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Full length article

The effect of androgen administration on in vitro fertilization outcome in poor responders undergoing ovarian stimulation with microdose protocol: A randomized clinical trial

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ABSTRACT

Objective(s): Patients with poor ovarian response who have reduced ovarian reserve sometimes despite the maximum dose of gonadotropins do not respond properly. Androgens have been shown to play an important role in the early follicular development and proliferation of granulosa cells. This study aimed to evaluate the effect of androgen administration on IVF outcome in poor responders.

Study Design: In this randomized clinical trial, 60 poor responder women candidate for controlled ovarian stimulation were randomly enrolled in two groups (n = 30/each). In the intervention group testosterone gel added to the interrupted microdose flare protocol. The control group received the conventional microdose flare protocol.

Results: The main outcome was clinical and chemical pregnancy; and the second outcomes were the number of mature oocytes, duration of cycle and total dose of gonadotropins. Basic clinical and demographic features were comparable between the groups. The total gonadotropin consumption were significantly higher in the control group than the intervention group ($p = 0.047$). In addition, the number of MII oocytes was higher (but not significant) in the intervention group than the control group ($p = 0.16$). The mean total duration of the cycle was equal in both groups. There were no significant differences in chemical and clinical pregnancy rates between the two groups ($p = 0.41$, $p = 0.67$).

Conclusion(s): The results of the current study showed that androgen administration in poor responders in in vitro fertilization reduces the total dose of gonadotropin, but it does not improve the pregnancy outcomes.

Introduction

Patients with poor ovarian response are a wide range of patients who have less than optimal response to ovarian stimulation [1] and are a major concern [2]. The reported prevalence of these patients is between 9 % and 26 % [2,3].

Various methods have been performed in trials to modify the response to gonadotropin stimulation and pregnancy outcome in poor responders. In common methods, the most commonly used drugs before and during ovarian stimulation include, aromatase inhibitor [4], recombinant LH [5], growth hormone [6,7], glucocorticoids [7–9] and recently injection of intraovarian platelet-rich plasma (PRP) [10]. Another treatment is the use of androgens to improve the ovarian response to gonadotropins. Over the years, several researchers have attempted to define poor response in the assisted reproductive system. In July 2011, the European Society of Embryology and Human Reproduction developed a simple and comprehensive definition of patients with poor ovarian response as the Bologna criteria [2].

According to Bologna criteria for diagnosis, there should be 2 characteristics of the following 3 criteria: 1- age $\geq$ 40 years or another risk factor, 2- previous cycle with poor ovarian response (3 or less oocytes in normal ovarian stimulation) 3-An abnormal ovarian reserve test (number of antral follicles or AFC less than 5–7 or anti mullerian hormone (AMH) less than 0.5 to 1.1 ng/ml). Two cycles with a previous poor response after maximal ovarian stimulation are also sufficient for diagnosis [11].

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Controlled ovarian stimulation (COS) is used to increase the number of developing follicles and oocytes to improve the pregnancy rate of women undergoing IVF. However, patients with poor ovarian response who have reduced ovarian reserve sometimes despite the maximum dose of gonadotropins do not respond properly [12] and the follicular response does not improve oocyte quality or outcome [1].

In fact, the maximum dose required in these patients is not known and they often receive large amounts of expensive drugs and have high cost cycles, poor response and cancellation [1]. It has been proven that the response to gonadotropin can be based on Serum FSH levels. Serum FSH levels >20 IU/L per day of 7 cycles or later may indicate saturation of FSH receptors in granulosa cells and therefore adding more exogenous FSH does not increase follicular response [1].

Antral follicles, especially in patients with poor response, have a wide range of FSH sensitivity. Contact with a set of follicles with different sensitivities to very high concentrations of FSH may lead to rapid growth of 1 or 2 Follicles that are more sensitive, while there is not enough time for the growth and development of the remaining follicles [1].

Androgens have been shown to play an important role in the early follicular development and proliferation of granulosa cells. In addition, increased intra-follicular androgen can increase FSH receptors in granulosa cells and the number of antral and preantral follicles [3,13,14] resulting in ovarian response, increase gonadotropin and follicular growth [12,15].

Given that patients with poor ovarian response often have slow or asynchronous follicular growth, studies have shown that initial treatment with transdermal testosterone can reduce ovarian sensitivity to FSH and follicular response to gonadotropin in patients with poor ovarian response who undergo IVF, slows down [13] and leads to an increase in the number of oocytes extracted, the rate of clinical pregnancy and live birth, we decided to do this project whether gonadotropin discontinuation and complementary treatment with transdermal testosterone can increase sensitivity as well as simultaneous follicular growth.

Materials and methods

Study design and sample collection

In this randomized clinical trial, 60 women with poor ovarian response to controlled ovarian hyperstimulation referred to Yazd Reproductive Sciences Institute, Yazd, Iran, for in vitro fertilization from October 23, 2018 to June 20, 2019 were enrolled.

Poor ovarian response is based on the Bologna criteria, which includes at least two of the following three criteria:

1) Age ≥ 40 years or another risk factor
2) Previous cycle with poor ovarian response (3 or fewer oocytes in normal ovarian stimulation)
3) An abnormal ovarian reserve test (number of antral follicles or AFC less than 7–5 or antimullerian hormone or AMH less than 0.5 to 1.1 ng/ml)

Two cycles with a previous poor response after maximal ovarian stimulation are also sufficient for diagnosis.

Table 1
Basal characteristics of participants in two groups.

Variable	Intervention group	Control Group	P-Value**
Female age (year)	35.38 ± 5.23	36.21 ± 3.01	0.47
Duration of infertility (year)	6.62 ± 1.05	6.01 ± 0.81	0. 65
AMH	0.88 ± 0.08	0.82 ± 0.08	0.60
AFC	5.34 ± 1.69	5.54 ± 2.13	0.71

Data were shown as mean ± SD ** student *t*-test.

Our exclusion criteria were:

- Uncontrolled endocrine disease
- Intrauterine disorders (intrauterine adhesions, submucosal fibroma, and uterine polyp)
- Azoospermia of the husband
- Severe endometriosis

The participants were randomly divided into two equal-sized groups.

First, using the random allocation software version 1.0, we generate a random sequence using a simple random allocation method. In this table, we specify from 1 to 60 and each number is assigned to an intervention group (A or B). Number 1 is assigned to the first qualified person, second person is assigned number 2, and so on up to 60 patients. In order to blindly randomly assign clients, we ask for help from a third person who is unaware of the interventions, and this table is given to them. When the person eligible for the study refers, based on the person's number of the intervention group, the third person will be asked over the phone.

Stimulation protocol

Intervention group: Since the 3rd day of menses begun GnRH agonist buserelin acetate (Cinafact, cinagen, Iran) at a dose of 50 μg subcutaneously twice daily and menotropin (BSV, Germany) 325 IU daily intramuscular. Follicular size with vaginal sonography and serum FSH level were measured on cycle day 7 and then every 2–3 days one time. When serum FSH levels exceeded 20 IU/L on cycle day 7 or any time thereafter and or follicular growth considered to be slow or asynchronous (follicular growth was considered to be asynchronous when one or two leading follicles were ≥4 mm (average diameter) larger than the rest of the cohort), gonadotropins discontinued for some days. After disruption of gonadotropin, 40.5 mg of daily transdermal testosterone (Androgel 1.62 %, Abbvie) was started. Then, every 2 days, we performed a vaginal ultrasound to determine the size of the follicles. When the size of the follicles matched on ultrasound, gonadotropins were restarted. Gonadotropins and transdermal testosterone were continued until the day of the ovulation trigger.

Control group: Since the 2d day of menses GnRH agonist buserelin acetate (Cinafact, cinagen, Iran) at a dose of 50 μg subcutaneously twice daily and since the 4th day menotropin (BSV, Germany) 325 IU daily intramuscular begun. Follicular size with sonography was measured on cyle day 9 and then every 2–3 days one time. In this group, gonadotropin did not interrupt and continued until trigger day.

In both groups when at least two dominant follicles will reach to size of ≥17 mm, serum FSH,LH,P and E2 levels were measured, 10,000 IU hCG (Pregnyl, Netherlands) was administered subcutaneously for ovulation trigger. Thirty-six hours later, the oocytes were retrieved by means of ultrasound-guided transvaginal needle aspiration. Following egg retrieval, intracytoplasmic sperm injection/IVF was done. The embryos (2 embryos) were transferred at the cleavage stage 2–3 days later. More embryos were frozen.

On the oocyte-retrieval day, progesterone suppositories (Cyclogest®, 400 mg) were administered vaginally-two times per day for the purpose of luteal-phase support. This was maintained until ultrasonography demonstrated fetal heart activity.

Fourteen days after embryo transfer, serum Beta-hCG (β-hCG) was examined. β-hCG > 50 IU/L was considered a positive pregnancy Test and was considered as the chemical pregnancy. If the heart is seen on a vaginal ultrasound, which is done 4 weeks after the embryo transfer it was considered as a clinical pregnancy.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS software), version 20.0, Chicago, Illinois. To

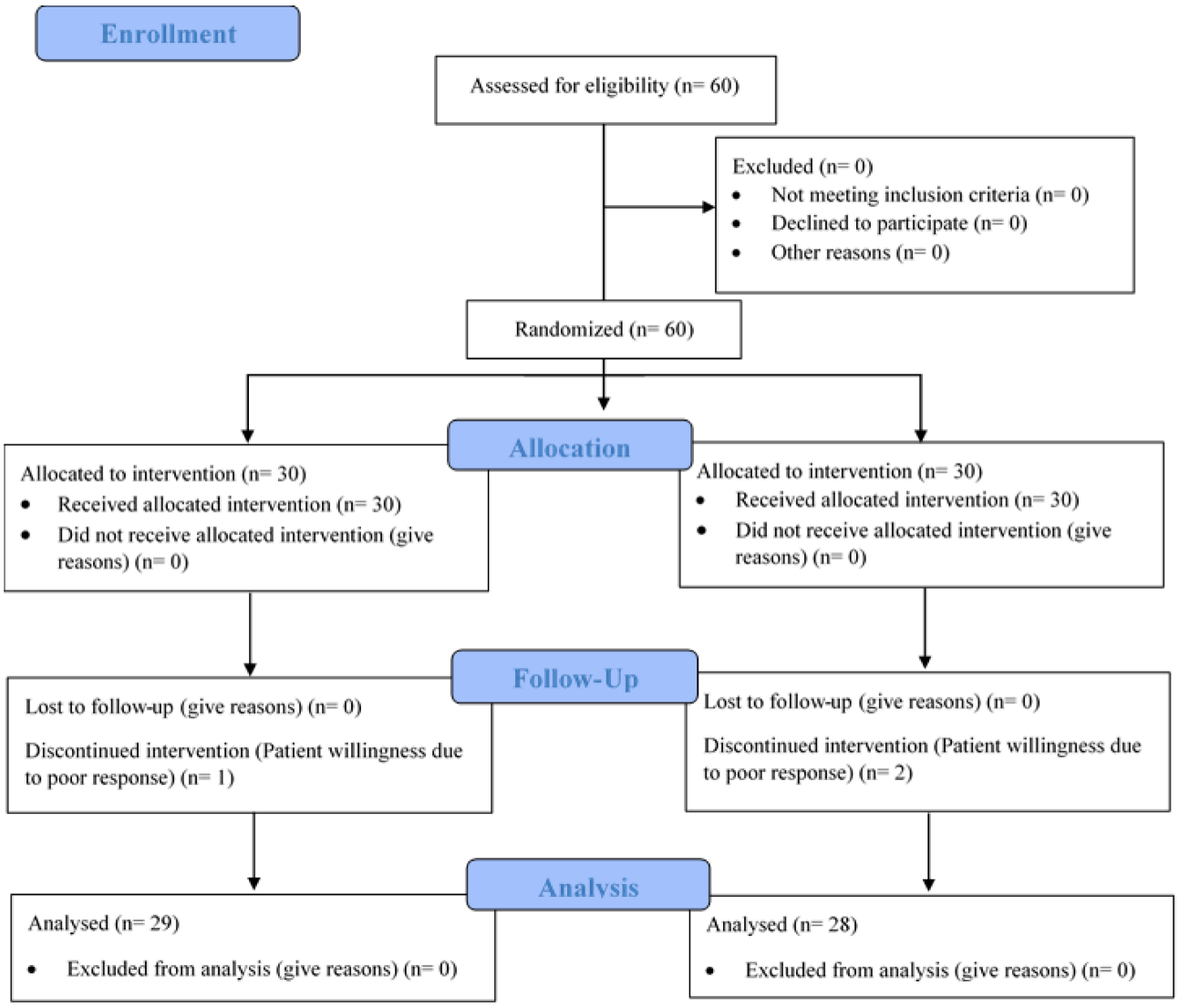

Fig. 1. The CONSORT flowchart of the study.

Table 2
Comparison of cycle characteristics in embryo transfer in two study groups.

Variable	Intervention group	Control group	P-Value**
Ovulation trigger injection (cycle day)	12.45 ± 0.57	12 ± 0.59	0.59
Total dose of gonadotropin (IU)	29.92 ± 2.01	35.67 ± 2.21	0.047
Estradiol level on the day of hCG (pg/mL)	1135.77 ± 175.37	1031.7 ± 114.72	0.63
No. of oocyte	4.1 ± 0.42	3.61 ± 0.52	0.46
No. of M II oocyte retrieved	3.34 ± 0.39	2.54 ± 0.41	0.164
No. of cleavage embryo	2.17 ± 0.31	1.61 ± 0.29	0.19

Data were shown as mean ± SD ** student *t*-test.

determine the significant differences between both groups, Student's *t* test and Chi-square test were employed with the significance level set at p-value <0.05.

Results

Totally, 60 participants were included in this study into two groups (n = 30/each). Two study groups were matched in the terms of baseline characteristics (Table 1).

Table 3
Comparison of ART outcomes in two group.

Variable	Intervention group	Control group	P-value*
Chemical pregnancy rate/ transfer	4 (13.4)	2 (7.1)	0.41
Clinical pregnancy rate/transfer	3 (10.3)	2 (7.1)	0.67

Data were shown frequency (percent) *: chi-square test.

29 patients in intervention group completed one cycle of ovarian stimulation with the use of the interrupted microdose flare protocol, and the 28 patients in the control group completed a conventional microdose flare protocol. Two of the 30 patients in the control group and 1 of the 30 patients in intervention group canceled the continuation of the cycle due to low chances (Fig. 1).

For the interruption group, gonadotropins were withdrawn on cycle day 8.59 ± 1.42. The mean duration of gonadotropin discontinuation period was 2.86 ± 1.02. The mean total duration of the cycle was equal in both groups (p = 0.59). There were two people in the intervention group and four people in the control group did not have m2. However, the mean number of m2 and embryo in the intervention group was higher but not significantly. One person in the control group did not

respond to treatment (Table 2).

There was no significant difference between chemical and clinical pregnancy rates in the two groups (p = 0.41, p = 0.67) (Table 3).

Gonadotropin intake in intervention group was significantly lower than control group (p = 0.047) (Table 2).

Discussion

Patients with poor ovarian response because of low oocyte count and undeveloped embryos are constantly discussed.

Throughout the long term, a few medicines have been proposed, however few have had the option to add many advantages to patient results.

The theory of the current examination showed that the utilizing adding androgen (testosterone) to gonadotropin in the ovarian stimulation protocol in poor responder patients could upgrade pregnancy outcomes and decline gonadotropin use.

Although the results showed no difference between the two groups in the clinical and chemical pregnancy rates, the gonadotropin dose decreased in the case group, significantly.

A number of studies have examined the effect of testosterone gel before ovarian stimulation in patients with poor response [2,3,11–15].

In some of these studies, like our study, the dose of gonadotropin consumed was reduced [2,3,12].

In 2010, Kim et al. prescribed 12.5 mg of transdermal testosterone for patients with poor response 21 days before the start of the ovarian stimulation cycle. The FSH level in the treatment group was clearly lower than in the control group. The number of extracted oocytes, mature oocytes, fertilized oocytes and good quality embryos as well as the rate of clinical pregnancy per cycle started were higher in the treatment group [12].

In 2006, Balasch et al., 25 patients who had had their previous 2 cycles canceled despite receiving a high dose of gonadotropin due to poor follicular response, were given 20 μg/kg transdermal testosterone for 5 days prior to gonadotropin initiation. Of these 25 patients, they had an 80 % >5-fold increase in recruited follicles and a pregnancy rate of 30 % for each retrieval oocyte [11].

A *meta*-analysis was performed in 2012 to evaluate the effect of transdermal testosterone before ovarian stimulation in patients with poor response undergoing IVF. Three studies with 113 patients were performed. In this study, live birth rate and clinical pregnancy rate were significantly higher. The dose required by FSH was clearly lower, although there was no significant difference in the number of oocytes [2].

These findings confirm the theoretical effect of the role of androgens and FSH in folliculogenesis. These and similar studies (Fabregues 2009 in Barcelona, Doan in 2017) concluded that pretreatment with transdermal testosterone could reduce ovarian susceptibility to FSH and follicular response to gonadotropin therapy in patients with a previous poor response [14,15].

In our study, the number of oocytes and pregnancy rate were higher in the testosterone group, but there was no significant difference. However, in our study, the required dose of gonadotropin was significantly reduced.

Perhaps the reason for the difference is in how testosterone is used that we used it in the ovulation stimulation cycle and these studies used it a few days before the stimulation cycle (in the previous cycle).

In 2016, Mitri et al. In Toronto studied 26 patients with poor response, aged 34–47 years. They stopped gonadotropin stimulation on day 7 or cycle 4 and started testosterone gel. They clearly had an increase in follicular growth during the gonadotropin discontinuation period and up to 2 days later. They had four pregnancies in the treatment group and more cases than in the control group. They also had more oocytes, M2 and more embryos in the treatment group, which was not significant [1].

In our study, similar to this study, testosterone was started during the treatment cycle by discontinuation of gonadotropin, which had similar results to Mitri et al study. However, in our study, gonadotropin intake was significantly lower, which was not significant in Mitri et al. study. They concluded that the FSH termination protocol and androgen initiation could improve the ovarian response to gonadotropin in cycles that may be canceled [1]. There was no clear difference in the number of oocytes between the two groups [1].

In the Keay et al. and Sipe et al. study, as in our study, there was no clear difference between the number of oocytes between the two groups [16,17].

Haydardedeoglu et al. tried to create a new method of pre-treatment and co-therapy by using drugs that are physiologically effective separately. They used DHEA administration with the addition of transdermal testosterone and GH before the onset of COH in DOR patients who had previously had cancelled or failed IVF/ICSI cycles. Clinical pregnancy rate, metaphase II oocyte count and fertilization rate increased [18].

In a study by Saharkhiz et al., The number of oocytes and embryos and the rate of pregnancy were significantly higher in the intervention group, perhaps due to the difference in the duration of gel use they administered to the patient throughout the ovarian stimulation with gonadotropin testosterone gel. In that study, there was no comparison of FSH consumption [19].

Adding testosterone gel to gonadotropin in women with poor ovarian response reduces the overall gonadotropin dose; however, it does not improve pregnancy outcomes.

Ethics approval

This trial was approved by the ethics committee of the Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Code: IR.SSU.RSI.REC.1397.001). In addition, the study proposal was registered at the Iranian Registry of Clinical Trials (IRCT) (Code: IRCT20180818040828N1). After approval in the IRCT, sampling was done.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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