was by sequentially numbered, opaque, sealed envelopes. All participants were blinded to group assignment; however, researchers were not blinded.

### 2.2 | Intervention

The two TTG groups were administered 12.5 mg transdermal testosterone 1% (AndroGel 50 mg, Besin Healthcare, Belgium) onto clean, dry, healthy skin over both upper arms, once daily in the evening. All women did not take any form of oral contraceptive pills before or during the pretreatment period. Patients were instructed to avoid washing the skin on which the gel had been applied for 5 hours after application. The duration of TTG pretreatment was 4 or 6 weeks, depending on the group. In the 4 week group, TTG was applied from the first day of the menstrual cycle until the first day of the next menstrual cycle. In the 6 week group, TTG application was started 2 weeks earlier than in the 4 week group and ended on the first day of the menstrual cycle. Ovarian stimulation was started the day after the last testosterone gel application. In the control group, controlled ovarian stimulation was started on day 2 of the menstrual cycle without any pretreatment.

In all groups, patients underwent controlled ovarian stimulation with gonadotropin-releasing hormone (GnRH) antagonist protocol, using rFSH (Gonal F<sup>®</sup>, 300 IU, Merck Serono, Italy). The injection was started on day 2 of the cycle. The FSH starting dose and FSH dose adjustment were based on patient's condition and individual ovarian response. GnRH antagonist (Cetrotide<sup>®</sup>, 250  $\mu$ g, Merck Serono, German) was started at 250 mcg per day on day 6 of FSH injection and maintained daily until the day of trigger. hCG 6.500 IU (Ovitrelle<sup>®</sup>, 250  $\mu$ g, Merck Serono, Italia) was administered subcutaneously when at least 2 follicles had reached  $\geq$ 18 mm. Oocyte retrieval was performed under transvaginal ultrasound guidance 36-37 hours after hCG injection, and the embryos were cultured in G1-PLUS<sup>™</sup>/G2-PLUS<sup>™</sup> sequential media (Vitrolife<sup>®</sup>, Västra Frölunda, Sweden). We classified embryos according to standard morphological scoring by Istanbul consensus,<sup>1</sup> depending on the number of cells, size of the cells, fragmentation, and embryo quality as: good, fair, and poor. One to four embryos were transferred into the uterus on day 3 after oocyte aspiration, depending on the patient's age, embryo quality, and previously unsuccessful embryo transfer. Luteal support with micronized progesterone (Utrogestan 200 mg, Besin Healthcare, France) was administered (800 mg/day) from the day of oocyte retrieval until 12 weeks of gestational age.

## 2.3 | Hormonal tests

Hormone concentrations were measured using commercially available kits. Serum estradiol concentrations were estimated by electrochemical enzyme immunosorbent assay (EIA) (Elecsys Estradiol III, catalog number: 06 656 021 190, Roche, Japan) with a sensitivity of 5 pg/mL. Serum FSH and luteinizing hormone (LH) concentrations were measured by an immunoenzymatic assay (Elecsys FSH, catalog number: 11 775 863 122, and Elecsys LH, catalog number 11 732 234 122, Roche, Japan) and the sensitivity of both the FSH and LH assays was 0.1 IU/L. Serum AMH concentrations were measured by an immunoenzymatic assay (Elecsys AMH, catalog number: 06 331 076 190, Roche, Japan), and the sensitivity was 0.01 ng/mL. Total beta-human chorionic gonadotropin (hCG) was measured by immunoassay (Elecsys hCG +  $\beta$ , catalog number: 0 327 179 190, Roche, Japan) with a sensitivity of 0.1 mIU/mL.

## 2.4 | Ultrasonography

Ultrasonic scans were performed using a GE Voluson p6 ultrasound machine (2018, Korea) equipped with a 5-7 MHz endovaginal probe. The AFC ultrasound procedure was performed on day 2 of the menstrual cycle. All follicles measuring 2-10 mm in diameter in both ovaries were counted and recorded with the total number of AFCs. In IVF cycles, patients were scanned by ultrasound at least three times on day 2 of the cycle, day 6 of FSH, and day 8 of FSH. The last ultrasound was performed on the day of the trigger. The follicular diameter was calculated as the average length of the longest follicular axis and that of the axis perpendicular to it in the same scanning plane.

## 2.5 | Sample size and power calculation

The sample size was calculated for the difference in 2 independent means as follows:

$$n = \frac{(\sigma_1^2 + \sigma_2^2) \cdot (z_{1-\alpha/2} + z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

Notably,  $\mu_1 \pm \sigma_1 = 5.4 \pm 1.9$  was considered the number of oocytes retrieved in the testosterone group, and  $\mu_2 \pm \sigma_2 = 3.8 \pm 1.4$  was the number of oocytes retrieved in the control group according to Kim's study.<sup>2</sup>  $Z_{1-\alpha/2} = 1.96$  corresponds to 95% confidence interval;  $Z_{1-\beta} = 1.64$  corresponds to 95% strength, and the minimum sample size was 28 cycles per group. In total, 80 cycles were recruited for the study population during the recruitment period.

## 2.6 | Outcome assessment

The primary outcome of this study was the total number of retrieved mature oocytes. The secondary outcomes were the ongoing pregnancy

rate per cycle, pregnancy rate, biochemical pregnancy rate, and clinical pregnancy rate per cycle. Similarly, we also evaluated and analyzed the total dose and days of rFSH administration. Pregnancy was defined as positive when the serum beta-hCG level was  $\geq 50$  IU/L 14 days after embryo transfer. Clinical pregnancy was defined as the presence of a gestational sac identified by transvaginal ultrasound 4 weeks after embryo transfer. Biochemical pregnancy was defined as the absence of an identifiable pregnancy on ultrasound examination despite a positive serum hCG pregnancy test, and ongoing pregnancy was defined as a viable intrauterine pregnancy after 12 weeks of gestation.

## 2.7 | Statistical analysis

Data were analyzed using STATA software version 14.0 (Stata Corporation, College Station, TX, USA) on an intention-to-treat basis. Proportions, means, and standard deviations (SDs) were examined and presented for selected baseline variables in the treatment group. Comparisons of baseline characteristics and outcomes by treatment group were performed using ANOVA for continuous variables and a chi-square test for proportions. A Bonferroni correction was used for multiple testing wherein the resulting *P*-value was multiplied by the number of tests measuring similar constructs. Multivariable logistic regressions were used to quantify the effects of the treatment group, adjusted for all potential confounders including maternal age, body mass index (BMI), infertility duration, primary or secondary infertility, and history of IVF treatment. All statistical tests were two-sided, and *P* values  $< 0.05$  were considered statistically significant.

## 2.8 | Ethical approval

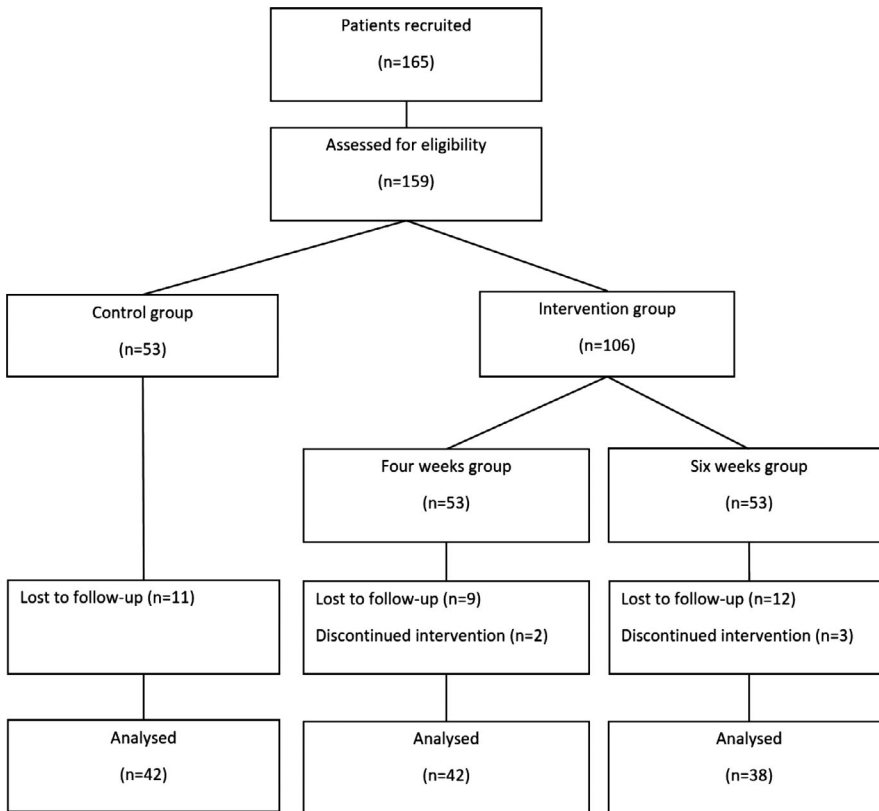
The protocol for this study was approved by the local Scientific and Ethical Committee in Biomedical Research, Hanoi Medical University (26NCS17/HMU IRB dated February 8, 2018) and the registration number at Clinical trials was NCT04602143. Written consent was obtained from all participants before data collection, following the Declaration of Helsinki.

# 3 | RESULTS

## 3.1 | Trial profile

We invited 165 potentially eligible women for screening, among which 6 were excluded before random assignment because they did not meet the study criteria. Among the 159 women who were eligible and agreed to participate, 37 were lost to follow-up because they received a donor egg or quit treatment before ovarian stimulation (Figure 1).

No differences were observed between the TTG pretreatment and control groups in general characteristics, including age, BMI,



**FIGURE 1** Study population enrollment flow chart From 165 women with poor ovarian response, 159 cases satisfied the inclusion and exclusion criteria. After screening, eligible patients were randomly allocated into two transdermal testosterone gel intervention groups (53 cases in 4 wk group and 53 cases in 6 wk group) and one control group (53 cases) through a manual lottery. During the time of study, loss of follow-up and discontinue of treatment results in the final data with 42, 38, and 42 women in 4 wk group, 6 wk group, and the control group, respectively

infertility duration, infertility classification, history of IVF treatment, AFC, and basal endocrine profile (Table 1). No difference in the characteristics of the study sample was observed among the groups.

Two cycles (4.7%) of the control group, two cycles (4.7%) of the 4 week group, and one cycle (2.6%) of the 6 week TTG group were canceled because of the lack of follicular development after stimulation or unproductive embryo obtained after culture (Table 2). No significant difference in the overall cycle cancellation rate was observed between the TTG pretreatment and control groups.

Compared to the control group, the treatment groups (either 4 or 6 weeks) have a significantly high endometrial thickness on the day of hCG injection ( $P = .00$ ), short time of recombinant follicle-stimulating hormone (rFSH) administration ( $P = .00$ ), and a low total rFSH dose ( $P = .00$ ) (Table 2). Similarity, all these differences are observed when comparing the 6 week group with the 4 week group on the day of hCG injection (Table 2).

Regarding the primary outcomes, no significant differences in the number of oocytes retrieved were observed between groups ( $5.5 \pm 2.4$  vs  $5.4 \pm 2.8$  and  $5.6 \pm 3.4$  in control and 4 week or 6 week group with  $P = .9$  and  $P = .88$ , respectively). Similar findings were also obtained in the secondary outcomes, including mature oocytes ( $4.5 \pm 1.9$  vs  $4.3 \pm 2.2$  and  $4.4 \pm 2.9$  with  $P = .6$  and  $P = .78$ , respectively), the number of good-quality embryos ( $3.8 \pm 2.1$  vs  $3.6 \pm 2.2$  and  $3.4 \pm 1.9$  with  $P = .8$  and  $P = .39$ , respectively), and the number of embryos transferred ( $2.3 \pm 1.0$  vs  $2.2 \pm 1.0$  and  $2.1 \pm 0.9$  with  $P = .39$  and  $P = .25$ , respectively), with no significant difference observed between the control group and the treatment groups (4 weeks and 6 weeks, respectively) (Table 3).

The hCG positive and ongoing pregnancy rates were significantly higher in the TTG pretreatment groups (32.5% and 30% in 4 week; 29.7% and 21.6% in 6 week group, respectively) than in the control group (only 10% and 7.5%, respectively) as shown in Table 3. Additionally, the clinical pregnancy rate was higher in the 4 week pretreatment groups (32.5% vs 10%) than in the control group ( $P = .01$ ). No differences in hCG positive, clinical, and ongoing pregnancy rates were observed between the 4 and 6 week TTG pretreatment groups. Only one case presented with light itching for a short time, which disappeared after a few days.

The effects of TTG treatment were further examined in multivariable logistic regression models, adjusted for all potential confounders (Table 4). The statistically significant differences were observed in the TTG treatment groups, unlike the BMI and female age groups. The 4 week pretreatment group has a better pregnancy outcome in hCG positive rate (odds ratio (OR): 4.14, 95% CI (confidence interval): 1.15-14.93,  $P = .03$ ), clinical pregnancy rate (OR: 4.63, 95% CI: 1.25-17.18,  $P = .02$ ), and ongoing pregnancy rate (OR: 5.84, 95% CI: 1.36-25.10,  $P = .02$ ) than the control group. Similarity, the 6 week pretreatment group yielded higher hCG positive rate (OR: 4.12, 95% CI: 1.15-14.85,  $P = .03$ ) than the control group; however, the magnitude of the effects is smaller than that in the 4 week group.

## 4 | DISCUSSION

This study was designed to evaluate the optimal duration of androgen supplementation before controlled ovarian stimulation using a GnRH

**TABLE 1** General characteristics of the study population

Characteristics	Control group n = 42	Treatment group		P*
		4 wk group n = 42	6 wk group n = 38	
Age (y)	36.4 ± 5.2	35.9 ± 5.4	36.2 ± 4.7	.92
BMI (kg/m <sup>2</sup> )	21.8 ± 2.3	21.1 ± 1.9	21.7 ± 2.7	.41
Underweight (<18.5)	4 (9.5)	3 (7.1)	4 (10.5)	
Normal (18.5-22.9)	25 (59.5)	28 (66.7)	22 (57.9)	
Overweight (23.0-24.9)	9 (21.4)	11 (26.2)	7 (18.4)	
Obesity (≥25)	4 (9.5)	0 (0)	5 (13.2)	.40
Infertility duration (mo)	65.4 ± 46.9	45.8 ± 26.7	56.8 ± 37.0	.05
Infertility classification				
Primary infertility	10 (23.8)	12 (28.6)	10 (26.3)	
Secondary infertility	32 (76.2)	30 (71.4)	28 (73.7)	.88
History of IVF treatment				
Yes	6 (14.3)	36 (85.7)	32 (84.2)	
No	36 (85.7)	6 (14.3)	6 (15.8)	.98
Endocrine tests				
Basal FSH (IU/L)	8.1 ± 2.0	7.5 ± 2.5	7.7 ± 3.8	.67
Basal LH (IU/L)	4.3 ± 1.8	4.3 ± 2.6	4.2 ± 1.8	.93
Basal E2 (pg/mL)	35.8 ± 12.8	40.3 ± 14.4	38.4 ± 19.8	.42
AMH (ng/mL)	1.0 ± 0.3	1.0 ± 0.4	0.9 ± 0.4	.55
AFC	5.9 ± 3.6	5.4 ± 2.6	4.9 ± 1.9	.32

Note: Data are presented as mean ± standard deviation or number (%).

Abbreviations: AFC, antral follicle count; AMH, anti-Mullerian hormone; BMI, body mass index; E2, estradiol; FSH, follicle-stimulating hormone; IVF, in vitro fertilization; LH, luteinizing hormone.

\*Comparison was performed between the treatment and control groups using the independent samples t test and chi-square test.

**TABLE 2** Comparison of ovarian response in the treatment groups and control group

Ovarian response	Control group	Treatment group			P1	P2	P3
		4 wk group	6 wk group				
No. of cycles recruited	42	42	38				
No. of cycles with oocyte retrieved and embryo transfer	40	40	37				
No. of cycles canceled	2 (4.7%)	2 (4.7%)	1 (2.6%)	.86	1.00	.62	
Starting dose of FSH (IU)	384.1 ± 55.7	373.2 ± 49.2	389.5 ± 61.7	.19	.34	.69	
Duration of rFSH (d)	10.0 ± 0.9	9.1 ± 0.6	9.5 ± 1.0	.03	.000	.04	
Total dose of rFSH (IU)	3803.8 ± 627.6	3369.6 ± 490.9	3707.2 ± 729.8	.01	.00	.4	
E2 level on day of hCG injection (pg/mL)	1771.1 ± 860.6	1841.5 ± 824.4	1909.8 ± 1137.4	.76	.70	.54	
EMT on day of hCG injection (mm)	10.3 ± 1.6	11.6 ± 1.9	10.7 ± 2.2	.05	.00	.38	

Note: P1: comparison between the 4-wk and 6-wk groups; P2: comparison between the 4-wk and control groups; P3: comparison between the 6-wk group and control group.

Abbreviations: CPR, clinical pregnancy rate; E2, estradiol; EMT, endometrial thickness; hCG, human chorionic gonadotropin; IU, international unit; No, number; rFSH, recombinant follicle-stimulating hormone.

antagonist in poor responders undergoing IVF. Our data confirms that androgen supplementation improves response to ovarian stimulation, regarding the number of retrieved oocytes. However, the 4 week protocol appeared to be as good as 6 week administration in term of

ovarian response and pregnancy outcomes. Therefore, it is unnecessary to extend the duration of androgen supplementation to 6 weeks.

The roles of androgens in folliculogenesis have been discussed more frequently in recent years. In essence, androgens are the basic

**TABLE 3** Comparison of IVF-ET outcomes between treatment groups and control group

Outcomes	Control group (n = 42)		Treatment group				P1	P2	P3
	n	Mean ± SD/%	4 wk group (n = 42)		6 wk group (n = 38)				
			n	Mean ± SD/%	n	Mean ± SD/%			
No. of oocyte retrieved	40	5.5 ± 2.4	41	5.4 ± 2.8	37	5.6 ± 3.4	.81	.90	.88
No. of mature oocyte	40	4.5 ± 1.9	41	4.3 ± 2.2	37	4.4 ± 2.9	.88	.60	.78
No. of embryos	40	3.8 ± 2.1	41	3.6 ± 2.2	37	3.4 ± 1.9	.56	.80	.39
No. of embryos transferred	40	2.3 ± 1.0	40	2.2 ± 1.0	37	2.1 ± 0.9	.69	.39	.25
hCG positive (%)	40	4 (10)	40	13 (32.5)	37	11 (29.7)	.79	.01	.03
CPR (%)	40	4 (10)	40	13 (32.5)	37	8 (21.6)	.28	.01	.16
Ongoing pregnancy rate (%)	40	3 (7.5)	40	12 (30.0)	37	8 (21.6)	.40	.00	.08

Note: P1: Comparison between the 4 wk and 6 wk groups; P2: Comparison between the 4 wk and control groups; P3: Comparison between the 6 wk group and control group. Data are presented as mean ± standard deviation or number (%).

Abbreviations: CPR, clinical pregnancy rate; hCG, human chorionic gonadotropin; No., number.

**TABLE 4** Effects of 4-wk and 6-wk transdermal testosterone gel pretreatment on pregnancy outcomes by multivariable logistic regression analysis

Factors	hCG positive			Clinical pregnancy rate			Ongoing pregnancy rate		
	OR	(95% CI)	P	OR	95% CI	P	OR	95% CI	P
Treatment groups									
Control	Ref								
4 wk	4.14	(1.15-14.93)	.03	4.63	(1.25-17.18)	.02	5.84	(1.36-25.10)	.02
6 wk	4.12	(1.15-14.85)	.03	2.59	(0.68-9.80)	.16	3.67	(0.85-15.89)	.08
Female's age									
<35 y	Ref								
≥35 y	1.00	(0.37-2.70)	.99	1.10	(0.39-3.11)	.86	1.33	(0.44-4.04)	.62
BMI									
Normal (18.5-22.9)	Ref								
Overweight (23.0-24.9)	1.33	(0.47-3.79)	.59	1.18	(0.40-3.54)	.77	1.38	(0.45-4.25)	.57
Obesity (≥25)	1.00	(0.16-6.15)	.99	1.52	(0.25-9.36)	.65	1.83	(0.27-12.25)	.53

Note: Abbreviations: BMI, body mass index; CI, confidence interval; hCG, human chorionic gonadotropin; OR, odd ratio; y, years.

substrate in the steroid making process of the ovaries based on the two-cell, two-gonadotropin theory.<sup>14</sup> Androgens supposedly play an important role in early follicular development and granulosa cell proliferation.<sup>15-17</sup> Additionally, they have been shown to increase the expression of FSH receptors in granulosa cells and increase the number of pre-antral and antral follicles.<sup>16</sup> In studies of human granulosa cells and follicular fluid of primary follicles, a significant correlation was observed between androgen receptors and FSH receptors in mRNA expression. Furthermore, follicle fluid testosterone concentration was observed to be highly correlated with FSH receptor mRNA expression.<sup>18</sup>

Although there have been many studies on the use of testosterone before ovarian stimulation, the results are limited and controversial. Similarly, studies have evaluated the efficacy of the bioactive androgen, testosterone, and its use in patients with POR to improve

ovarian response and IVF outcomes.<sup>9-11,19</sup> Our study found that androgen supplementation did not improve the retrieved oocytes number, however, better pregnancy outcomes were recognized in testosterone treatment groups. Although the number of embryos transferred was similar, clinical and ongoing pregnancy rates were lower in the control group than in the treatment groups. Because the endometrium thickness was higher in the 4 week group than in the control group, the "quality" of the oocytes and, therefore, quality of the embryos might be improved with testosterone supplement.

The duration and total dose of testosterone supplementation were suggested as key factors before controlled ovarian stimulation.<sup>12</sup> The ovarian bioavailability of systemic androgen is recommended to be studied further.<sup>20</sup> Similarly, the appropriate timing of androgen supplementation should be carefully considered. Considering the effect of androgen in the early stages of follicular

development, supplementation can be taken for 1-3 weeks before ovarian stimulation.<sup>12,17,21,22</sup> To evaluate the effect of a longer duration of androgen supplementation, the present RCT compared 4 and 6 week interventions to a control group to evaluate the optimum duration of testosterone supplementation in patients with POR. Our data showed that the duration of FSH administration and the total FSH dose in the 4 week group were significantly lower than those in the 6 week group. Positive effects of TTG pretreatment on ovarian stimulation were observed in the pregnancy and ongoing pregnancy rates in the treatment groups compared to the control group. The main findings of this study were the nonsignificant differences observed in pregnancy rate, CPR per cycle recruited, and ongoing pregnancy rates per cycle recruited between the 4 and 6 week TTG pretreatment groups. Our results highlight the advantages of androgen supplementation for 4 weeks rather than 6 weeks. The strategy of exclusively adopting the 4 week supplementation may offer the advantages of lower cost, patient-friendly prescription to patients, and reduction in the risk of therapy interruption.

However, does the shorter application of TTG under 4 weeks provide more benefits? Previously, Saharkhiz et al reported that testosterone gel pretreatment (25 mg/day for 12 days) significantly improved the number of oocytes retrieved.<sup>23</sup> However, in another study by Kim et al<sup>12</sup> with different androgen duration protocols (no pretreatment and 2, 3, and 4 weeks of testosterone pretreatment), 2 weeks of androgen pretreatment did not show any significant effects. However, patients treated for 3 and 4 weeks showed increases in AFC and ovarian stromal blood flow and significantly improved ovarian response (more oocytes retrieved); however, only patients pretreated for 4 weeks showed a significant increase in clinical pregnancy and live birth rates.<sup>12</sup> Recently, four RCTs (two with a placebo control) evaluating the effects for transdermal testosterone use for 5-20 days in POR patients showed no significant effect of testosterone pretreatment therapy.<sup>24</sup> In humans, the follicle progresses from a pre-antral stage to an early antral stage with a follicle diameter of 180-250  $\mu\text{m}$ . Through the accumulation of fluid in the antral cavity and the proliferation of granulosa and theca cells, the follicle progresses through the subsequent stages of development until it reaches 2 mm in size, a process that takes approximately 70 days.<sup>13</sup> Therefore, a short course of androgens has no significant effect on ovarian response and IVF outcome.

A possible limitation of this study is that it was not double-blinded as the clinicians were aware of the groups the patients were classified. This study was conducted in a tertiary hospital, a national center for assisted reproduction; therefore, clinical activities were monitored closely by the principal investigator, and the study design was strictly followed. The study sample was homogeneous (by random selection using sealed envelopes), based on general characteristics between groups. Although the sample size of 122 patients can be considered a strength of this study, we did not include severe POI cases into the inclusion criteria because they all received donated oocytes after consultation. Furthermore, several previous studies reported that there was a positive association between testosterone levels and the number of oocytes retrieved<sup>4,5</sup>; however, we did not

collect data on serum testosterone. Therefore, this relation was not revealed by our data. In this study, some cases were lost to follow-up because they withdrew from treatment to have their oocytes donated and some other cycles were paused due to the need to adhere to the social distancing guideline because of the Covid-19 pandemic.

In conclusion, this study confirmed that TTG pretreatment could improve IVF outcomes in poor responders undergoing IVF/ICSI. Particularly, a 4 week dose of 12.5 mg androgen supplementation appeared to be a better approach than an extended 6 week treatment as it guarantees a similar number of retrieved mature oocytes and pregnancy outcomes with shorter application time and lower cost.

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## DISCLOSURES

**Conflict of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. **Human rights statements and informed consent:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written consent was obtained from all participants before data collection. **Approval by Ethics Committee:** The protocol for this study was approved by the Scientific and Ethical Committee in Biomedical Research, Hanoi Medical University, 26NCS17/HMU IRB dated February 8, 2018.

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