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Testosterone Supplementation in Women with Diminished Ovarian Reserve

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Abstract

Objective: Women with Diminished Ovarian Reserve (DOR) have extremely low pregnancy rates after *In Vitro* Fertilization (IVF) cycles and there are currently few treatment options available. Testosterone supplementation prior to controlled ovarian hyperstimulation may improve IVF outcomes via enhanced folliculogenesis.

Methods: This retrospective cohort study analyzed 83 IVF cycles based on inclusion criteria of age <42 and diagnosis of DOR. Cycles in the control group were carried out using the standard IVF protocol while cycles in the treatment group were supplemented with transdermal testosterone prior to ovarian stimulation. Four primary outcomes were evaluated: total number of oocytes retrieved, number of mature oocytes retrieved, number of embryos generated, and pregnancy potential of the generated embryos.

Results: Pretreatment with transdermal testosterone had no impact on the total number of eggs retrieved after ovarian stimulation. Testosterone had a negative impact of the number of mature oocytes retrieved, but had no impact on the number of embryos generated from those oocytes. Pregnancy rates between the treatment and control groups were no different per embryo transfer, but were lower per cycle initiation with testosterone therapy.

Conclusion: Within this study population, retrospective analysis of testosterone therapy revealed no improvement in IVF outcomes. A randomized controlled trial is recommended to further investigate this association.

Introduction

In Vitro Fertilization (IVF) produces the highest pregnancy rate per cycle among infertility treatment options [1]. It consists of ovarian stimulation, oocyte retrieval, fertilization, embryo culture, and embryo transfer to the uterus. The standard ovarian stimulation protocol involves the use of exogenous gonadotrophins along with a Gonadotrophin-Releasing Hormone (GnRH) agonist or antagonist to induce pituitary suppression [2]. This prompts the maturation of multiple follicles, and thus allows for the retrieval of multiple oocytes per cycle [3]. Though this protocol has achieved successful ovarian stimulation and subsequent pregnancy in many patients, there remains a subset for which this treatment is ineffective. Women with Poor Ovarian Response (POR) have reduced oocyte production, higher rates of cycle cancellation, and overall low likelihood of pregnancy [4].

The diagnosis of POR is defined by the presence of at least two of the following criteria: i) advanced maternal age or any condition associated with reduced number of resting follicles; ii) previous POR (≤ 3 oocytes with standard stimulation protocol); iii) an abnormal ovarian reserve test [5]. Diminished Ovarian Reserve (DOR) is a major underlying cause of POR, and while its exact definition remains under debate, it is generally characterized by a reduction in the quantity and quality of oocytes remaining in the ovaries [6,7]. It is often seen in women in their late thirties, presumably due to acceleration in the normal physiological decline of ovarian function [8]. Women with DOR have extremely low pregnancy rates, even with the use of assisted reproductive technologies such as IVF. Current treatment options are sparse, and oocyte adoption is often suggested to these patients as the only effective therapy [7-9,11]. There is need for an alternative treatment option for women with DOR who desire biological children.

The use of testosterone supplementation prior to ovarian stimulation has been suggested as a potential treatment modification to improve the IVF outcomes of women with DOR. While the exact role of testosterone in the ovaries is unknown, it is clear that androgens play an important role in ovarian function both directly and as estrogen precursors [12,13]. Primate studies involving

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rhesus monkeys have demonstrated that testosterone may promote folliculogenesis, both through synergy with Follicle-Stimulating Hormone (FSH) to promote ovarian responsiveness to stimulation as well as through enhanced initiation of primordial follicle growth [14,15]. With this in consideration, it is thought that transient increases in testosterone levels prior to gonadal stimulation during IVF may amplify sensitivity to FSH and improve follicular maturation, therefore providing a novel therapeutic option for women with DOR. There is currently limited data on the success of testosterone therapy in IVF protocols, and previous studies examining its efficacy have yielded conflicting results. The present retrospective cohort analysis investigates the effect of testosterone pretreatment in IVF patients with DOR, evaluating both the number and quality of oocytes obtained as well as the potential impact on embryo development and pregnancy rates.

Materials and Methods

Patient selection and study design

A total of 48 female patients who underwent IVF at the University of Kansas Center for Advanced Reproductive Medicine between 2014 to 2018 were retrospectively analyzed based on the inclusion criteria of age <42 and diagnosis of DOR. DOR was defined by the presence of at least one of the following: basal FSH >10, anti-Mullerian Hormone (AMH) <1, Antral Follicle Count (AFC) <6 or history of poor response to stimulation (<4 follicles). Exclusion criteria included partner history of severe male factor (initial sperm concentration <5 million/mL and initial motility <20%), BMI >40, or history of >3 previous IVF stimulation cycles.

A total of 83 IVF cycles were evaluated and were separated into 2 primary groups for analysis. Cycles in the control group (n=49) were carried out using the standard IVF protocol. Cycles in the treatment group (n=34) involved the standard IVF protocol supplemented with transdermal testosterone prior to ovarian stimulation.

To confirm the similarity of treatment and control groups at baseline, age, Body Mass Index (BMI), AMH level, number of previous cycle cancellations, and partner sperm characteristics were assessed. Response to gonadal stimulation was measured by cycle cancellation rate, total amount of stimulation medications required, length of stimulation, and peak estradiol and progesterone levels. Stimulation outcomes were assessed using the total number of eggs retrieved, number of mature eggs, and number of embryos, embryo utilization, and rate of culture arrest. Pregnancy rates were reported per cycle initiation and per embryo transfer. There were 4 primary outcomes of the study. They included the effect of transdermal testosterone supplementation on the total number of retrieved oocytes, number of mature oocytes, number of embryos generated, and clinical pregnancy rate.

IVF Stimulation protocols

Cycles in the control group began with initiation of a GnRH antagonist for pituitary suppression during the midluteal phase of the previous cycle, starting with a lead follicle of ≥ 14 mm. Ovarian stimulation was achieved with recombinant Human Follicle Stimulating Hormone (r-hFSH) and Human Menopausal Gonadotropin (HMG), administered on an individual basis according to transvaginal ultrasound and serum estradiol levels. Cycles in the treatment group were carried out using the same protocol as those in the control group with the addition of testosterone therapy for 5 days prior to gonadal stimulation. Testosterone was administered transdermally via patch with a delivery rate of 2 mg/day. Low-dose oral contraception (Loestrin) was used for 10 days beginning in the luteal phase of the previous cycle. Ovarian stimulation with r-hFSH and hMG was then initiated the day after the final testosterone patch was applied.

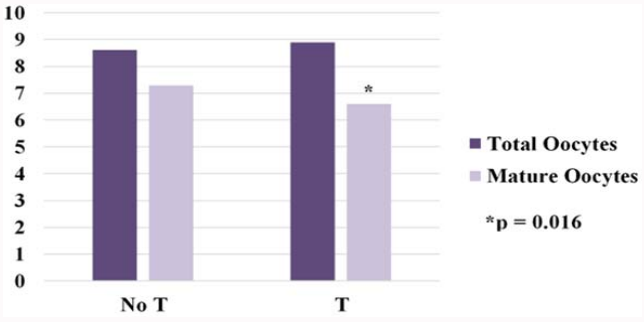

Figure 1: Comparison of the fraction of mature *vs.* total oocytes between treatment groups.

In both control and treatment groups, trigger injection with HCG or Lupron occurred when at least 2 follicles ≥ 18 mm in diameter were seen on ultrasound. Cycles were cancelled if <2 follicles were seen after 8 days of gonadal stimulation. Egg retrieval was performed under transvaginal ultrasound guidance 35 h after trigger injection. Eggs were then fertilized by either standard IVF or Intracytoplasmic Sperm Injection (ICSI). After fertilization and embryo culture, patients chose to either undergo a fresh embryo transfer 5 days following egg retrieval or cryopreserve the embryo(s) for future frozen transfer. Clinical intrauterine pregnancy was confirmed by the presence of fetal cardiac activity on ultrasound.

Monitoring

In groups, baseline estradiol and progesterone levels were measured via blood draw on day 3 of the menstrual cycle preceding stimulation. Partner sperm characteristics were evaluated via semen analysis. Ultrasounds and serum estradiol levels were collected at baseline, on medication day 5, and additionally per provider discretion. AFC was measured on day 1 of ovarian stimulation; follicles with a diameter ≤ 10 mm were recorded.

Statistical analysis

Data were analyzed using the ANOVA test and Fisher's exact test. $p<0.05$ was considered significant. The study was adequately powered based on a calculated necessary sample size of 43.

Results and Discussion

There were no statistically significant differences between the treatment and control groups at baseline based on age, BMI, AMH, number of previous cycle cancellations, and male sperm characteristics (Table 1). Response to gonadal stimulation was also similar between treatment and control groups, evaluated by cycle cancellation rate, total amount of stimulation medication used, length of stimulation, and peak estradiol and progesterone levels (Table 2). Transdermal testosterone pretreatment had no impact on the total number of eggs retrieved, total number of embryos generated, embryo utilization, or rate of culture arrest. However, it had a negative impact on the number of mature oocytes retrieved (Table 3 & Figure 1). Pregnancy rates between the groups were no different per embryo transfer but were lower per cycle initiation with testosterone therapy (Table 4).

This retrospective cohort analysis revealed no improvement

Table 1: Comparison of baseline characteristics between treatment groups. AMH-Anti-Mullerian Hormone.

	No T (n=49)		T (n=34)		P value
	Average	Range	Average	Range	
Age	35.7	26 to 41	35.8	27 to 42	0.867
BMI	25.8	18 to 33.8	25.9	19.4 to 33	0.891
AMH (ng/mL)	0.7	0.12 to 1.24	0.6	0.12 to 1.36	0.458
Previous cancellations	0.3	0 to 2	0.5	0 to 2	0.233
Initial sperm volume (mL)	3.7	0.9 to 8.4	3.2	0.5 to 6.9	0.274
Initial sperm concentration	54.2	2.8 to 317.2	31.7	8.8 to 75.2	0.092
Initial sperm motility	0.5	0.21 to 0.76	0.5	0.07 to 0.67	0.077

Table 2: Comparison of response to stimulation between treatment groups.
FSH: Follicle Stimulating Hormone; HMG: Human Menopausal Gonadotropin

	No T (n=49)		T (n=34)		P value
	Average	Range	Average	Range	
Cycle cancellation	15/49		14/34		0.356
Total FSH+HMG dose (IU)	5402.2	2700 to 7800	5832.5	3600 to 7650	0.205
Length of stimulation	10.8	9 to 15	10.9	9 to 13	0.747
Peak estradiol (ng/mL)	1836.8	286 to 3967	1973	1025 to 3491	0.553
Peak progesterome	1.1	0.4 to 3	1.1	0.4 to 2	0.83

Table 3: Comparison of stimulation outcomes between treatment groups.

	No T (n=34)		T (n=20)		P value
	Average	Range	Average	Range	
Total Oocytes	8.6	1 to 20	8.9	5 to 19	0.876
Mature Oocytes	7.3	1 to 18	6.6	3 to 14	0.016
Total Embryos	5.5	0 to 14	4.8	0 to 10	0.184
Embryo Utilization	2.3	0 to 7	2.5	0 to 8	0.054
Culture Arrest	5/34		2/20		1

Table 4: Comparison of pregnancy potential between treatment groups.

	No T	T	P value
Pregnant per Cycle Initiation	15/49	2/34	0.006
Pregnant per Embryo Transfer	15/32	2/11	0.154

in IVF outcomes when transdermal testosterone was used at 2 mg/day for 5 days prior to ovarian stimulation in women with DOR. Testosterone did not increase the total number of oocyte retrieved, number embryos generated, or clinical pregnancy rate per embryo transfer. It also resulted in significantly fewer mature oocytes retrieved and a lower clinical pregnancy rate per cycle initiation. Therefore, in this study population, testosterone supplementation did not appear to enhance response to ovarian stimulation and in fact had a negative impact on oocyte maturity and pregnancy potential.

Women with DOR comprise a challenging infertility population. Despite investigation of multiple treatment options, a consistently effective therapy has yet to be discovered. It is possible that testosterone pretreatment has an impact on ovarian function, but its role in the management of DOR remains unclear due to inconclusive results in the current literature. A randomized controlled trial by Massin et al. [9] in 2006 showed no improvement in ovarian response when DOR patients undergoing IVF received testosterone [11]. A 2012 meta-analysis by Bosdou et al. [16] demonstrated an increase in the number of retrieved oocytes as well as the clinical pregnancy and live birth rates in poor ovarian responders treated with testosterone prior to ovarian stimulation [16]. In contrast, a meta-analysis by Jeve et al. [17] in 2016 revealed higher clinical pregnancy and live birth rates but no improvement in the number of retrieved oocytes with this intervention [17]. The largest meta-analysis on this issue to date was conducted in 2019 by Noventa et al. [18] it showed testosterone supplementation in poor ovarian responders undergoing IVF yielded improvements in live birth rates as well as the number of retrieved oocytes [18].

The exact role of androgens in the human ovary remains unclear, and the potential therapeutic benefit of testosterone in poor ovarian responders is still under investigation. Though testosterone appears to be a positive regulator of folliculogenesis in rhesus monkeys, its effects in other animal models is controversial. For example, previous studies have reported a negative impact of androgens on follicular development in murine oocytes [19,20]. In addition, androgen excess in humans, such as in Polycystic Ovarian Syndrome (PCOS), is associated with decreased oocyte quality [21]. It is possible that testosterone has both dose-specific and species-specific effects and does not exert the same beneficial action on human ovaries as it does in monkeys.

The present study is limited by the use of retrospective data *vs.* a prospective randomized controlled study design. Because IVF can be performed multiple times in the same woman, there are multiple

patients whose cycles appear in both treatment and control groups. In addition, the IVF protocols were not entirely standardized; both GnRH antagonist and microdose flare protocols were included, trigger shots occurred with either hMG or Lupron, fertilization was achieved with either standard IVF or ICSI, and embryo transfers occurred with both fresh and frozen embryos. While the inclusion of cycles with these features is more broadly representative of DOR cycles in general, it somewhat limits our ability to reliably isolate testosterone as being responsible for the changes observed.

This study was adequately powered to detect a difference in clinical pregnancy rate, but the very low numbers of pregnancies in this study population make it difficult to draw clinically significant conclusions. This is may be the explanation behind the discrepancy in pregnancy rates per cycle initiation *vs.* per embryo transfer. However, since decreased clinical pregnancy rate is itself a feature of DOR, this will likely be a challenge in any investigation involving women with this diagnosis.

Our current conclusion is that testosterone therapy at 2 mg/day for 5 days prior to ovarian stimulation does not improve IVF outcomes in this study population, and may negatively impact oocyte maturity and pregnancy potential. Additional data through a randomized controlled trial is needed to further investigate the possible role of testosterone in enhancing ovarian response to stimulation and providing an effective intervention for women with DOR who desire pregnancy.

References

1. Van Voorhis BJ. *In Vitro* Fertilization. New Engl J Med. 2007;356(4):379-86.
2. Balasch J, Fábregues F, Peñarrubia J, Carmona F, Casamitjana R, Creus M, et al. Pretreatment with transdermal testosterone may improve ovarian response to gonadotrophins in poor-responder IVF patients with normal basal concentrations of FSH. Hum Reprod. 2006;21(7):1884-93.
3. Farquhar C, Marjoribanks J, Brown J, Fauser BCJM, Lethaby A, Mourad S, et al. Management of ovarian stimulation for IVF: narrative review of evidence provided for World Health Organization guidance. Reprod Biomed Online. 2017;35(1):3-16. Badawy A, Wageah A, Gharib ME, Osman EE. Prediction and Diagnosis of Poor Ovarian Response: The Dilemma. J Reprod Infertil. 2011;12(4):241-8.
4. Badawy A, Wageah A, Gharib ME, Osman EE. Prediction and Diagnosis of Poor Ovarian Response: The Dilemma. J Reprod Infertil. 2011;12(4):241-8.
5. Ferraretti AP, La Marca A, Fauser BCJM, Tarlatzis B, Nargund G, Gianaroli L, et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for *in vitro* fertilization: the Bologna criteria. Hum Reprod. 2011;26(7):1616-24.
6. Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in *in vitro* fertilization: a prospective study comparing basal markers of ovarian reserve. Fertil Steril. 2002;77(2):328-36.
7. Jahromi BN, Sadeghi S, Alipour S, Parsanezhad ME, Alamdarloo SM. Effect of Melatonin on the Outcome of Assisted Reproductive Technique Cycles in Women with Diminished Ovarian Reserve: A Double-Blinded Randomized Clinical Trial. Int J Mol Sci. 2017;42(1):73-8.
8. Jirge PR. Poor ovarian reserve. J Hum Reprod Sci. 2016;9(2):63-9.
9. Levi AJ, Raynault MF, Bergh PA, Drews MR, Miller BT, Scott RT. Reproductive outcome in patients with diminished ovarian reserve. Fertil Steril. 2001;76(4):666-9.
10. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest. 1998;102(12):2622-9.
11. Vendola K, Zhou J, Wang J, Famuyiwa OA, Bievre M, Bondy CA. Androgens promote insulin-like growth factor I expression and initiation of follicle development in the primary ovary. Biol Reprod. 1999;61(2):353-7.
12. Luo S, Li S, Li X, Qin L, Jin S. Effect of pretreatment with transdermal testosterone on poor ovarian responders undergoing IVF/ICSI: A meta-analysis. Exp Ther Med. 2014;8(1):187-94.
13. Hugues JN. Impact of androgens on fertility-physiological, clinical and therapeutic aspects. Reprod Biomed Online. 2005;11(5):570-80.
14. Massin N, Cedrin-Durnerin I, Coussieu C, Galey-Fontaine J, Wolf JP, Hugues JN. Effects of transdermal testosterone application on the ovarian response to FSH in poor responders undergoing assisted reproduction technique-a prospective, randomized double-blind study. Hum Reprod. 2006;21(5):1204-11.
15. Fábregues F, Peñarrubia J, Creus M, Manau D, Casals G, Carmona F, et al. Transdermal testosterone may improve ovarian response to gonadotrophins in low-responder IVF patients: a randomized, clinical trial. Hum Reprod. 2008;24(2):349-59.
16. Bosdou JK, Venetis CA, Kolibianakis EM, Toulis KA, Goulis DG, Zepiridis L, et al. The use of androgens or androgen-modulating agents in poor responders undergoing *in vitro* fertilization: a systematic review and meta-analysis. Hum Reprod Update. 2012;18(2):127-45.
17. Jeve YB, Bhandari HM. Effective treatment protocol for poor ovarian responders: a systematic review and meta-analysis. J Hum Reprod Sci. 2016;9(2):70-81.
18. Noventa M, Vitagliano A, Andrisani A, Blaganje M, Viganò P, Papaelo E, et al. Testosterone therapy for women with poor ovarian response undergoing IVF: a meta-analysis of randomized controlled trials. J Assist Reprod Genet. 2019.
19. Smith DM, Tenney DY. Effects of steroids on mouse oocyte maturation *in vitro*. J Reprod Fertil. 1980;60(2):331-8.
20. Anderiesz C, Trounson AO. The effect of testosterone on the maturation and development capacity of murine oocytes *in vitro*. Hum Reprod. 1995;10(9):2377-81.
21. Sahu B, Ozturk O, Ranierri M, Serhal P. Comparison of oocyte quality and intracytoplasmic sperm injection outcome in women with isolated polycystic ovaries or polycystic ovarian syndrome. Arch Gynecol Obstet. 2008;277(3):239-44.