Efficacy of transdermal testosterone in assisted reproduction outcome of poor responders

Milal Muhammad Al-Jeborry

1. University of Babylon, Department of Obstetrics, Gynecology and Infertility, Babylon, Iraq

Email: drmelal@yahoo.com. Mobile +9647706326600

Abstract

**Background:** Testosterone had a synergistic action with follicular stimulating hormone to enhance early stages of requirement and growth of ovarian follicles to enhance outcome in assisted reproduction program cycles. The aim of current study was to assess whether transdermal testosterone treatment before assisted reproduction cycle is effective in women with poor response. **Methods:** A prospective randomized trial performed in 132 low prognosis women for two years and at two IVF centers in Iraq. Participants were divided randomly into two groups; Group 1 involved 71 poor responders received transdermal testosterone in previous cycle and Group 2 women did not receive testosterone transdermal. In both groups, high dose gonadotrophins imitated from second day of cycle following baseline vaginal ultrasound and baseline hormonal investigations after obtaining written consents from all participants and ethical approval from assisted reproduction committee in Baghdad. The treatment regime was flexible antagonist in dose of 0.25mg corticosteroid when transvaginal ultrasound revealed dominant follicle size 13-14mm given daily subcutaneously till day of HCG triggers. Number of retrieved and mature oocytes and pregnancy rate were primary outcomes while secondary outcomes were fertilization and implantation rates, and rate of cancellation of cycle of IVF in addition to required doses of administered gonadotrophins and duration of stimulation. **Results:** There was no significant variation regarding the demographic parameters and basal hormonal assessment ($P$-value $>$ 0.05%). The testosterone gel group required less days of stimulation and fewer ampoules of gonadotrophins than non-testosterone group ($P$-value $<$ 0.05%). There were more collected oocytes, mature and fertilized oocytes with more transferred embryos with reduced cancellation of IVF cycle in testosterone gel group compared to non-testosterone gel group ($P$-value $<$ 0.05%). The pregnancy rate was none statistically higher in testosterone gel-treated group compared to non-testosterone treated group ($P$-value $>$ 0.05). **Conclusions:** In poor responders the transdermal testosterone treatment in the preceding cycle of IVF improves the clinical outcome with more collected and mature oocytes, higher pregnancy rate and less cancellation.

**Keywords:** transdermal testosterone, assisted reproduction outcome, poor responders, IVF.
Introduction

Poor responders represent a subgroup of IVF patients who did not respond well to ovarian stimulation by gonadotrophin. Although there was no accurate definition for poor responders, these women are of advanced age ≥40 years, had history of decreased ovarian response to conventional stimulation regimens or had reduced ovarian reserve \(^{(1)}\). In premenopausal women, the level of serum testosterone declines with advancing age in parallel with declining AMH value and declining AFC. It was postulated that secretion of testosterone from theca cells which surround the follicles declines with age. So, it was concluded that ovarian testosterone had a vital action in response of follicles to FSH, and the declining of response of oovaries with advanced age partly related to decrease production of androgens from ovaries. So, it was suggested that treatment with testosterone might enhance the action of FSH on ovaries \(^{(2-6)}\). Androgens were believed to play a valuable role in early stages of development of growing follicles and in proliferation of granulosa cells. Moreover, they enhance the expression of receptors of FSH in granulosa cells and elevating number of follicles in antral and pre-antral stages \(^{(7-9)}\). In addition, supplementation of androgens in form of testosterone gel or DHEA in cycle preceding IVF may enhance the outcome of assisted reproduction particularly in low prognosis women although there was controversy about the benefits of testosterone treatment on IVF \(^{(10-12)}\). Transdermal testosterone was most attractive and suitable treatment owing to its easy to apply, more convenient to patient, painless and safer than oral androgens as it avoids the first pass metabolism in liver \(^{(13)}\). It was reported that testosterone gel in preceding cycle will enhance the sensitivity of ovaries to stimulation by gonadotrophins \(^{(10)}\). Two studies also demonstrated a positive relationship between level of serum testosterone with improvement of some criteria of ovarian stimulation in assisted reproduction cycles \(^{(14, 15)}\). Therefore, the aim of current study was to investigate the effectiveness of testosterone gel applied transdermally in previous IVF cycle on outcome in low prognosis women.

Materials and Method

A prospective randomized trial includes 132 women with reduced ovarian reserve and diminished response to controlled stimulation in previous ICSI cycle to participate in this study after obtaining written consents from all participants during the period from November 2017 to October 2019. The study was conducted in two IVF centers; AL Sadder IVF center and higher institute for infertility and assisted reproduction in Baghdad after approval of research from committee of Arab Board and Assisted reproduction committee in Baghdad. A full history was obtained from all participants in the study regarding age, type, duration and etiology of infertility, previous ICSI cycle or previous cancelled cycle. Exclusion criteria were women older than forty three years, obese women with BMI more than thirty two, presence of...
endocrine diseases, women diagnosed as grade three or four endometriosis, those with premature ovarian failure and presence of male with azoospermia. Women diagnosed as low responders were randomly divided into two groups; Group 1 (N=71) they were instructed to apply 10mg testosterone gel 1% (Androtas) each gram contains testosterone USP 10mg, ethanol IP 67.0% and gel base. The testosterone gel applied daily to lower abdomen each night and leaving the area uncovered for 10-15 minutes and without friction of area to maximize absorption of the gel. The gel was applied for 21 days starting from day 5 of previous menstrual cycle. Group 2 (N=61) involved women as low responders according to POSEIDON classification but without receiving testosterone gel. The women asked to attend the IVF center on day two of cycle where baseline ultrasound by vaginal route (VENO 20) was performed to both groups to ensure absence of ovarian cyst, with the thickness of endometrium should be less than 5mm and counting the antral follicles in both ovaries. On same day, baseline hormonal investigations to both groups involving AMH, TSH, FSH, LH and serum estradiol after withdrawing 5-10ml of venous blood using VIDAS assay for performing the analysis. On second or third day of cycle, controlled stimulation initiated in both groups in form of recombinant FSH Merck Serono utilizing high dose ranging from 300-4500IU each day with or without addition of Menogon 75-150IU daily. Then, follow up of patients by assessment through transvaginal ultrasound every few days to monitor follicular growth and to assess the response of ovaries and titration of dose of gonadotrophins accordingly, with serial estradiol level in serum. When vaginal ultrasound revealed 13-14mm follicular size of dominant follicle then antagonist in form of cetrotide 0.25mg injection daily through subcutaneous route was given to both groups according to flexible regime. When two-three follicles more than 17-18mm were shown through vaginal ultrasound then triggering of final maturation of follicles by giving pregnyl in dose of 5000-10 000IU followed by aspiration of follicles under general anesthesia after 34-35 hours with the assistance of vaginal ultrasound utilizing double wash needle. Then assessment of maturity of oocytes under inverted microscope and all M11 oocytes (characterized by appearance of polar body) after stripping the cumulus cells through mechanical method or chemical method by utilizing hyaluronidase enzyme were injected by prepared sperm under inverted microscope. Identification of occurrence of fertilization was performed following eighteen hours through observation of second polar body and two pro-nucleoli under inverted microscope. Following three days culture of embryos in a special commercial media and after assessment of quality of developing embryos through observation of percentage of fragmentation and counting number of blastomeres and assess blastomeres for regularity, then transferring the embryos using Cook or Gynetics Medical with aid of ultrasound to guide replacement of embryos one centimeter under uterine fundus.

Support of luteal phase was performed through cyclogest progesterone suppository (Actavis, UK) inserted vaginally in dose of 400mg twice daily starting from day of oocyte collection up to 10-12 weeks after confirmation of biochemical pregnancy by quantitative pregnancy test for beta subunit of HCG which is performed two weeks beyond embryos transfer and after another two weeks we confirm clinical pregnancy by observing gestational sac and observing heartbeat. The number of collected oocytes, maturity
of oocytes and pregnancy rate were the primary outcomes, whereas length of stimulation, required dose of gonadotrophins, fertilization, implantation rates and rate of cancellation were the secondary outcomes.

**Statistical analysis**

The analysis of data was performed by using SPSS version 18. The independent \( t \)-test was used to compare between the two groups. Also, Data will be presented as mean±SD. \( P \)-value less than 0.05% will be considered significant.

**Results**

A randomized controlled trial enrolled 132 poor responder women defined according to POSEIDON criteria. The study population divided into two groups: 71 women take testosterone gel, whereas 61 women did not take testosterone gel. There was no statistically significant variation in demographic and day three basal hormonal and ultrasound parameters including age, BMI, period of infertility, previous cycle of IVF or cancelled previous IVF, AFC, AMH and day 3 estradiol, FSH, LH and TSH (Table 1).

Table (1) Demographic and basal hormonal criteria (day 3) of both study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test. gel Mean±SD</th>
<th>No Test. gel Mean±SD</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>36.6338±5.57094</td>
<td>35.7377±5.59733</td>
<td>.360</td>
</tr>
<tr>
<td>BMI</td>
<td>26.9168±1.84498</td>
<td>27.3331±1.80396</td>
<td>.194</td>
</tr>
<tr>
<td>Duration of infertility (year)</td>
<td>9.8944±5.12690</td>
<td>8.5328±4.95637</td>
<td>.125</td>
</tr>
<tr>
<td>FSH</td>
<td>10.2396±3.26079</td>
<td>10.6018±4.09184</td>
<td>.573</td>
</tr>
<tr>
<td>Estradiol</td>
<td>36.9886±8.08201</td>
<td>43.5074±5.351636</td>
<td>.313</td>
</tr>
<tr>
<td>AMH</td>
<td>.6052±0.29266</td>
<td>0.6166±0.25685</td>
<td>.815</td>
</tr>
<tr>
<td>AFC</td>
<td>5.5211±1.68064</td>
<td>5.0164±1.42000</td>
<td>.067</td>
</tr>
<tr>
<td>Previous IVF</td>
<td>0.5070±0.89240</td>
<td>0.3607±0.65911</td>
<td>.292</td>
</tr>
<tr>
<td>Previous cancelled cycle</td>
<td>0.2394±0.57233</td>
<td>0.1639±0.41554</td>
<td>.394</td>
</tr>
</tbody>
</table>

There was statically significant less required days of stimulation in testosterone gel group compared to non-testosterone gel groups (10.4085±1.14115 versus 12.5410±1.11913, respectively). With less required gonadotrophin dose (3811.2676±1020.84339 versus 4930.3279±875.70346, respectively) and more endometrial thickness (10.4873±1.91885 versus 9.1387±1.85333, respectively) in day of triggered the ovulation by HCG. There were statically significant higher collected oocytes (5.3662±2.72680 versus
3.4754±2.27014, respectively), higher mature oocytes (4.0563±2.72680 versus 3.4754±2.27014, respectively) and more transferred embryos (2.6901±1.36896 versus 1.6066±1.38177, respectively) in women take testosterone gel compared to non-testosterone gel group. There was significant variation among both groups regarding peal estradiol on day of trigger by HCG as revealed in Table (2).

Table (2) Stimulation parameters of both testosterone and non-testosterone gel groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test. gel Mean±SD</th>
<th>No Test. gel Mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of stimulation</td>
<td>10.4085±1.14115</td>
<td>12.5410±1.11913</td>
<td>.000</td>
</tr>
<tr>
<td>Gonadotrophins total dose</td>
<td>3811.2676±1020.84339</td>
<td>4930.3279±875.70346</td>
<td>.000</td>
</tr>
<tr>
<td>Peak estradiol</td>
<td>2917.6490±15124.59548</td>
<td>884.3177±669.85223</td>
<td>.296</td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>10.4873±1.91885</td>
<td>9.1387±1.85333</td>
<td>.000</td>
</tr>
<tr>
<td>Oocyte number</td>
<td>5.3662±2.72680</td>
<td>3.4754±2.27014</td>
<td>.000</td>
</tr>
<tr>
<td>Mature oocytes</td>
<td>4.0563±2.72680</td>
<td>3.4754±2.27014</td>
<td>.000</td>
</tr>
<tr>
<td>Transferred embryos</td>
<td>2.6901±1.36896</td>
<td>1.6066±1.38177</td>
<td>.000</td>
</tr>
</tbody>
</table>

There was statically significant higher fertilization rate in testosterone gel group (77.85% versus 60%, respectively) in non-testosterone gel group, with more implantation (16.20% versus 6.15%, respectively) and significantly less cancellation of cycle of IVF (9.8% versus 29.5%, respectively).

The pregnancy rate was not significantly higher in testosterone gel group than non-testosterone gel group (25.35% versus 13.11%, respectively; Table 3).

Table (3) Clinical outcomes of both testosterone gel and non-testosterone gel groups

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Test. gel N=71</th>
<th>Non Test. gel N=61</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization rate</td>
<td>77.85%</td>
<td>60%</td>
<td>.008</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>16.20%</td>
<td>6.15%</td>
<td>.022</td>
</tr>
<tr>
<td>Cancellation rate</td>
<td>9.8%</td>
<td>29.5%</td>
<td>.005</td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>25.35%</td>
<td>13.11%</td>
<td>.078</td>
</tr>
</tbody>
</table>
Discussion

Several treatment strategies had been adopted to enhance the outcome of assisted reproduction of low prognosis women, but still no agreement on the most effective regime and management of women with reduced ovarian reserve and remain a serious challenge in ART. Numerous studies had revealed that addition of testosterone to treatment regime in the preceding cycle might improve the stimulation outcome in low prognosis women (16-18). The mechanism beyond that is the expression of mRNA of androgen receptors which enhances expression of mRNA of FSH receptors of granulosa cells of follicles and this in turn augments the effects of FSH for growth of ovarian follicles (7,19). The current study showed improved oocyte number, maturity of oocyte and more transferred number of embryos in women received testosterone gel compared to non-testosterone gel group which is statistically significant. These findings were in concordance with RCT of (16) who involved more patients (110) and utilized higher dose of testosterone gel (12.5mg) per day lasting 21 days and showed significant elevation of oocyte number in testosterone group than non-testosterone group.

The same author designed second RCT in 2014, involved 120 women with varying duration of testosterone treatment and demonstrated statistically increase in oocytes number after 3 or 4 weeks of treatment (20). Our findings also agreed with a prospective study of (6) who included 35 women with prior decreased response and demonstrated more oocyte collected, enhanced AFC and more embryos cryopreserve with resultant increased cumulative pregnancy rate. These results disagreed with study of (21) on 49 low prognosis women and RCT and study of (22) on 50 poor responders who revealed no significant variation in oocytes collected number between women pre-treated with testosterone gel in dose of 10mg per day lasting up to 15-21 days.

Current study showed that in testosterone gel group there was less days of stimulation with less required gonadotrophins needed with improved fertilization and implantation rates, and reduced cancellation rate. There was no statistically significant higher clinical pregnancy rate compared to non-testosterone gel group. These results were in concordance with (12) who reported significant reduction of total required dose of gonadotrophins following three or four weeks of treatment with increased pregnancy rate and live birth rate.

Also, these results agreed with a meta-analysis conducted by (23) and revealed significantly increased clinical pregnancy rate and live birth rate with reduced doses of recombinant FSH in women treated by
testosterone (113 women) compared to women not received testosterone treatment (112 women). These results supported the additive action of testosterone with FSH in the process of folliculogenesis \(^{(23)}\).

While these results did not go with \(^{(22)}\) who reported similar days of FSH stimulation needed, with no variation in fertilization rate and live birth rate among both testosterone gel and non-testosterone treated groups.

On the other hand, \(^{(21)}\) failed to show enhancing action of testosterone treatment on ovarian sensitivity to gonadotrophins which might be related to many etiologies such as dose of testosterone given, duration and/or timing of treatment of testosterone. The differentiation of particular subgroup of low responding women with reduced blood androgen value who will get benefit from addition of testosterone transdermal treatment should be covered in subsequent studies.

**Conclusions**

In poor responders, the transdermal testosterone treatment in the preceding cycle of IVF improves the clinical outcome with more collected and mature oocytes, higher pregnancy rate with less cancellation.

**Ethical Clearance**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest**

The authors declare that they have no conflict of interest

**Funding:** Self-funding.

**References**


