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Effect of Pretreatment with Transdermal Testosterone on Controlled Ovarian Stimulation and Outcome in Poor Ovarian Responders Undergoing ICSI

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ABSTRACT

Background: Controlled ovarian stimulation (COS) has contributed to improving the pregnancy rate of women who undergo in vitro fertilization (IVF) by increasing the number of developing follicles and oocytes. Poor ovarian response to controlled ovarian stimulation (COS) remains a major problem in assisted reproduction.

Objective: To investigate the effectiveness of treatment with transdermal testosterone gel (TTG) before controlled ovarian stimulation (COS) in low responders undergoing intracytoplasmic sperm injection (ICSI).

Patients and Methods: A total of 300 patients who were defined as poor responders using BOLOGNA criteria were randomized into TTG pretreatment group and control group. For TTG pretreatment group, 10 g of TTG (5 % testosterone) for 15–20 days with a 5 mg/d nominal delivery rate of testosterone were applied daily for 15-20 days in the cycle preceding COS for IVF.

Main Outcome Measure(s): COS results and IVF outcome.

Result(s): There were no differences in patients' characteristics between the two groups. Total dose and days of human menopausal gonadotrophins (HMG) used were significantly lower in the TTG pretreatment group than in the control group. The numbers of oocytes retrieved, mature oocytes, fertilized oocytes, and good-quality embryos were similar in both groups. Embryo implantation rate, clinical pregnancy rate per cycle and live birth rate did not show any statistically significant difference between the two groups.

Conclusion: Transdermal testosterone pretreatment did not significantly increase the number of retrieved oocytes, clinical pregnancy rate or live birth rate in poor responders undergoing ICSI trials, however it reduces the total dose and number of days of HMG administration.

Keywords: Transdermal testosterone gel, controlled ovarian stimulation, IVF, low responders, ICSI

INTRODUCTION

The treatment success of in vitro fertilization (IVF) is based on various factors, including the number of retrieved oocytes. Failure to recruit adequate follicles, from which the oocytes are retrieved, is called a "poor response." The incidence of poor ovarian response (POR) in controlled ovarian hyperstimulation (COH) has been reported in 9–24% of intracytoplasmic sperm injection-embryo transfer (ICSI-ET) cycles [(1)].

Since POR represents several controversial issues in the clinical, scientific and psychological sense, an ESHRE Campus Workshop was organized in Bologna, 19 – 20 March 2010 involving all the ESHRE Special Interest Groups (SIGs) and the majority of research groups who have significantly contributed to the field. The main objective of the workshop was to reach a consensus on the definition and diagnosis of POR[(2)].

A definition was proposed in which a patient must exhibit two of the following: (1) Being over the age of 40 (≥40 age) or any other risk factor for POR (pelvic infection, ovarian endometrioma, ovarian surgery, chemotherapy, and short menstrual cycle). (2) Previous POR (with conventional stimulation protocol ≤ 3 oocytes). (3) Abnormal ovarian reserve test (the number of antral follicles < 5–7 or anti-Mullerian hormone (AMH) < 0.5–1.1 ng/mL) [(2)].

Clinicians have attempted to improve the ovarian response in poor ovarian responders by using androgens or androgen modulators prior to in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment [(3)].

Androgens, produced primarily by theca cells, play a critical role for an adequate follicular steroidogenesis and for a correct early follicular and granulosa cell development. They are the substrate for the aromatase activity of the granulosa cells, which converts the androgens to estrogens. Moreover, androgens may increase FSH receptor expression in granulosa cells amplifying the effects of FSH and thus potentially enhance responsiveness of ovaries to FSH. Furthermore, inadequate levels of endogenous androgens are associated with decreased ovarian sensitivity to FSH and low pregnancy rates after IVF [(3)].

The main androgen treatments involve direct androgen supplementation [including dehydroepiandrosterone (DHEA) and testosterone] and the indirect increase of intra-ovarian androgen levels. The latter has been achieved through aromatase inhibitors (anastrozole and letrozole), which are able to elevate intra-ovarian androgen levels by blocking the conversion of androgen substrate to estrogen. Among the androgens and androgen modulators mentioned above, transdermal testosterone treatment is most attractive due to its easy application, convenience, painlessness to the patient and most importantly its safety as, in contrast to oral androgen treatment, androgen is not first metabolized in the liver [(4)].

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The purpose of this study was to assess the effect of transdermal testosterone pretreatment on poor responders undergoing ovarian stimulation for ICSI in terms of number and quality of oocytes and embryos, total human menopausal gonadotrophins (HMG) dose, total duration of ovarian stimulation, the cancellation rate, pregnancy rate and live birth rate.

MATERIALS AND METHODS

This prospective randomized controlled trial included a total of 300 women who were seeking conception and underwent ICSI cycles, attending at ART unit, International Islamic center for population studies and researches, Al-Azhar university. This study was conducted between March 2015 and August 2018.

Ethical consideration and written informed consent:

An approval of the study was obtained from Al-Azhar University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the operation.

Subjects included in this study, and classified as poor responders by the BOLOGNA criteria were randomized into 2 groups by random allocation:

Group A: (intervention group): Patients receiving pre-treatment with testosterone gel preceding gonadotrophins stimulation (10 g of TTG (5 % testosterone) for 15–20 days with a 5 mg/d nominal delivery rate of testosterone were applied daily for 15-20 days in the cycle preceding COS for IVF).

Group B: (control group): Patients receiving standard gonadotrophin ovarian stimulation protocols without the administration of transdermal testosterone during the period of follicular stimulation.

Random allocation: three hundred opaque envelopes were numbered serially, inside each envelope written a letter either A or B denoting the allocated group according to a randomization table (where A is the study group and B is the control group), then all the envelopes were closed and put in one box, when the first patient arrived the first envelope was opened and the patient was allocated according to the letter inside. Randomization was done using a computer-generated randomization sheet.

A detailed history, including menstrual, obstetrical and past medical and surgical histories were taken, counseling the patients for the risks and benefits of undergoing ICSI and if there are alternatives.

General examination including BMI and local examination including Cusco speculum examination and vaginal ultrasound were done to all patients. Hormonal profile (FSH, LH, PRL, E2, TSH and AMH) and basic investigations (CBC, RH, RBS, coagulation profile, liver and kidney function tests and hepatitis markers) were done for all patients.

Flexible antagonist protocol for controlled ovarian stimulation was prescribed using highly purified Human Menopausal Gonadotrophins (Merional 75 IU, IBSA Institute Biochimique SA) In a dose of 375 IU (5 ampoules) intra-muscularly (IM) per day at a fixed dose regimen with individual adjustments according to ovarian response. Cetrolix acetate (Cetrotide 0.25 mg, EMD Serono) sub-cutaneous daily injection was started for patients when the leading follicle reached 14 mm. The ovarian response was monitored by vaginal ultrasound measurements of follicular growth (using Samsung Medison X6 ultrasound Machine) and serum level of estradiol every other day till the mean follicular diameter of most of the follicles reached 17-20-mm. Ovulation was triggered by administration of HCG (Choriomon 5000 I.U., IBSA Institute Biochimique SA), two ampoules intramuscularly.

Oocytes pick-up was done 36 hours later, incubated for one hour then injected using the selected sperms. Embryo transfer on day 2 or 3 was done for patients as part of the ICSI cycle.

Serum Quantitative B-HCG was done 14 days after the embryo transfer and repeated 48 hours later if needed. Trans-vaginal ultrasound was done 14 days after the pregnancy test for the pregnant women to check viability and number of the embryos

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance was used when comparing between two means.
- Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters.
- The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:
- Probability (P-value)
- P-value <0.05 was considered significant.
- P-value <0.001 was considered as highly significant.
- P-value >0.05 was considered insignificant.

RESULTS

Both groups were similar in regards to the patient characteristics (age, BMI, infertility duration, type of infertility, number and quality of oocytes and embryos), although the clinical pregnancy rate and the live birth rate were higher in the intervention group and the cancellation rate was higher in the control group, the differences were not statistically significant.

However, both HMG total dose and number of days of HMG administration showed statistically

significant difference between the intervention group and control group.

Human Menopausal Gonadotropins (HMG) administration:

Table 1 summarize the HMG administration for women enrolled in the study. There were no significant differences in HMG daily dose between the intervention group and control group. However, both HMG total dose and number of days of HMG administration showed statistically significant difference between the intervention group and control group (P = 0.025 and 0.038 respectively).

Table (1): HMG administration for women enrolled in the study:

	Intervention group (n=150)	Control group (n=150)	p-value
HMG daily dose (IU)	433.82 ± 35.99	438.97 ± 26.69	0.247 (NS)
HMG total dose (IU)	5057.35 ± 896.19	5319.85 ± 756.08	0.025*
HMG days	11.65 ± 1.66	12.09 ± 1.34	0.038*

Unpaired Student's t-test was used for continuous variables; Values are represented as mean±SD
*: Statistically significant (p<0.05) NS: Statistically not significant (P>0.05)

Outcome of ICSI:

Table 2 summarize the number of follicles at trigger, number of oocytes and number of embryos. The mean number of follicles at trigger in both groups were similar; 3.82 ± 1.21 in intervention group and 3.88 ± 1.09 in control group. The mean number of oocytes in both groups were similar; 2.99 ± 1.31 in intervention group and 2.74 ± 1.23 in control group. The mean number of embryos in both groups were also similar; 1.43 ± 0.84 in intervention group and 1.26 ± 0.83 in control group. There were no significant differences regarding them between the intervention group and control group (P = 0.716, 0.153 and 0.132 respectively).

Table (2): Number of follicles at trigger, oocytes and embryos of women enrolled in the study:

	Intervention group (n=150)	Control group (n=150)	p-value
Number of follicles at trigger	3.82 ± 1.21	3.88 ± 1.09	0.716 (NS)
Number of oocytes	2.99 ± 1.31	2.74 ± 1.23	0.153 (NS)
Number of embryos	1.43 ± 0.84	1.26 ± 0.83	0.132 (NS)

Unpaired Student's t-test was used for continuous variables; Values are represented as mean±SD
NS: Statistically not significant (P>0.05)

Table 3 summarize the quality of oocytes that produced. Apart of the number of atretic oocytes (P = 0.015), there were no significant differences between the intervention group and control group regarding the number of each oocyte grade (M2, M1 and GV).

Table (3): The quality of oocytes of women enrolled in the study:

	Intervention group (n=150)	Control group (n=150)	p-value
Number of M2	1.12 ± 1	1.1 ± 0.94	0.885 (NS)
Number of M1	0.97 ± 0.74	1.06 ± 0.74	0.395 (NS)
Number of GV	0.59 ± 0.62	0.49 ± 0.59	0.249 (NS)
Number of Atretic oocytes	0.33 ± 0.65	0.15 ± 0.41	0.015*

Unpaired Student's t-test was used for continuous variables; Values are represented as mean±SD
*: Statistically significant (p<0.05)
NS: Statistically not significant (P>0.05)

Table 4 summarize the quality of embryos. There were no significant differences between the intervention group and control group regarding the number of embryos grades A, B and C (P = 0.546, 0.216 and 0.702 respectively).

Table (4): The quality of embryos of women enrolled in the study:

	Intervention group (n=150)	Control group (n=150)	p-value
Number of Embryos grade A	1.06 ± 0.84	0.99 ± 0.78	0.546 (NS)
Number of Embryos grade B	0.32 ± 0.47	0.25 ± 0.43	0.216 (NS)
Number of Embryos grade C	0.04 ± 0.2	0.03 ± 0.17	0.702 (NS)

Unpaired Student's t-test was used for continuous variables; Values are represented as mean±SD
NS: Statistically not significant (P>0.05)

Table 5 summarize the rate of clinical pregnancy and the rate of cancellation. Although the clinical pregnancy rate is higher in the intervention group (12.7% vs. 10%) and the cancellation rate is higher in the control group (16.7% vs. 10.7%), the differences were not statistically significant (P = 0.42).

Table (5): Rate of clinical pregnancy and Rate of cancellation:

	Intervention group (n=150)	Control group (n=150)	p-value
Clinical Preg. +ve	19 (12.7%)	15 (10%)	0.420 (NS)
Clinical Preg. -ve	115 (76.6%)	110 (73.3%)	
Cycle is cancelled	16 (10.7%)	25 (16.7%)	

Pearson Chi square test was used for categorical variables; Values are represented as № (%)
NS: Statistically not significant (P>0.05)

Table 6 summarize the rate of live birth. Although the rate of live birth is higher in the intervention group (8.7% vs 6.7%), the differences were not statistically significant (P = 0.602).

Table (6): Rate of live birth:

	Intervention group (n=150)	Control group (n=150)	p-value
Live birth	13 (8.7%)	10 (6.7%)	.602 (NS)
No Live birth	137 (91.3%)	140 (93.3%)	

Pearson Chi square test was used for categorical variables; Values are represented as № (%)
NS: Statistically not significant (P>0.05)

DISCUSSION

Our study has shown that there were no statistically significant differences between the two groups as regards the mean number of follicles at trigger, the mean number and quality of the oocytes and embryos.

Although the clinical pregnancy rate is higher in the intervention group and the cancellation rate is higher in the control group, the differences were not statistically significant (P = 0.42). Live birth was higher in the intervention group however the differences were not statistically significant (P = 0.602).

Human Menopausal Gonadotropins total dose and number of days of HMG administration showed statistically significant difference between the intervention group and control group (P = 0.025 and 0.038 respectively).

Bosdou *et al.* [5], in a study included 50 patients revealed that transdermal testosterone pretreatment at a dose of 10 mg/day for 21 days does not increase the number of oocytes retrieved by more than 1.5 in poor responders undergoing ICSI stimulated by recombinant FSH in a long GnRH agonist protocol, also in agreement with our work, revealed a live birth rate of 10% in pretreatment group (2 out of 26) and 9.5% in control group (2 out of 24), he reported that the clinical pregnancy rate was found to be 7.7 %(two cases out of 26) in the pretreatment group meanwhile the control group had a pregnancy rate of 8.3% (two cases out of 24) (p value 1) which is in agreement with our study.

In contrary to our study, he reported that there was no statistical significant difference in the HMG dose (3750 versus 3600)(p value0.65) or duration (12 versus 12.5 days) (p value 0.65) between the pretreatment and the control group.

Kim *et al.* [6], in a large RCT including 110 patients showed that testosterone pretreatment (12.5 mg/day for 21 days) significantly increased the number of oocytes retrieved (mean difference: +1.60 COCs, 95% CI: +0.97 to +2.23) in poor responders as compared with no pretreatment. The same author in 2014 (Kim et al., 2014) studied the effect of testosterone pretreatment in 120 poor responders relatively to the duration of its administration (no pretreatment, 2, 3, and 4 weeks of testosterone pretreatment). This study suggested that testosterone has a beneficial effect on the number of oocytes retrieved, which however, is statistically detectable as compared with no treatment only after 3 or 4 weeks of administration. The mean±SD of oocytes retrieved in the four groups studied were: no pretreatment: 3.9+1.3, 2 weeks pretreatment: 4.3+1.6, 3 weeks pretreatment: 5.3+2.0, 4 weeks pretreatment: 5.8+1.9.

Kim *et al.* [6], found that the clinical pregnancy rates per cycle initiated and per cycle ET were also significantly higher in the TTG pretreatment group than in the control group (P=. 041 and P=.045, respectively). (Kim et al. 2014) reported a rising clinical pregnancy rate with longer testosterone pretreatment, so the clinical pregnancy rate was highest after 4 weeks therapy 36.7% (11/30) in comparison to 10.0% (3/30) , 16.7% (5/30) , 30.0 %(9/30) after the 1st ,2nd 3rd weeks respectively. They reported that the total dose of rhFSH required for oocytes were significantly lower in the pretreatment group than in the control group (2,552.3 ± 397 versus 3,000.8 ± 449) both(P<.001), and the duration of GnRH antagonist administration was also shorter in the pretreatment group (P=.001)

Kim *et al.* [7] stated that the doses of HMG were significantly lower in the intervention groups after 4weeks,3weeks and 2weeks pretreatment in comparison to the control group (2,765.7±567.8 , 2,643.5±389, 2,596.7±335.3 versus 3,025.0±425.9)(p value <0,001). and the duration of HMG administration was also shorter in the pretreatment group (P<.001)

Fabregues *et al.* [8], in a study that included 62 poor responder patients who were randomized in two treatment groups in their second IVF attempt. In patients in Group 1 (n = 31), transdermal application of testosterone preceding standard gonadotrophin ovarian stimulation under pituitary suppression was used. In Group 2 (n = 31 patients), ovarian stimulation was carried out with high-dose gonadotrophin in association with a mini-dose GnRH agonist protocol. The number of patients with ovum retrieval tended to be higher in Group 1 than in Group 2 (80.6% versus 58.1% ,P = 0.09), the difference reaching statistical significance (81.2% versus 41.1%, P < 0.05) when only patients having normal basal FSH levels (16 and 17 patients in Groups 1 and 2, respectively) were considered. He observed six live births in the intervention group (n = 31) compared with four live births in the control group (n = 31), whereas (Kim et al. 2011) reported 15 (n = 55) and seven (n = 55), respectively.(Kim et al.2014), reported that The numbers live birth rates were significantly higher in 4 weeks TTG treatment group than those in control group in the previous 1,2 and 3 weeks 30.0% (9/30) versus 6.7% (2/30),13.4% (4/30) and 20.0% (6/30) respectively. He reported six clinical pregnancies in the intervention group (n=31) compared with four clinical pregnancies in the control group (n=31) (p value=1) a possible explanation was presented on the basis of low FSH values in the patients of this study. He revealed that the total dose of FSH used was 2924 ± 987 in the pretreatment group in comparison to 3785 ± 1321 in the control group (p value <0.05) . In addition, the duration of the gonadotrophin therapy needed to achieve the criteria for hCG injection were significantly lower in Group 1 this could be explained by the beneficial effect of testosterone application on the ovarian response among patients having normal basal FSH levels.

CONCLUSION AND RECOMMENDATIONS

After reviewing reports in the literature regarding the efficacy and safety of the use of transdermal testosterone patches prior to ICSI cycles in poor responders compared to the usual protocols, and after presenting and discussing the results of the current study, we can conclude the following:

The current study has shown that transdermal testosterone pretreatment did not significantly increase the number of retrieved oocytes, clinical pregnancy rate or live birth rate in poor responders undergoing ICSI trials.

This study suggests that androgen pretreatment with transdermal testosterone gel significantly reduces the total dose and number of days of Human Menopausal Gonadotropins administration.

Transdermal testosterone should not be offered for poor ovarian responders as part of the routine induction protocols for Intracytoplasmic sperm injection (ICSI) cycles as till now there is no evidence of its beneficial effect as regards clinical and live birth rate.

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