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ORIGINAL ARTICLE ***Endocrinology***

Transdermal testosterone pretreatment in poor responders undergoing ICSI: a randomized clinical trial

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STUDY QUESTION: Does pretreatment with transdermal testosterone increase the number of cumulus-oocyte complexes (COCs) retrieved by more than 1.5 in poor responders undergoing intracytoplasmic sperm injection (ICSI), using recombinant follicle stimulating hormone (FSH) and gonadotrophin releasing hormone agonists (GnRHa)?

SUMMARY ANSWER: Testosterone pretreatment failed to increase the number of COCs by more than 1.5 as compared with no pretreatment in poor responders undergoing ICSI (difference between medians: 0.0, 95% CI: −1.0 to +1.0).

WHAT IS KNOWN ALREADY: Androgens are thought to play an important role in early follicular development by enhancing ovarian sensitivity to FSH. In a recent meta-analysis, testosterone pretreatment resulted in an increase of 1.5 COCs as compared with no pretreatment. However, this effect was based on the analysis of only two randomized controlled trials (RCTs) including 163 patients. Evidently, there is a need for additional RCTs that will allow firmer conclusions to be drawn.

STUDY DESIGN, SIZE, DURATION: The present RCT was designed to detect a difference of 1.5 COCs (sample size required = 48 patients). From 02/2014 until 04/2015, 50 poor responders fulfilling the Bologna criteria have been randomized (using a randomization list) to either testosterone pretreatment for 21 days ($n = 26$) or no pretreatment ($n = 24$).

PARTICIPANTS/MATERIALS, SETTING, METHODS: All patients underwent a long follicular GnRHa protocol. Recombinant FSH stimulation was started on Day 22 following GnRHa initiation. In the testosterone pretreatment group, a daily dose of 10 mg of testosterone gel was applied transdermally for 21 days starting from GnRHa initiation. Results are expressed as median (interquartile range).

MAIN RESULTS AND THE ROLE OF CHANCE: No differences in baseline characteristics were observed between the two groups compared. Testosterone levels [median (interquartile range)] were significantly higher in the testosterone pretreatment on the day of initiation of FSH stimulation [114 (99.5) ng/dl versus 20 (20) ng/dl, respectively, $P < 0.001$]. Duration of FSH stimulation [median (interquartile range)] was similar between the groups compared [12.5 (3.0) days versus 12 (3.0) days, respectively, $P = 0.52$]. The number of COCs retrieved [median (interquartile range)] was not different between the testosterone pretreatment and the no pretreatment groups [3.5 (4.0) versus 3.0 (3.0), 95% CI for the median: 2.0–5.0 versus 2.7–4.3, respectively; difference between medians: 0.0, 95% CI: +1.0 to −1.0). Similarly no differences were observed regarding fertilization rates [median (interquartile range)] [66.7% (32.5) versus 66.7% (42.9), respectively, $P = 0.97$] and live birth rates per randomized patient (7.7% versus 8.3%, respectively, rate difference: −0.6%, 95% CI: −19.0 to +16.9).

LIMITATIONS, REASONS FOR CAUTION: The study was not powered to detect differences less than 1.5 COCs, although it is doubtful whether these differences would be clinically relevant. Moreover, due to sample size restrictions, no conclusions can be drawn regarding the probability of live birth.

WIDER IMPLICATIONS OF THE FINDINGS: The results of this randomized clinical trial, suggesting that pretreatment with 10 mg of transdermal testosterone for 21 days does not improve ovarian response by more than 1.5 oocytes, could be used to more accurately consult patients with poor ovarian response. However, an improvement in IVF outcome using a higher dose of testosterone or a longer pretreatment period cannot be excluded.

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Introduction

Poor ovarian response (POR) remains one of the most challenging problems in reproductive medicine. It is present in a variable proportion of women undergoing ovarian stimulation for *in vitro* fertilization (IVF), ranging from 9 to 24% (Ben-Rafael *et al.*, 1991; Jenkins *et al.*, 1991; Surrey *et al.*, 2000). This variation is mainly attributed to the different criteria used for its definition and led to a consensus meeting in Bologna in 2010, following which standardized criteria for the definition of POR were published (Ferraretti *et al.*, 2011).

Over the years, various stimulation protocols have been proposed for the management of poor responders with controversial, however, results (Kyrou *et al.*, 2009; Venetis *et al.*, 2010). Limited data originating from randomized clinical trials (RCTs) indicated a potential benefit from growth hormone (GH) addition to follicle stimulating hormone (FSH) (Kolibianakis *et al.*, 2009) as well as from testosterone administration prior to ovarian stimulation (Massin *et al.*, 2006; Kim *et al.*, 2011).

The rationale behind the latter intervention is that androgens appear to play an important role in early follicular development and granulosa cell proliferation (Vendola, *et al.*, 1998; Weil, *et al.*, 1999). In addition, they have been shown to augment FSH receptor expression in granulosa cells and to increase the number of pre-antral and antral follicles (Hillier and De Zwart, 1981; Harlow *et al.*, 1986; Vendola *et al.*, 1998).

By synthesizing the existing evidence regarding the use of testosterone pretreatment in poor responders undergoing IVF, an increase in the number of cumulus oocyte complexes (COCs) retrieved, as well as in clinical pregnancy and live birth rates has been suggested (Bosdou *et al.*, 2012). However, this beneficial effect was based on the analysis of only two RCTs including 163 patients. Evidently, there is a need for additional studies that will allow firmer conclusions to be drawn.

The aim of this RCT was to evaluate whether pretreatment with transdermal testosterone increases the number of COCs by at least 1.5 in poor responders undergoing intracytoplasmic sperm injection (ICSI) using recombinant gonadotrophins and gonadotrophin releasing hormone (GnRH) agonists.

Materials and Methods

Study population

Fifty poor responders fulfilling the Bologna criteria were enrolled in this prospective, randomized, single blind, clinical trial. The study was conducted at the Unit for Human Reproduction of the 1st Department of Obstetrics and Gynecology at Aristotle University of Thessaloniki in cooperation with the Unit for Human Reproduction of the Department of Obstetrics and Gynecology at the University of Thessaly, from 02/2014 until 04/2015. Patients were allocated by a study nurse to either testosterone pretreatment for 21 days ($n = 26$) or no pretreatment ($n = 24$), according to a computer-generated randomization list. Physicians and embryologists involved in oocyte retrieval and embryo culture were not aware of patient allocation. Patients could participate in the study only once.

Patients who fulfilled at least two of the following three Bologna criteria (Ferraretti *et al.*, 2011) were included in the study: (i) advanced maternal age ($\geq$40 years) or any other risk factor for POR, (ii) a previous POR ($\leq$3 oocytes with a conventional stimulation protocol), (iii) an abnormal ovarian reserve test (i.e. AFC $<$ 5–7 follicles or AMH $<$ 0.5–1.1 ng/ml). Exclusion criteria were: body mass index (BMI) of $\geq$32 kg/m^2, endometriosis stage III-IV, history of previous ovarian surgery, endocrine or metabolic disorders and use of sperm from testicular sperm extraction, fine needle aspiration or cryopreservation.

The study was approved by the Ethics Committee Review Board of Aristotle University of Thessaloniki and the Local Ethics Committee Review Board. Written consent was obtained from all patients (NCT01961336).

Ovarian stimulation

All patients underwent a long follicular protocol with GnRH agonist triptorelin (Arvekap, Ipsen Ltd, France) 3.75 mg depot, starting on the first day of the menstrual cycle, followed by daily injections of triptorelin (Arvekap, Ipsen Ltd, France) 0.1 mg, if necessary. In the testosterone group, a daily dose of 10 mg of testosterone gel (Tostran 2% Gel, ProStrakan) was applied transdermally onto the inner thigh daily, for 21 days, as suggested by Kim *et al.* (2011), starting from the GnRH agonist initiation. Testosterone was supplied in a canister with a dosing pumping mechanism, which delivered one half gram of gel containing 10 mg of testosterone each time the piston was depressed.

Ovarian stimulation with follitropin alpha (Gonal-F; Merck Serono Europe Ltd, London, UK) was started on Day 22, the day after the last testosterone gel application. Patients in the no pretreatment group underwent the same protocol without receiving, however, testosterone pretreatment. Triggering of final oocyte maturation was performed using 250 μg of recombinant hCG (Ovitrelle, Merck Serono Europe Ltd, London, UK), as soon as at least two follicles reached 17 mm in diameter or if this was not possible, when the maximum number of follicles were present.

IVF procedure and luteal phase support

Oocyte retrieval was performed by ultrasound-guided transvaginal follicular aspiration 36 h after hCG administration. Intracytoplasmic sperm injection (ICSI) was performed in all patients. The culture media (Sydney IVF Cleavage Medium and Sydney IVF Fertilization Medium) were provided by COOK (COOK Medical, Ireland, Ltd.), while the culture conditions used were: 37°C, 6% CO_2 and 5% O_2 in N_2.

Up to three embryos, in women ≤40 years of age and up to four embryos, in women >40 years of age were transferred on Day 2 of *in vitro* culture, if available (Kyrou *et al.*, 2009), according to the Greek law of reproduction. Luteal phase was supported by administration of 600 mg/day (vaginal tablets, 200 mg t.i.d) of micronized progesterone (Utrogestan; Basins Iscovesco. Paris, France) starting on the day of oocyte retrieval and continued up to the day of pregnancy test or in case of a positive test until the seventh week of pregnancy.

Hormonal measurements and ultrasound assessment of follicular development

Luteinising hormone (LH), estradiol (E2), progesterone, testosterone, dehydroepiandrosterone (DHEA), sex-hormone binding globulin (SHBG), D4-androstenedione (Δ4-A) were assessed on the first day of the menstrual cycle, on Day 22 and on the day of hCG administration. Serum LH, E2, and progesterone levels were measured by means of the automated Elecsys immunoanalyser (Roche Diagnostics, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation were <3% and <4% for LH, <5% and <10% for E_2, and <3% and <5% for progesterone, <4.6% and <8% for AMH, <9% and <9.6% for testosterone, <8% and <12% for Δ4-androstenedione, respectively.

Outcome measures

The primary outcome measure was the number of COCs retrieved. Secondary outcome measures included duration of stimulation, number of follicles ≥11 mm and ≥17 mm on the day of triggering final oocyte maturation, serum hormonal levels of LH, E2, progesterone, testosterone, DHEA, SHBG, D4-A, free androgen index (FAI), number of metaphase II (MII) oocytes, maturation rate, number of 2-pronuclei (2pn) oocytes, fertilization rate, quality of embryos on Day 2 of *in vitro* culture, proportion of patients with top quality embryos, number of embryos transferred, proportion of patients with embryo transfer, positive hCG, clinical pregnancy and live birth rate (per randomized patient and per embryo transfer) and miscarriage rate per positive hCG.

Free androgen index was calculated as the ratio of total testosterone levels divided by SHBG levels. Maturation rate was defined as the number of MII oocytes divided by the number of COCs retrieved. Fertilization rate was calculated by dividing the number of 2pn oocytes by the number of MII oocytes (2pn/MII) or by dividing the number of 2pn oocytes by the number of COCs retrieved (2pn/COCs). Embryo quality was assessed according to morphological criteria based on the assessment of the blastomeres and the degree of blastomeric fragmentation (Ziebe *et al.*, 1997). Clinical pregnancy was defined as the presence of intrauterine sac with fetal heart activity at 6–8 weeks of gestation. Miscarriage rate was defined as pregnancy loss before 24 weeks of gestation.

All patients were monitored for the occurrence of testosterone associated side effects, such as skin reaction at the site of application, increased hair growth, nausea, acne or voice deepening, from initiation of testosterone treatment until the day of pregnancy test or until the seventh week of gestation, in cases where pregnancy was achieved.

Sample size

Sample size estimation showed that 24 patients were required to be included in each group in order to detect a difference of 1.5 COCs, using a two-sided, Mann-Whitney test with 80% power given a standard deviation (SD) of 1.9 (Kim *et al.*, 2011) and a significance level of 0.05. The difference of 1.5 in the number of COCs retrieved, on which the power analysis was performed, was based on the results of the meta-analysis by Bosdou *et al.* (2012), which showed that testosterone pretreatment increased the number of COCs by 1.5. Secondly, the difference of 1.5 COCs would be likely to result in approximately one embryo difference between groups, assuming a fertilization rate of 65%. A difference of 1.5 COCs and thus of one embryo is likely to be crucial in a proportion of poor responders, since it may differentiate those who will proceed to embryo transfer and thus retain the possibility to achieve pregnancy from those who will not.

Statistical analysis

Intention-to-treat (ITT) analysis was performed, analyzing patients according to their randomization. Parametric (independent sample student t-test) and non-parametric (Mood's median test) tests were used for continuous variables, depending on their distribution, as assessed by Shapiro-Wilk test. Fisher's exact test was used for analyzing binary variables. All analyses were performed in STATA v13 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP). Statistical significance was considered at $P < 0.05$.

Results

No statistically significant differences were observed between the testosterone pretreatment and no pretreatment groups regarding baseline patient characteristics (Table I).

Primary outcome measure

Using analysis per intention to treat (ITT), the median (interquartile range) of COCs retrieved was not statistically different between the testosterone pretreatment group and the no pretreatment groups (Table II). Using per protocol analysis, excluding the three patients who did not have oocyte retrieval (as shown in the flow diagram—Fig. 1), the median (interquartile range) of COCs retrieved did not change the direction of the results obtained (Table II).

Secondary outcome measures

Endocrine profile and ultrasound data on Day 22 after GnRH agonist administration between the testosterone pretreatment and the no pretreatment group are presented in Table III. Serum testosterone levels and FAI were significantly higher in the testosterone pretreatment group as compared with the no pretreatment group ($P < 0.001$) (Table III).

Duration of stimulation and total dose of gonadotrophins required were similar between the testosterone pretreatment and the no pretreatment groups (Table III).

Table I Baseline characteristics of patients randomized in the testosterone pretreatment and the no pretreatment groups.

	Testosterone pretreatment (n = 26)	No pretreatment (n = 24)
	Demographic characteristics	
	Median (interquartile range, 95% confidence intervals)	
Age (years)	41.5 (3.0, 41.0–43.0)	42.5 (4.0, 41.0–44.0)
BMI (kg/m^2)	24.2 (10.3, 21.3–28.6)	24.8 (6.9, 22.7–28.1)
Duration of infertility (years)	4.0 (2.0, 3.5–4.5)	5.0 (6.0, 4.0–8.3)
Age of first menstruation (years)	13.0 (2.0, 12.0–13.5)	13.0 (2.0, 12.0–14.0)
Duration of menstruation (days)	4.0 (1.0, 4.0–5.0)	4.0 (1.0, 4.0–5.0)
	% (n, 95% CI)	
Alcohol consumption	0 (0, 0.0–13.2)	4 (1, 0.1–21.1)
Smoking	31 (8, 14.3–51.8)	1 (5, 7.1–42.2)
Cause of infertility besides poor ovarian response:		
Tubal factor	15 (4, 4.4–34.9)	25 (6, 9.8–46.7)
Male factor	35 (9, 17.2–55.7)	29 (7, 12.6–51.1)
Tubal and male factor	4 (1, 0.1–19.6)	13 (3, 26.6–32.4)
Fibroid uterus	4 (1, 0.1–19.6)	0 (0, 0–14.2)
Other/unexplained	42 (11, 23.4–63.1)	33 (8, 15.6–55.3)
	Baseline endocrine profile and antral follicle count	
	Median (interquartile range, 95% confidence intervals)	
FSH (IU/l)	10.7 (5.7, 8.1–12.8)	8.3 (3.6, 7.2–9.6)
LH (IU/l)	5.3 (2.9, 3.8–6.1)	3.5 (3.0, 2.9–4.9)
Estradiol (pg/ml)	38.9 (30.7, 32.2–53.7)	37.5 (32.6, 29.2–51.9)
Progesterone (ng/ml)	0.53 (0.3, 0.5–0.6)	0.45 (0.4, 0.3–0.7)
Testosterone (ng/dl)	27.0 (19.5, 20.0–38.0)	25.0 (27.3, 14.3–35.3)
Sex hormone binding globulin (nmol/l)	64.0 (37.1, 52.3–75.2)	81.2 (58.8, 51.5–101.5)
Free androgen index	1.2 (1.7, 0.9–2.0)	1.1 (1.0, 0.8–1.4)
Dehydroepiandrosterone sulfate (μg/dl)	141.0 (89.3, 107.5–174.9)	125.8 (138.5, 81.1–186.2)
Δ4-Androstenedione (ng/ml)	1.9 (1.5, 1.3–2.7)	1.8 (1.3, 1.3–2.3)
Anti-Müllerian hormone (ng/ml)	0.97 (0.65, 0.54–1.19)	0.65 (0.78, 0.34–1.23)
Mean ovarian volume (ml)	5.4 (3.3, 4.3–6.6)	4.8 (2.9, 3.5–6.3)
Antral follicle count	6.0 (4, 5.0–7.3)	5.0 (3, 4.7–6.0)

Table II Embryological outcome in the testosterone pretreatment and the no pretreatment groups.

	Testosterone pretreatment ($n = 26$)	No pretreatment ($n = 24$)	95% CI of the difference between medians	*P*-value
Primary outcome measure				
Cumulus oocyte complexes (COCs), Intention to treat (ITT) analysis	3.5 (4.0, 2.0–5.0)	3.0 (3.0, 2.7–4.3)	−1.0 to +1.0	0.76
COCs per protocol analysis	4.0 (4.0, 2.0–5.3)	3.0 (3.0, 3.0–4.7)	−1.0 to +2.0	0.66
Secondary outcomes				
Metaphase II oocytes (MII) ITT analysis	3.0 (2.0, 2.0–3.5)	3.0 (2.0, 1.7–3.0)	−1.0 to +1.0	0.63
Maturation rate (MII/COCs) % per patient with COCs retrieved	100.0 (25.0, 78.3–100.0)	80.4 (50.0, 65.5–100.0)	0.0 to +25.0	0.77
2-pronuclei oocytes (2pn) ITT analysis	2.0 (3.0, 1.0–3.0)	2.0 (2.0, 1.0–2.3)	−1.0 to +1.0	0.50
Fertilization rate (2pn/COCs) % per patient with COCs retrieved	66.7 (32.5, 50.0–78.5)	66.7 (42.9, 48.8–75.0)	−16.7 to +25.0	0.73
Fertilization rate (2pn/MII) % per patient with COCs retrieved and treated by ICSI	85.4 (38.3, 66.7–100.0)	81.7 (33.3, 66.7–100.0)	−15.9 to +10.0	0.99
Number of embryos transferred	2.0 (1.0, 2.0–3.0)	2.0 (2.0, 1.0–2.0)	0.0 to +1.0	0.27
Embryo quality on Day 2 of *in vitro* culture:				
Top quality embryos	0.0 (0, 0.0–0.0)	0.0 (0, 0.0–0.1)	0.0 to 0.0	0.93
Medium quality embryos	2.0 (2, 1.0–3.9)	1.0 (2, 1.0–2.0)	0.0 to +2.0	0.14
Low quality embryos	0.0 (1, 0.0–0.0)	0.0 (0, 0.0–0.0)	0.0 to 0.0	0.45

Data are median (interquartile range, 95% confidence intervals).

Similarly, endocrine profile and number of follicles ≥11 mm or ≥17 mm were not significantly different between the two groups compared on the day of hCG administration (Table III).

As shown in Table II, there was no significant difference between the testosterone pretreatment and the no pretreatment group in the number of MII oocytes or the number of 2PN oocytes. Similarly, no differences were observed between the two groups regarding maturation rate, fertilization rate and the number of embryos transferred.

The number of embryos classified as top, good or low quality (Table II) and the proportion of patients with at least one top quality embryos (Table IV) were similar in both groups on Day 2 of *in vitro* culture. Twenty (83.3%) and twenty one (91.3%) (difference: −8.0; 95% CI: −28.2 to +12.7) patients had embryo transfer in the testosterone pretreatment and the no pretreatment group, respectively. In patients with embryo transfer, the proportions (*n*, %; 95% CI) of patients with single embryo transfer (3, 15.0%; 95% CI: 3.2–37.9 versus 7, 33.3%; 95% CI: 14.6–57.0), the proportion of patients with two (8, 40.0%; 95% CI: 19.1–63.9 versus 9, 42.9%; 95% CI: 21.8–66.0), three (7, 35.0%; 95% CI: 15.4–59.2 versus 5, 23.8%; 95% CI: 8.2–47.2) or four embryos transferred (2, 10.0%; 95% CI: 1.2–31.7 versus 0, 0.0%; 95% CI: 0–16.1) did not differ significantly between the testosterone pretreatment and the no pretreatment group, respectively.

No significant difference was observed regarding the probability of clinical pregnancy or live birth rate between the testosterone pretreatment and the no pretreatment groups (Table IV). No multiple pregnancies occurred in either group.

No side effects, such as skin reaction at the site of application, increased hair growth, nausea, acne or voice deepening, were reported in testosterone treated patients for the duration of follow-up.

Discussion

The current RCT has shown that transdermal testosterone pretreatment at a dose of 10 mg/day for 21 days did not increase the number of COCs retrieved in poor responders undergoing ICSI, stimulated by recombinant FSH in a long GnRH agonist protocol.

Despite the presence of a significantly higher testosterone level, as expected after 21 days of testosterone administration compared with no pretreatment, no statistically significant increase in the median number of COCs retrieved was observed (3.5 versus 3.0, respectively). No such difference was also present in the number of antral follicles at initiation of stimulation between the two groups compared.

Endometrial thickness was normal in both groups on the day of triggering final oocyte maturation however, it was significantly higher in the testosterone pretreatment group. This difference might be attributed to the higher but not significantly so estradiol levels in the

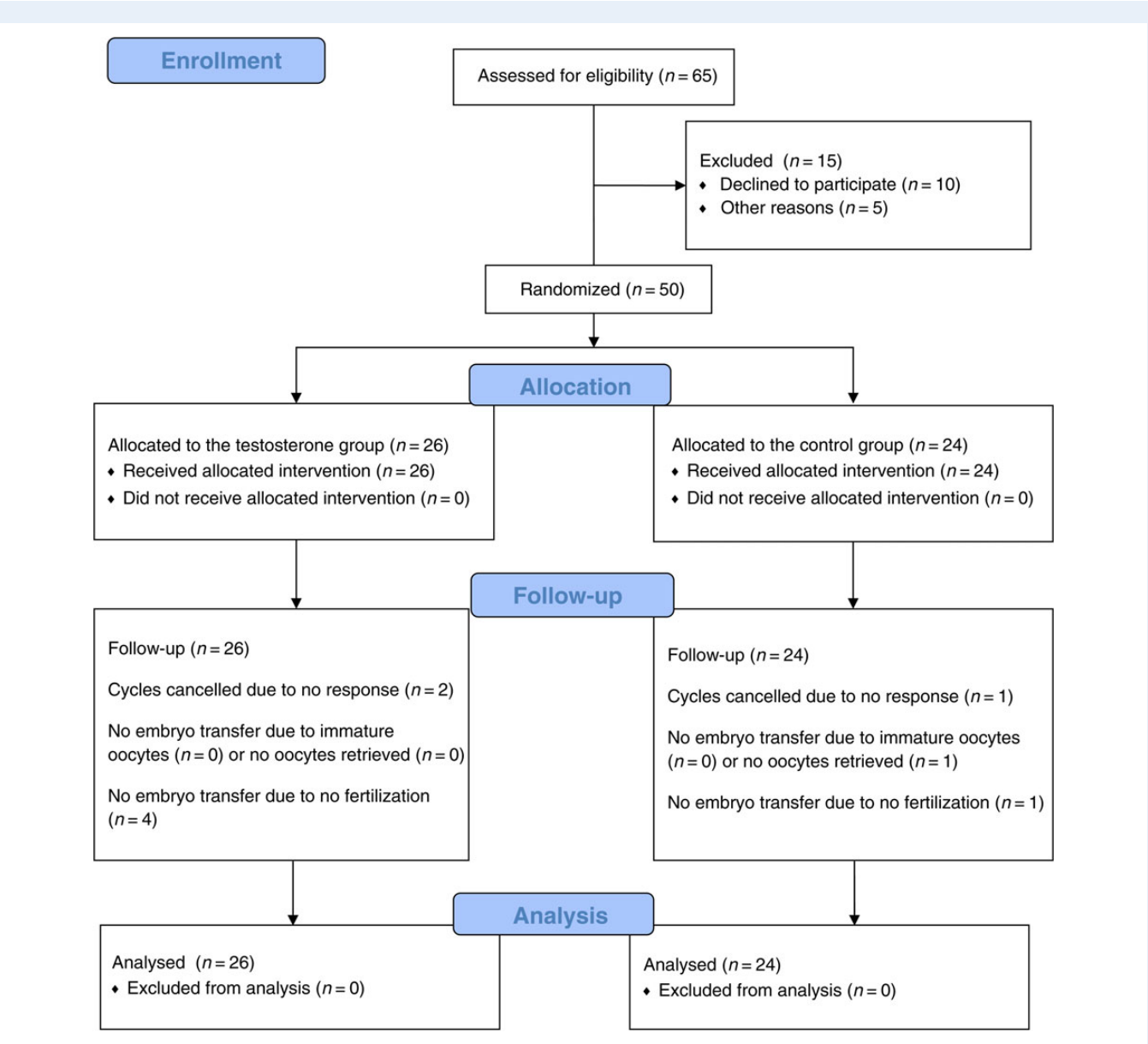

Figure 1 CONSORT 2010 flow diagram.

testosterone pretreatment group (Table II). The clinical importance of this difference as well as whether it could be explained by a direct effect of testosterone on endometrium, needs to be further explored. Nevertheless, it should be noted that testosterone levels on the day of triggering oocyte maturation were similar between the two groups compared.

Testosterone supplementation is considered as a simple and inexpensive treatment, yet, only a few randomized controlled studies, assessing its effectiveness, have been published so far. Currently, only three RCTs evaluating testosterone pretreatment in poor responders have been published (Massin *et al.*, 2006; Kim *et al.*, 2011, 2014). In agreement with the current study, the first relevant RCT (Massin *et al.*, 2006), including 49 patients, showed a non-significant increase with testosterone pretreatment (10 mg/day for 15–21 days) as compared with no pretreatment on the number of COCs retrieved (mean difference: +0.31 COCs, 95% CI: −1.64 to +2.26). On the other hand, a larger RCT (Kim *et al.*, 2011) including 110 patients, showed that testosterone pretreatment (12.5 mg/day for 21 days) significantly increased the number of COCs retrieved (mean difference: +1.60 COCs, 95% CI: +0.97 to +2.23) in poor responders as compared with no pretreatment. The same authors 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). That study suggested that testosterone has a beneficial effect on the number of COCs retrieved, which however, is statistically detectable as compared with no treatment only after 3 or 4 weeks of administration. The mean ± SD of COCs 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.

Table III Endocrine profile and stimulation data after testosterone pretreatment on Day 22 and on the day of hCG administration between the testosterone pretreatment and the no pretreatment groups.

	Testosterone pretreatment ($n = 26$)	No pretreatment ($n = 24$)	*P*-value
Endocrine profile and antral follicle count after testosterone pretreatment			
FSH (IU/l)	1.8 (2.2, 1.5–3.5)	3.5 (2.8, 2.4–4.2)	0.67
LH (IU/l)	1.2 (0.7, 1.0–1.7)	1.1 (0.6, 0.7–1.3)	0.65
Estradiol (pg/ml)	20.2 (10.6, 17.6–23.9)	19.0 (11.2, 11.8–20.9)	0.20
Progesterone (ng/ml)	0.3 (0.3, 0.2–0.4)	0.4 (0.3, 0.2–0.4)	0.58
Testosterone (ng/dl)	114.0 (99.5, 88.1–151.4)	20.0 (20.0, 11.3–29.2)	**<0.001**
Sex hormone binding globulin (nmol/l)	64.2 (42.9, 43.8–79.8)	74.6 (60.5, 50.6–99.5)	0.56
Free androgen index	5.7 (17.6, 3.9–11.4)	1.0 (1.4, 0.6–1.7)	**<0.001**
Dehydroepiandrosterone sulfate (μg/dl)	145.0 (102.2, 111.1–181.9)	112.5 (140.0, 76.3–177.3)	0.56
Δ4-Androstenedione (ng/ml)	2.0 (1.8, 1.3–2.9)	1.6 (1.1, 1.3–2.0)	0.77
Antral follicle count (AFC)	8.0 (4.0, 7.0–9.0)	6.0 (4.0, 5.0–8.0)	0.11
Difference in AFC between Day 22 and baseline assessment	2.0 (4, 0.0–3.0)	1.0 (3, 0.0–2.0)	0.39
Endocrine profile and stimulation data on the day of hCG administration			
FSH (IU/l)	21.0 (7.7, 17.7–24.4)	18.9 (7.8, 15.7–23.0)	0.61
LH (IU/l)	0.6 (0.5, 0.5–0.8)	0.6 (0.4, 0.4–0.6)	0.77
Estradiol (pg/ml)	719.7 (733.1, 487.1–1160.3)	559.5 (643.0, 415.8–915.7)	0.66
Progesterone (ng/ml)	0.8 (0.4, 0.6–0.9)	0.6 (0.6, 0.5–0.8)	0.15
Testosterone (ng/dl)	30.0 (19.0, 20.5–36.0)	23.0 (18.0, 16.8–31.5)	0.77
Sex hormone binding globulin (nmol/l)	72.1 (56.8, 51.8–92.0)	79.9 (50.5, 57.3–97.7)	0.76
Free androgen index	1.3 (1.6, 0.7–1.8)	1.1 (1.2, 0.8–1.4)	0.76
Dehydroepiandrosterone sulfate (μg/dl)	146.2 (102.4, 117.9–206.8)	148.0 (144.4, 85.0–208.2)	0.76
Δ4-Androstenedione (ng/ml)	1.6 (1.1, 1.3–2.2)	1.6 (1.2, 1.1–2.1)	0.75
Follicles ≥ 11 mm	8.0 (5.0, 4.0–9.0)	7.0 (3.0, 5.3–8.0)	0.30
Follicles ≥ 17 mm	3.0 (3.0, 2.0–4.7)	3.0 (3.0, 2.7–4.3)	0.83
Endometrial thickness (mm)	10.7 (2.5, 9.8–11.8)	9.0 (3.0, 8.1–9.7)	**0.01**
Duration of stimulation (days)	12.5 (3.0, 11.0–13.3)	12.0 (3.0, 11.0–13.0)	0.65
Units of gonadotrophins required (IU)	3750 (900, 3300–4001)	3600 (900, 3300–3900)	0.65

Data are median (interquartile range, 95% confidence intervals). *P*-values indicating statistical significance appear in bold. Statistical significance was considered at $P < 0.05$.

Table IV Clinical outcome between the testosterone pretreatment group and the no pretreatment group.

	Testosterone pretreatment (n = 26)	No pretreatment (n = 24)	Difference % 95% CI *P*-value
	% (n)		
Proportion of patients with at least one top quality embryos	20.0 (4)	23.8 (5)	−3.8 −28.2 to +21.5 0.72
Patients with embryo transfer	83.3 (20)	91.3 (21)	−8.0 −28.2 to +12.7 0.47
Cancelation rate	7.7 (2)	4.2 (1)	+3.5 −13.5 to +20.3 1.00
Positive hCG (intention to treat [ITT] analysis)	7.7 (2)	8.3 (2)	−0.6 −19.0 to +16.9 1.00
Positive hCG per embryo transfer	10.0 (2)	9.5 (2)	−0.5 −20.2 to +21.7 1.00
Clinical pregnancy (ITT analysis)	7.7 (2)	8.3 (2)	−0.6 −19.0 to +16.9 1.00
Clinical pregnancy per embryo transfer	10.0 (2)	9.5 (2)	−0.5 −20.2 to +21.7 1.00
Live birth rate (ITT analysis)	7.7 (2)	8.3 (2)	−0.6 −19.0 to +16.9 1.00
Live birth per embryo transfer	10.0 (2)	9.5 (2)	−0.5 −20.2 to +21.7 1.00

All three previously published studies (Massin *et al.*, 2006; Kim *et al.*, 2011, 2014) on testosterone pretreatment have used different definitions for poor ovarian response. The current study is the first RCT exploring the role of transdermal testosterone in poor responders who have been defined according to the Bologna criteria (Ferraretti *et al.*, 2011). It should be noted, however, that although a uniform definition of poor ovarian response is mandatory to enhance our knowledge in this area, concerns have been expressed in terms of the homogeneity of the population achieved following the application of the Bologna criteria for defining poor responders (Venetis, 2014).

It should be noted that the current study was not powered to detect differences less than 1.5 COCs, although it is doubtful whether these differences would be clinically relevant. Moreover, the study was not powered to detect differences in probability of pregnancy, and thus no solid conclusions can be drawn regarding this outcome. Nevertheless, an improvement in IVF outcome using a higher dose of testosterone or a longer pretreatment period cannot be excluded as suggested by data presented by Kim et al. (2014). Interestingly, no side effects have been reported in that study (Kim *et al.*, 2014) after a 4-week period of testosterone pretreatment. However, it is not known whether such side effects would occur by using an increased testosterone dosage or a longer pretreatment period.

The results of this randomized clinical trial can be used to advise poor responders. The non-significant increase in the number of COCs (3.5 versus 3.0 COCs, respectively), following pretreatment with 10 mg of transdermal testosterone for 21 days, was not associated with the probability of embryo transfer, or the proportion of patients with at least one top quality embryos which were higher but not significantly so in the no pretreatment group (Table IV). Moreover, it was accompanied by a higher although not significantly so cycle cancelation rate (Table IV).

In conclusion, the current RCT suggests that transdermal testosterone pretreatment at a dose of 10 mg/day for 21 days does not increase the number of COCs retrieved by more than 1.5 in poor responders undergoing ICSI, stimulated by recombinant FSH in a long GnRH agonist protocol.

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Authors' roles

J.K.B.: contributed toward the analysis and the interpretation of the data and drafted the manuscript. E.M.K.: conceived the idea of the study, contributed toward the analyses and interpretation of the data and drafted the manuscript. C.A.V.: contributed in the analyses and interpretation of the data and revised the manuscript for important intellectual content. K.D., L.Z., K.C., G.A., A.Mi., A.Ma., I.E.M. and B.C.T contributed in the interpretation of the data and revised the manuscript for important intellectual content. All authors approved the final version of the manuscript.

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Conflict of interest

C.A.V. reports personal fees and non-financial support from Merck, Sharp and Dome, personal fees and non-financial support from Merck Serono, personal fees and non-financial support from IPSEN Hellas S.A., outside the submitted work. B.C.T. reports grants from Merck Serono, grants from Merck Sharp & Dohme, personal fees from Merck Serono, personal fees from Merck Sharp & Dohme, personal fees from IBSA & Ferring, outside the submitted work.

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