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Role of decreased androgens in the ovarian response to stimulation in older women

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Ovarian testosterone increases the response of antral follicles to stimulation, declines with age, and has effects mediated or potentiated by insulin-like growth hormone I (IGF-I). Increased circulating insulin and IGF-I, exogenous testosterone, and increased local ovarian testosterone concentrations due to aromatase inhibition or exogenous luteinizing hormone/human chorionic gonadotropin are all associated with an increased ovarian response to gonadotropins. These factors should be further investigated alone or in combination for enhancing oocyte yield with fertility treatments, particularly in older reproductive-age women. (Fertil Steril® 2013;99:5–11. ©2013 by American Society for Reproductive Medicine.)

Key Words: Age, controlled ovarian hyperstimulation, female, growth hormone, IGF-I, testosterone

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EFFECTS OF TESTOSTERONE ON PREANTRAL AND ANTRAL FOLLICLES

Serum testosterone (T) decreases as age advances in premenopausal women (1–4), paralleling a similar age-related decline of antral follicle count (AFC) and level of antimüllerian hormone (AMH). Because it has been found that the T response to human chorionic gonadotropin (hCG) decreases with age (5), it is assumed that there is an age-related decrease of T secretion from the theca tissue surrounding ovarian follicles. In a study of 425 normally cycling women (1), baseline T remained correlated with the number of retrieved oocytes after adjusting by logistic regression analysis for age, body mass index (BMI), smoking, and timing of the sample during the menstrual cycle. These findings suggest that ovarian T plays a role in the ability of follicles to respond to follicle-stimulating hormone (FSH), and that part of the decreased ovarian response with aging may be due to declining ovarian androgen production.

Dickerson et al. (6) found in normal cycling women that the free androgen index and insulin resistance correlated with the follicle count after stimulation. Nardo et al. (7) similarly found a positive relationship of AMH with T, free androgen index, and insulin resistance in both nonobese polycystic ovary syndrome (PCOS) patients and normally cycling controls. Barbieri et al. (8) first observed that insulin stimulates T secretion from theca tissue cultured from the normal human ovary. These findings suggest that bioavailable T within the ovary may increase follicular response in a continuum from insulin-sensitive low-responding women without PCOS through to obese, insulin-resistant women with severe PCOS, who are at high risk of an excessive response to stimulation.

The effect of T on follicular response appears to be mediated by increasing FSH-receptor activity and by stimulating insulin-like growth factor I (IGF-I). In studies in subhuman primates, androgen receptor gene expression was shown to correlate with follicle growth, and T treatment significantly increased granulosa cell (GC) FSH receptor messenger RNA (mRNA) (9). Growing preantral and small antral follicles were significantly and progressively increased in number and theca layer thickness in T-treated monkeys (10). Testosterone has also been shown to stimulate earlier stages of follicular

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growth. Vendola et al. (11) showed that T increased the number of primordial follicles, increased IGF-I by threefold, and increased IGF-I receptor mRNA by fivefold in primordial follicle oocytes ($P<.0001$). These investigators hypothesized that IGF-I may stimulate primary follicle development, and IGF-I has been shown to enhance oocyte metabolic activity and maturation in vitro. Very strong correlations of follicular fluid T and GC androgen receptor mRNA with FSH receptor expression were also found in 3 to 9 mm antral follicles in adult human ovaries (12).

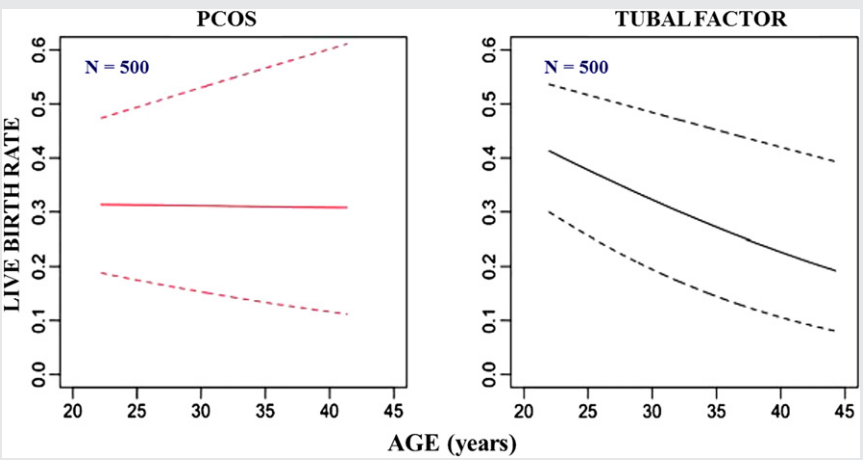
In the human ovary, IGF-II is primarily produced by the granulosa, whereas IGF-I is expressed in the theca (13). Regulation of IGF action in the ovary is complex, with FSH regulating IGF-II in granulosa, growth hormone (GH) regulating IGF-I systemically, and a family of IGF-binding proteins (IGFBPs) and IGFBP proteases within the follicle maximizing IGF action in the dominant follicle and the cohort recruited during ovulation induction with gonadotropins. The adjunctive use of GH as a co-gonadotropin in infertility therapy is believed to be due to its action on the liver, increasing IGF-I systemically and only secondarily within the follicle for oocyte maturation and enhanced follicle growth and steroidogenesis (13). Growth hormone does not have any direct action to increase expression of insulin-like growth factors or their receptor genes in the premenopausal human ovary (14).

In this review, we add to the present evidence supporting a hypothesis that the intraovarian effects of bioavailable T act to cause a continuum of antral follicle number and response from the poor responder—who is often more insulin-sensitive, has lower circulating insulin levels, and has a limited number of FSH-insensitive antral follicles (15)—through to women with severe PCOS, who are generally overweight or obese, insulin-resistant, hyperinsulinemic, and have a profusion of highly FSH-sensitive antral follicles. That concept in turn supports the premise that the decreasing thecal androgen production due to advancing age causes a progressive impairment of the aging ovary's ability to respond to stimulation for fertility treatments.

Women whom we would consider in optimal health paradoxically can be worse candidates for in vitro fertilization (IVF) with advancing age than our PCOS patients, whose success with IVF is relatively maintained (Fig. 1) in part because oocyte yield falls less with age in the women with PCOS compared with controls (not shown) (16). The birth rate with PCOS may also have been maintained with advancing age because of improved oocyte quality. In a large series of IVF patients studied by Holte et al. (17), AFC was found to predict success even after adjusting for oocyte yield and age, suggesting that AFC predicts oocyte quality and not just quantity. As we will outline, T improves GC health and therefore may be the link between a higher AFC and improved oocyte and embryo quality.

From a therapeutic perspective for women who have responded poorly to ovarian stimulation, the dilemma has been how to increase intraovarian androgen exposure to promote FSH receptor expression and an increased number of FSH-sensitive antral follicles. Vendola et al. (10) clearly showed in the subhuman primate model that an amount of systemically applied T (50 μg/kg per day for 5 days), which raised the circulating T concentration into the low male range, was capable of increasing preantral and antral follicles (Table 1); administration of dihydrotestosterone confirmed that the androgen receptor was responsible for the changes observed (10). Granulosa cell proliferation and health (decreased apoptosis) were also increased. Note that increasing the amount and duration (10 days) of T, achieving a circulating T concentration in the high male range, further increased antral follicles. That amount appears to raise the intraovarian concentration of T sufficiently to simulate a state similar to polycystic ovaries (PCO), but it is clearly impractical because of the expected androgenic side effects.

FIGURE 1



Live-birth rate versus age with in vitro fertilization in 500 women with polycystic ovary syndrome (PCOS) compared with 500 women with tubal factor infertility. The delivery rate remained unchanged with PCOS ($P=.96$) compared with a statistically significant decline with age in controls ($P=.03$) (16).

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TABLE 1

Number of antral follicles, percentage staining for Ki57 (a nuclear protein associated with cellular proliferation), percentage showing apoptosis, and the circulating testosterone (T) level (ng/dL) in monkeys treated with dihydrotestosterone, 20 μg/kg per day of T for 5 days, and 400 μg/kg per day of testosterone for 10 days.

Parameter	Antral follicles (no.)	Proliferation (Ki67, %)	Apoptosis (%)	T level (ng/dL)
Control	3.2	20.0	60.7	38.3
Dihydrotestosterone	8.3 ($P=.038$)	53.3[a]	17.7[a]	46.4
T 20 μg/kg for 5 d	8.7 ($P=.018$)	44.5[a]	16.8[a]	443.0[a]
T 400 μg/kg for 10 d	15.5[a]	40.0[a]	19.8[a]	1,345.0[a]

[a] $P<.001$.

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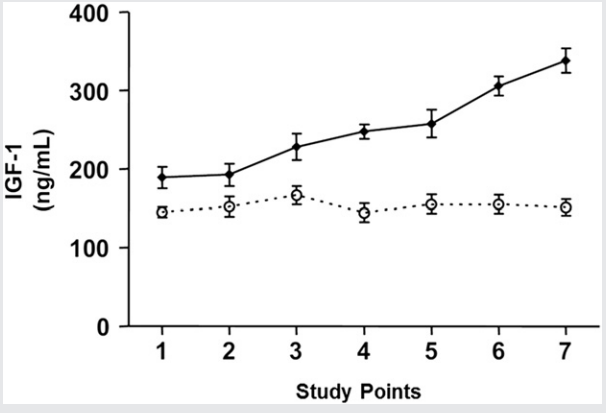
TESTOSTERONE ADMINISTRATION AND OVARIAN RESPONSE

Balasch et al. (18) used 20 μg/kg per day of T for 5 days in a series of poor responders using a 2.5 mg T patch and confirmed a significant increase in ovarian response. Their initial study was not randomized, but the identical poor response in two prior cycles argued strongly against regression to the mean as an explanation for the marked increase of follicles observed (mean 1.6 to 8.5, $P<.005$). It is very interesting that just 5 days of T caused a progressively increasing level of circulating IGF-I throughout the ovarian stimulation for more than a week after T was discontinued (Fig. 2; note that numbers refer to study points, not cycle days), and that IGF-I levels were significantly lower in the patients with canceled cycles. They then published a further study of 62 women whose cycles had been canceled due to a poor response, who were randomized to the T patch compared with increased gonadotropin and reduced gonadotropin-releasing hormone (GnRH) agonist administration. The T group showed a statistically significantly decreased incidence of poor response in spite of receiving significantly less gonadotropin (19).

FIGURE 2



Mean ± standard error of the mean for circulating insulin-like growth factor I (IGF-I) levels (ng/mL) in women with canceled cycles (*interrupted line*) compared with women who underwent retrieval (*solid line*). Study point 3: day 2 of 5 days of testosterone (T) supplementation. Study point 4: day after T. Study points 5, 6, and 7: days 5, 7, and 9 to 10 of ovarian stimulation. The area under the curve was different at $P<.05$ (18). For this review, further analysis of the data confirmed a statistically significant increase among the women receiving T supplementation (Kruskal-Wallis analysis of variance, $P<.001$, with each point 3 through 7 being increased compared with the baseline, $P=.026$ to $P<.001$) and a statistically significantly lower level before T supplementation in the patients with canceled cycles both at baseline and after agonist suppression ($P=.012$ and .048, Kruskal-Wallis test for multiple independent comparisons). The levels of IGF-I showed no statistically significant variation throughout the study period in the group of patients with canceled cycles.

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Another approach has been to provide a lower level of systemic T for a more extended duration. Testosterone administration using a gel applied to the skin for 3 weeks appears to make up for the lower T level achieved with that modality (about half as high as with the T patch). Kim et al. (20) randomized 110 women who had three or fewer oocytes retrieved in their prior cycle to 12.5 mg of T gel or no treatment and observed not only a significant increase of retrieved oocytes, but also significant increases in grade 1 embryos, implantation rate, and a more than doubled clinical pregnancy rate. Although more lengthy administration could cause changes in hair growth, voice pitch, or libido, the investigators reported that "no patients reported any systemic or local adverse effects." With this lower level of systemic T, these investigators had previously observed that at least 3 weeks were required to increase the ovarian response (21); consistent with that observation, a prior randomized trial by Massin et al. (22) of 15 days of the same transdermal gel product had failed to show a significant impact on stimulation. Also in the latter study, 10.0 g rather than 12.5 g of product was applied to the outer thigh rather than to the upper arms, and the T level rose from a higher pretreatment level (53–58 versus 20 ng/dL), suggesting an assay measuring higher to about 150 ng/dL compared with 190 ng/dL in the study by Kim et al.

Unfortunately, the levels achieved by transcutaneous T gel appear to vary with the product. For example, the levels reported in the U.S. *Physician's Desk Reference* (23) with 10.0 g of the generic T gel are lower than with the same dose of a widely used brand of gel (Androgel; Abbott Laboratories). It does appear that the dose of 12.5 g for 3 weeks is very close to the threshold producing a response, and the site and method of application could also affect the therapeutic response. Because of these variables it is difficult to recommend a 10.0 g dose for 3 weeks as the sole method to increase androgen exposure to the ovary.

These studies together show that the therapeutic effect of systemic T on growing ovarian follicles depends on the

duration and level of T exposure to the ovary. It is likely mediated or facilitated by IGF-I.

DHEA ADMINISTRATION AND OVARIAN RESPONSE

Another maneuver aimed at increasing ovarian T exposure has been systemic administration of dehydroepiandrosterone (DHEA). In gonadotropin-stimulated follicles, almost 50% of follicular fluid T was shown to be from circulating DHEA sulfate (24). Because adrenal production of DHEA drops about 50% from age 25 to age 45, the age-related decline of DHEA could be contributing to the reduced circulating and intraovarian T in older infertile women. In older women, 50 mg of DHEA doubled the circulating level of T into the high normal range (25). In one case-control study, 75 mg of DHEA was associated with a modest increase in ovarian response, although marked heterogeneity of the treatment group made a firm conclusion on therapeutic efficacy difficult (26).

Unfortunately, the only randomized study published on oral administration of DHEA and ovarian response to stimulation (27) was terminated after randomization of fewer than 20 women in each group. It was based on a secondary outcome measure that was uncorrected for multiple observations per patient, and the statistical significance level was not appropriate for an interim analysis. Because the investigators were not blinded, it was also unclear whether the interim analysis was planned to be performed at such an early point when statistical significance would not have been expected with such small numbers of patients. Large, properly designed and executed studies of DHEA treatment of poor responders are needed.

AROMATASE INHIBITION AND OVARIAN RESPONSE

A further way to increase intraovarian T is by blocking the conversion of T to estradiol using an aromatase inhibitor. In a case-control study of poor responders, adding letrozole to gonadotropin stimulation increased the number of retrieved oocytes from 4.3 to 6.1 ($P<.03$) (28). The most striking finding of that study was the increase in the implantation rate from 9% to 25% ($P<.009$). Follicular fluid levels of androstenedione and T were almost double those of controls (both $P<.004$), even though letrozole was discontinued at least a week before oocyte retrieval. The follicular fluid IGF-I levels were unfortunately not measured.

Ozmen et al. (29) randomized 70 women whose cycles had been canceled or had three or fewer oocytes retrieved in their prior cycle to either 5 days of 5 mg/day of letrozole or no treatment. They found that addition of letrozole to the gonadotropin/GnRH antagonist cycle statistically significantly reduced the amount of gonadotropin used, and the cancelation rate decreased from 28.6 to 8.6% (both $P<.05$). In spite of oocyte retrieval being achieved by 20% more patients with letrozole, the clinical pregnancy rate per transfer was 25.8% with letrozole compared with 20% in the controls (not statistically significant). In that study, the initiation of gonadotropin was delayed, whereas in the study by Garcia-Velasco et al. (28) gonadotropin stimulation was begun simultaneously with the letrozole.

LH/HCG AND OVARIAN RESPONSE

Finally, thecal androgen production can be increased by small daily doses of luteinizing hormone (LH) or hCG; as well described by Menon et al. (30), large doses of hCG or even the LH surge cause down-regulation of the LH/hCG receptor. Luteinizing hormone is a prime stimulator of androgens by the theca, with the response further potentiated by insulin and IGF-I (31), and increased basal LH levels have been correlated with higher IVF success (32).

Using a long GNRH agonist protocol, Durnerin et al. (33) randomized 76 normally cycling, nonobese women to a daily dose of 300 IU of recombinant LH administered for 7 days before ovarian stimulation compared with 71 women receiving no treatment. Small antral follicles increased from 7.3 to 8.8 ($P<.007$) and fertilized oocytes from 5.5 to 7 ($P<.03$). One of the authors (D.R.M.) of our study has shown that a single dose of 50–75 IU of hCG returns bioactive LH/hCG to above baseline in women suppressed with a GnRH antagonist (34). Filicori et al. (35) used 50 IU of hCG daily in a woman with hypogonadotropic hypogonadism to restore the ovarian response to pure FSH, resulting in a triplet pregnancy. Based on those experiences, and because recombinant LH is no longer clinically available in the United States, a daily dose of 50 IU of hCG should be examined to increase antral follicles in low responders.

GROWTH HORMONE/IGF-I AND OVARIAN RESPONSE

Insulin-like growth factor I synergizes with FSH in its effect in inducing GC aromatase activity and also stimulates thecal androgen production, synergizing with LH (13, 31). If IGF-I is a mediator or facilitator of the effects of T on the growth of preantral follicles, treatment with GH before initiating ovarian stimulation would be expected to improve the ovarian response. In the only such study to date, 61 women who had responded poorly to high-dose gonadotropin in their first cycle were randomized to receive a relatively high dose of GH or no additional treatment from the midluteal administration of GnRH agonist, amounting to at least a week before the onset of stimulation, and continuing until the administration of hCG (36). In spite of requiring less gonadotropin ($P<.001$), the number of mature oocytes doubled from 3.2 to 6.5, and the number of fertilized oocytes increased from 1.5 to 4.4 ($P<.001$). The pregnancy rate doubled, but the study was not sufficiently powered to examine that outcome. Further large, placebo-controlled studies should be conducted to confirm their study's provocative findings, although administration of T may be a less costly way to improve ovarian response. A combination of T and a lower, more affordable dose of GH could also be considered.

The levels of follicular fluid IGF-I have been reported to be markedly lower in women with five or fewer follicles at least 14 mm in diameter (mean $\pm$ standard error: 42 $\pm$ 4.7 ng/mL, $P<.001$) compared with women who produced 10 or more (85.4 $\pm$ 7.8) (37). Both GH and IGF-I levels in follicular

fluid were also observed to be statistically significantly lower in normal responding women under age 35 years who failed to establish an ongoing pregnancy via IVF (38). Tesarik et al. (39) randomized very low prognosis women over age 40 years to supplementary GH or placebo starting on day 7 of gonadotropin stimulation and continuing until oocyte retrieval. With GH, the delivery rate increased over fivefold, and the GH levels in follicular fluid approached but did not reach the levels observed in their group's previous study of young women achieving ongoing pregnancies with IVF (38).

In a meta-analysis of studies using GH in poor responders, the delivery rate was increased over threefold, although the extreme heterogeneity among dose regimens and protocols makes a conclusion regarding the method of choice difficult (40). Because GH and IGF-I act to improve GC function, one would expect that an increased implantation rate of embryos with GH would be accompanied by improvements in embryo quality, although the increased delivery rate could also be attributed to improved endometrial receptivity as IGF-I is considered an estromedin in the endometrium (41).

Mendoza et al. (38) observed that in women who established a successful pregnancy the follicular fluid GH levels were higher in association with embryos chosen by morphology for transfer, compared with embryos that were cryopreserved. In the study by Tesarik et al. (39), embryo quality was improved if the data are analyzed by a *t*-test. Those investigators elected to be conservative in using a nonparametric test, showing a lack of statistical significance, but the *t*-test is fairly robust regarding minor deviations of data from normality.

It is important to also analyze studies that seem to yield data opposing the concept we are presenting. In a very small group of normal women averaging 42 years of age with a mean AFC of over 12, luteal administration of a T patch for 12 days failed to affect the response to a small dose of FSH (75 IU) (42). Cai et al. (15) have shown that only poor responders to a stronger gonadotropin stimulus have reduced GC FSH receptor activity, and (as noted earlier) poor responders have a marked decrease of follicular fluid IGF-I (37). The finding that older normal women with good ovarian reserve did not experience any response to an adequate T stimulus adds further emphasis to defining the role of reduced follicular IGF-I in poor responders for mediating or facilitating T effects on follicle responsiveness.

DISCUSSION

All of these studies indicate that IGF-I appears to mediate or facilitate the effect of T on early follicle development, and also improves oocyte and embryo quality when levels are raised by administering GH during stimulation. Stimulation of IGF-I by T may explain the unusually high implantation rates reported in some studies with treatments aimed at increasing the exposure of T to ovarian follicles in poor responders (20, 28). Further research should focus on factors that could explain the lower IGF-I levels in poor responders and women failing to conceive with IVF, both prominent characteristics of the older woman, and also examine whether the lower levels are due to lower circulating GH or reduced hepatic IGF-I production, as suggested with the subset of women failing to respond to T (Fig. 2). Stimulation of IGF-I by systemic T administration provides an important opportunity for correction of these critical defects experienced by older women undergoing IVF. Further research should also focus on the mechanism of action of IGF-I and the roles of IGF-II, IGFBPs, and IGFBP proteases on follicle maturation from the primordial stage to the fully developed preovulatory follicle.

Table 2 summarizes the interventions designed to increase ovarian T that have been shown by randomized, controlled trials to improve ovarian response. The ages of the patients and the definitions of what constitutes a poor responder have varied among studies. More direct approaches with systemic administration of T or GH appear to have produced the clearest results, compared with androgen-modulating agents like LH/hCG or aromatase inhibitors. Further controlled trials should be conducted to examine whether the interventions increase delivery rates.

Understandably, each therapeutic maneuver we have described has been studied alone to verify its efficacy. Because the affects are likely to be additive, future studies should also examine combinations of those interventions, such as

TABLE 2

Interventions shown by randomized controlled trials to improve ovarian response to gonadotropin stimulation.

Intervention	Study (reference)	End points	*P* value
T patch	Fabregues et al. (19)	Decreased poor response	< .05
		Reduced gonadotropin use	< .001
		Fewer days of stimulation	< .01
T gel	Kim et al. (20)	Increased mature oocytes	< .001
		Increased good quality embryos	< .001
		Increased implantation rate	< .019
Letrozole	Ozmen et al. (29)	Reduced gonadotropin use	< .05
		Reduced cancellation	< .05
Recombinant LH	Durnerin et al. (33)	Increased small antral follicles	.007
		Increased fertilized oocytes	.03
GH	Kucuk et al. (36)	Reduced gonadotropin use	< .001
		Increased mature/fertilized oocytes	< .001

Note: GH = growth hormone; LH = luteinizing hormone; T = testosterone.

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low-dose hCG with letrozole, or T gel followed by a T patch and with added GH during final follicle maturation. It is important to limit such studies to poor responders because, as previously mentioned, they have deficient systemic IGF-1 levels (37), lower intraovarian T levels (above), and reduced FSH receptor expression (15). For example, Lossl et al. (43) found in a randomized study of normal infertile women that a combination of hCG and anastrozole had minimal impact on the recruitment of antral follicles and negative effects on oocyte fertilization, with a trend toward fewer top-quality embryos compared with a control group receiving neither hCG nor anastrozole. It is hoped that interventions such as those that have been described herein develop into widely accepted ways to improve both the response to stimulation and the quality of oocytes and embryos in older women undergoing IVF.

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