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Original Research Articles

Use of transdermal testosterone in women Poseidon IV group under high complexity assisted reproduction treatment

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Poor ovarian reserve (POR) is considered a frequent cause of infertility and is still considered one of the significant challenges in reproductive medicine. Numerous studies have suggested that androgens (dehydroepiandrosterone and testosterone) may play a critical role in follicular development by increasing the number of follicles and, consequently, the number of oocytes, ultimately leading to an increased pregnancy rate. Testosterone is a sex steroid hormone that originates from cholesterol and is considered an obligatory precursor of estradiol biosynthesis. It contributes to greater follicular recruitment, leading to the consideration that exogenous administration could increase the number of recovered oocytes.

Methodology

A historical, quantitative, observational, longitudinal, retrolective cohort study was carried out in the clinical area of assisted reproduction; 2 groups were formed where all women over 35 with primary or secondary infertility who met the criteria were included. Classification criteria of the POSEIDON IV group (age > 35 years with CFA < 5 follicles and/or AMH < 1.2 ng/mL), during the period from October 2021 to September 2022, the first group of patients had received supplementation with transdermal Testosterone 50mg daily one month before ovarian stimulation, the second group did not receive any treatment before ovarian stimulation. Women with a history of diagnosis of endometriosis, pelvic surgery or oophorectomy were excluded. This study has the approval of the ethics committee of the HISPAREP clinic. Each patient was given an informed consent which they signed before the study. We declare that we have no conflict of interest.

The data from the records of all the patients who met the inclusion criteria were collected, including the antral follicle count on the first three days of the menstrual cycle by transvaginal ultrasound one month before ovarian stimulation and after one month of supplementation and without supplementation when starting controlled ovarian stimulation. The number of metaphase II oocytes obtained in each group was also analyzed.

Results

A total of 20 women were included; 10 underwent controlled ovarian stimulation with prior administration of transdermal testosterone at a dose of 50 mg every 24 hours for one month. The other 10 patients did not receive any supplementation or treatment before ovarian stimulation for highly complex assisted reproduction treatment due to various causes of infertility.

The average age of the women was 40.2 ± 2.5 years in the study group and 43.3 ± 2 in the control group; normal weight in 80% of the group with testosterone and 90% without testosterone.

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The baseline conditions of the patients revealed an average anti-Müllerian hormone (AMH) level of 0.65 ± 0.28 ng/dL in the testosterone group and 0.84 ± 0.49 ng/dL in the non-testosterone group. The infertility factor was ovarian endocrine dysfunction, present in 60% of the testosterone group and 40% of the non-testosterone group; this factor was the predominant cause in both groups. The most common protocol stimulation was with (300/150 U) FSH/LH recombinant (Pergoveris, Merck) and GnRH antagonist (Cetrotide, Merck).
The antral follicular count observed by ultrasound in each group after treatment with testosterone was 6.4 ± 2.4, and without testosterone was 6 ± 3.47; p<0.778. Without observing significant differences. The number of metaphase II oocytes obtained (mean ± standard deviation) after testosterone administration was 4.5 ± 2.37 and 1.5 ± 1.62 in participants who did not receive testosterone; p=0.04886, that is p<0.05, so the results were statistically significant in favor of testosterone administration.

Conclusions

Transdermal testosterone supplementation can be used as an adjuvant in controlled ovarian stimulation treatments in women of the POSEIDON IV group to improve the reproductive prognosis of this group of women. The 50mg dose of transdermal testosterone every 24 hours 1 month prior to the ovarian stimulation in highly complex treatments increases the number of metaphase II oocytes recovered statistically.

INTRODUCTION

The reproductive aging process in a woman occurs with the depletion of the number of oocytes or ovarian reserve.[1] Decreased ovarian reserve refers to the reduced number and quality of the remaining oocytes in the ovary.[2]

The incidence of low ovarian reserve ranges from 6 to 64% in infertile women of different ages. In these patients, not only a reduction in the number and quality of remaining oocytes is observed, but also a decrease in the ovarian response to the administration of gonadotropins in controlled ovarian stimulation, high rate of cycle cancellation, increase in the dose of ovulation stimulants, decrease in the number of oocytes retrieved, decrease in the rate of clinical pregnancy and live birth, and high rate of spontaneous abortion in reproductive treatments highly complex assisted.[2]

Although a decrease in reproductive potential is considered part of the natural ovarian aging process, it can also be found in young women.[2]

Subsequently, various studies were carried out where it was defined that the previous diagnostic criteria were based on a heterogeneous population with different reproductive results, age was not taken into account in terms of oocyte quality and to use them it was necessary to have completed 1 stimulation cycle, reason whereby in 2019 a group of experts created a more precise classification that takes into account patient-oriented strategies according to the number of oocytes obtained individually called POSEIDON for its acronym in English. This panel of experts suggests a novel, more detailed stratification for patients with BRO, adding to the already mentioned factors, ovarian reserve in relation to the number of oocytes and aneuploidy rate in relation to maternal age, sensitivity to external gonadotrophins.[3]

With this objective, POSEIDON classifies patients with poor response into four groups:

- Group 1: patients under 35 years of age with normal ovarian reserve parameters (AMH ⩾1.2 ng/mL; AFC ⩾5) and unexpectedly poor or suboptimal outcome to ovulation stimulation (⩽9 oocytes in the 1st cycle of stimulation)
- Group 2: patients older than 34 years with normal ovarian reserve parameters (AMH ⩾1.2 ng/mL; AFC ⩾5) and unexpectedly poor or suboptimal outcome to ovulation stimulation (⩽9 oocytes in the 1st cycle of stimulation)
- Group 3: patients under 35 years of age with low ovarian reserve parameters (AFC <5, AMH <1.2 ng/mL).
- Group 4: patients older than 35 years with low ovarian reserve parameters (AFC <5, AMH <1.2 ng/mL).

With these criteria, it was possible to individualize the treatment used based on the individual characteristics of each patient, and to propose focused therapeutic strategies.

In patients classified in the POSEIDON III and IV group, multiple adjuvant treatments have been investigated to increase the number of antral follicles at the beginning of the cycle and thus increase the number of oocytes obtained in follicular capture, some of these are the administration of growth hormone, dehydroepiandrosterone and testosterone of which indisputably effective recommendations are not yet available.[3] While the effects of androgens (dehydroepiandrosterone and testosterone) on follicle maturation in humans remain controversial, due to lack of direct investigations, experiments in rodents strongly demonstrate an essential function of androgens mediated by the androgen receptor (AR) on granulosa cells, especially in early stages of follicle maturation up to preantral stages.[4]

The hormone Dehydroepiandrosterone (DHEA) is the most potent androgen and the one with the highest affinity to bind to the androgen receptor, in women it is converted to testosterone and to a lesser extent to estradiol. DHEA supplementation for a minimum of 6 weeks is sufficient to be able to observe the beneficial effects that prove the pos-

itive effect during the early stages of follicular maturation, also coinciding with the presence of androgen receptors.[4] After the administration of DHEA, Testosterone (T) levels rise, this elevation being statistically associated with the chances of pregnancy after IVF. Increased androgen levels have previously been associated with elevated anti-Müllerian hormone (AMH) levels, which are favorable for improving functional ovarian reserve.[5]

Testosterone, the main circulating androgen in women, is a naturally occurring steroid secreted by the ovaries and adrenal glands (100 to 400 mcg/day).[5] Peak serum testosterone concentrations are reached 24-36 hours after administration, with wide interindividual variability. Testosterone is primarily metabolized in the liver by CYP3A4.

Circulating testosterone is strongly bound to plasma proteins, with about 66% bound to sex hormone-binding globulins and 33% to albumin. The free fraction of testosterone is determined by the rate of testosterone production, the rate of metabolic clearance, and the level of sex hormone-binding globulins.[5]

So far, the review of both clinical and preclinical evidence that the use of androgens can have a positive effect on follicular maturation is too limited to standardize their use; however, multiple published studies have shown that within of the therapeutic ranges can be clinically beneficial, mainly in those patients with low ovarian reserve and in assisted reproduction treatments.[3-5]

The low ovarian response is one of the daily challenges of the clinician in the infertility consultation. It is estimated that the incidence of low responders in the population undergoing IVF is 9-24% 7,10. These patients have worse pregnancy rates when compared to patients with a normal response. Numerous strategies have been proposed for the treatment of low responders, but up to now no advantages of some treatments over others have been achieved. Some recent published studies support the use of androgens as a supplement for women in these conditions, since they increase the expression of the FSH receptor in granulosa cells, promote the initiation of primordial follicle growth, and increase the number of pre-antral and antral follicles.[6,7]

OBJECTIVES

Main objective: To evaluate the increase in the antral follicular count in patients of the POSEIDON IV group after the administration of 50mg daily for 1 month of transdermal testosterone prior to ovarian stimulation.

Secondary objective: To evaluate the number of metaphase II oocytes after treatment with 50mg of transdermal testosterone daily for one month compared to patients who did not receive any treatment prior to ovarian stimulation.

STATISTICAL ANALYSES

A quantitative, descriptive, observational, longitudinal, retrolective study was carried out in which a historical cohort was analyzed in the assisted reproduction clinical research area at the Hisparep Clinic of Hospital Español de México.

An analysis with descriptive statistics was performed for the baseline characteristics of both groups. Then a chi-square test was performed to obtain statistically significant variables. A p value <0.05 was used to be considered significant, for the descriptive analysis, frequencies and proportions were used for the categorical variables and measures of central tendency and dispersion for the numerical variables. According to normality and homogeneity, parametric or non-parametric tests were used to compare before and after.

RESULTS

The women who will be included in the protocol must be over 35 years of age who have attended Hisparep Assisted Reproduction Clinic for assisted reproduction treatment, either with primary or secondary infertility and meet the classification criteria of the POSEIDON IV group. (age > or = 35 years with CFA < 5 follicles or AMH < 1.2 ng/mL) and in the first group of patients they have been given supplementation with transdermal Testosterone 50 mg one month prior to ovarian stimulation. In the second group, they have not received treatment before ovarian stimulation.

Material and methods:
The treatment protocol consisted of providing a fixed dose of transdermal testosterone which was 50 mg (1 gel) applied to the forearm every 24 hours, 30 days before starting the next menstrual cycle in conjunction with ovarian stimulation.

This study has the approval of the ethics committee of the HISPAREP clinic. Each patient was given an informed consent which they signed before the study. We declare that we have no conflict of interest.

Twenty patients belonging to the POSEIDON IV group were selected, of which 10 received supplementary treatment with testosterone 50 mg transdermal daily for one month prior to ovarian stimulation and 10 did not receive testosterone; whose general characteristics are mean age of 40.2 ± 2.5 years of the group that received testosterone and 43.3 ± 2 years for the group that did not receive testosterone, while normal weight was found in 80% and 90% (overweight of 20% and 10%) of each group, respectively (**Table 1**).

The initial conditions of the patients are described below: AMH (mean ± standard deviation) of 0.65 ± 0.28 ng/dL in those who received testosterone and 0.84 ± 0.49 ng/dL in those who did not receive testosterone (**Figure 1**); the altered infertility factor was ovarian endocrine in 60% (n=6) of the participants who were administered testosterone and in 40% (n=4) of those who were not administered, in both this cause represented mode (**Figure 2**).

The most common type of stimulation received was with recombinant FSH/LH in both groups with GnRH antagonist.

The antral follicular count observed by ultrasound after testosterone treatment was 6.4 ± 2.4, without testosterone 6 ± 3.47; p<0.778. Without observing significant differences (**Figure 3**).

Table 1. General characteristics of the study patients (N = 20).

Characteristics	With testosterone n= 10 (%)	Without testosterone n= 10 (%)
Average age	40.2 ± 2.5	43.3 ± 2
Physical constitution		
Normal weight	8 (80%)	9 (90%)
Overweight	2 (20%)	1 (10%)

Source: Hisparep Clinic at Spanish Hospital

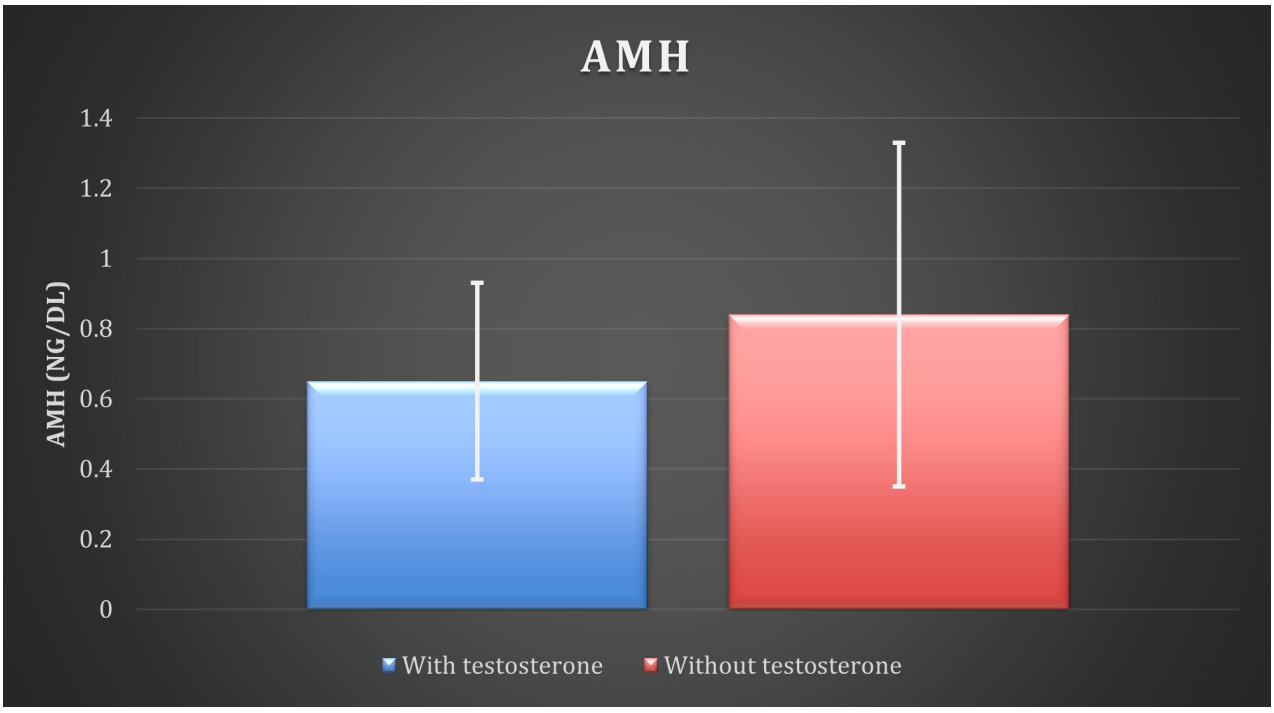

Figure 1. Serum AMH concentration in initial conditions (N = 20).

Source: Hisparep Clinic at Spanish Hospital

The number of metaphase II oocytes obtained (mean ± standard deviation) after testosterone administration was 4.5 ± 2.37 and 1.5 ± 1.62 in the participants who did not receive testosterone (**Figure 4**); When carrying out a statistical analysis in which the count of oocytes in metaphase II observed after the administration of testosterone and the count in the absence of testosterone was compared through the Chi square, with 7 degrees of freedom, it was obtains that $p=0.04886$, that is $p<0.05$, so the results were statistically significant in favor of the administration of testosterone.

DISCUSSION

The decrease in ovarian reserve has become one of the leading causes of infertility, found in 9 to 24% of patients undergoing assisted reproduction treatment,[8] this is mainly due to the current tendency to delay pregnancy at older ages. A close relationship has been found between patients with decreased ovarian reserve and low response to stimulation treatment.[9] In our clinic, the patients with decreased ovarian reserve were around 60% of all patients because most of them were more than 40 years old.

Gleicher mentions in his study that women over the age of 35 may benefit from a more direct administration of testosterone (transdermal testosterone) due to the less efficient conversion rate of DHEA to testosterone.[4] A meta-analysis by Bosdou et al. reports that the clinical pregnancy rate was significantly increased by 15% in patients who were pretreated with transdermal testosterone compared with those who were not (+15%, 95% CI).[10] Similarly, the live birth rate increased by 11% in patients pre-treated with transdermal testosterone (11%, 95% CI).[10]

According to the theory underlying the literature, intraovarian androgens promote FSH sensitivity in growing follicles and, therefore, could increase performance and oocyte maturity after ovarian stimulation and improve pregnancy rates.[11] In this context, it should be noted that in all the interventions analyzed in the Bosdou meta-analysis, the effect in terms of clinical outcomes of pregnancy rates is only observed as statistically significant in the case of transdermal testosterone compared to other therapies, such as the addition aromatase inhibitors or hCG.[10]

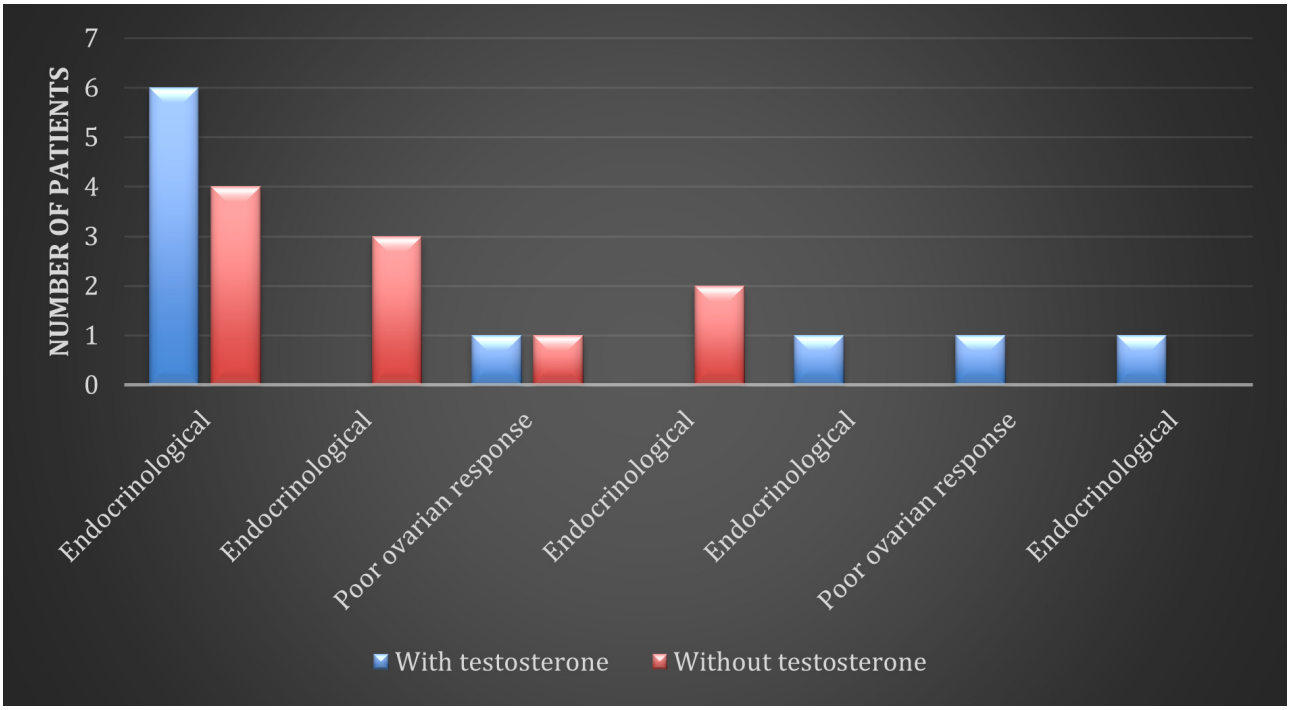

Figure 2. Altered infertility factor (N = 20).

*Percentage calculated with respect to the total number of patients who received testosterone.
**Percentage calculated with respect to the total number of patients who did not receive testosterone
Source: Hisparep Clinic at Spanish Hospital

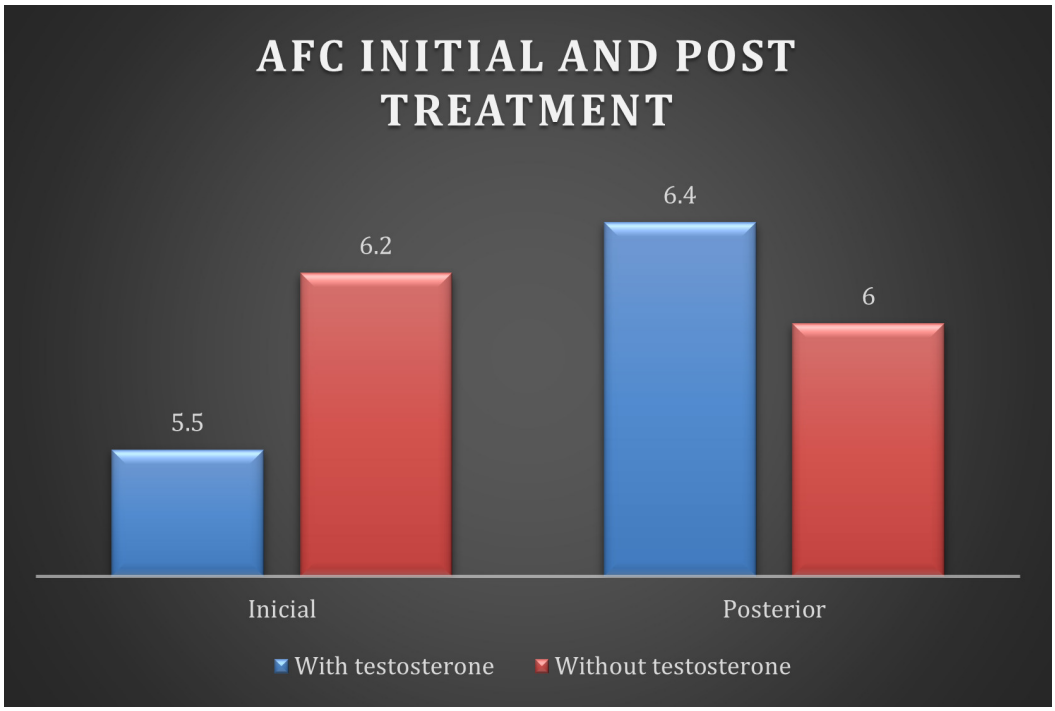

Figure 3. Antral follicular count of the patients in the group with use of testosterone and without testosterone (N = 40).

Source: Hisparep Clinic at Spanish Hospital

Two small studies[6,7] built on the proposed theory to enhance follicular development with increased intraovarian androgens.[11] The hypotheses that are postulated and evaluated for effective treatment in patients with BRO are:

1. Increase the number of oocytes retrieved
2. decrease of the total dose of gonadotropins required
3. Decrease the duration of ovarian stimulation.

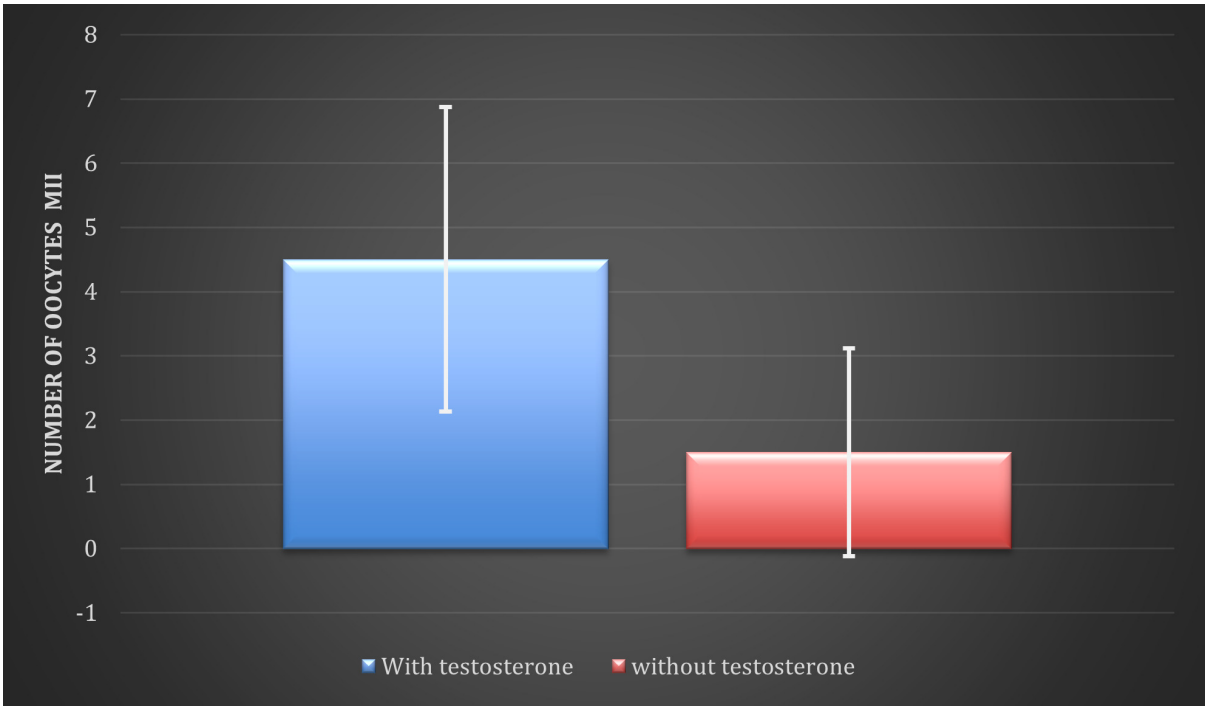

Figure 4. Number of oocytes in metaphase II after testosterone administration and without drug administration (N = 20).

Source: Hisparep Clinic at Spanish Hospital

Regarding the antral follicular count, an increase was also observed. However, this result was not statistically significant, so it is still necessary to carry out randomized clinical trials with a larger sample size to increase the evidence of this type of treatment and use it in a more effective way routine in this group of patients, which continues to be a challenge for the doctor in the area of assisted reproduction.

Only in the case of transdermal testosterone administrations were all these hypotheses confirmed. Currently, based on the limited evidence available, transdermal testosterone pretreatment appears to increase clinical pregnancy and survival relative to birth rates in poor responders undergoing ovarian stimulation for in vitro fertilization.[9,12]

Some of the limitations of this study were the relatively small sample size (20 participants), it was conducted at a single breeding center, the long-term effects of testosterone supplementation on reproductive results and possible risks or side effects and results in clinical pregnancy rate and live birth rate were not evaluated.

CONCLUSIONS

The poor response to an ovarian stimulation cycle in women in the POSEIDON IV group could be improved with supplementary treatments such as transdermal testosterone. The dose of 50 mg every 24 hours for a month before the ovarian stimulation increases the number of metaphase II oocytes recovered statistically significantly.

For this reason, we propose that the reproductive results in women with poor ovarian reserve within group IV of POSEIDON will be favorably modified after the administration of 50mg of testosterone administered dermal daily for one month before ovarian stimulation. However, it is still necessary to carry out randomized clinical trials that can support its routine use in this group of patients who continue to be a challenge for doctors in assisted reproduction treatment.

AUTHORS' CONTRIBUTION

Conceptualization: Martha Esmeralda Espinosa Esparza; *Data curation*: Jorge Luis Lezama Ruvalcaba; *Formal Analysis*: José Carlos Salazar Trujillo; *Funding acquisition*: Martha Esmeralda Espinosa Esparza; *Investigation*: Martha Esmeralda Espinosa Esparza; *Methodology*: Martha Esmeralda Espinosa Esparza; *Project administration*: Carlos Gerardo Salazar López Ortíz; *Resources*: Martha Esmeralda Espinosa Esparza; *Software*: Jorge Luis Lezama Ruvalcaba; *Supervision*: Carlos Gerardo Salazar López Ortíz; *Validation*: Jorge Luis Lezama Ruvalcaba; *Visualization*: Martha Esmeralda Espinosa Esparza; *Writing – original draft*: Martha Esmeralda Espinosa Esparza; *Writing – review & editing*: Jorge Luis Lezama Ruvalcaba.

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