Hypoandrogenism in association with diminished functional ovarian reserve

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STUDY QUESTION: Is diminished functional ovarian reserve (DFOR) associated with low androgen levels?

SUMMARY ANSWER: Low androgen levels are associated with DFOR at all ages.

WHAT IS KNOWN ALREADY: Androgen supplementation via dehydroepiandrosterone (DHEA) has been reported to improve functional ovarian reserve (FOR); pregnancy rates in IVF cycles are associated with how well DHEA converts to testosterone (T); and androgen effects through the androgen receptor have been demonstrated in mice to beneficially affect early stages of follicle maturation.

STUDY DESIGN, SIZE, DURATION: In a controlled cohort study we investigated consecutive women presenting to our center with two forms of DFOR, premature ovarian aging/occult primary ovarian insufficiency (POA/OPOI) and physiologic diminished ovarian reserve (DOR). As controls for POA/OPOI patients, infertile women with normal age-specific FOR were recruited.

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: The study involved 140 women with POA/OPOI, defined as age <38 years and abnormally low FOR by age-specific FSH and/or anti-Muellerian hormone (AMH), 166 women with DOR, defined as women age >40 years. Forty-nine control patients <38 years demonstrated normal FOR by FSH and/or AMH. In all three patient groups dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS), total testosterone (TT) and free testosterone (FT) at the time of initial presentation to our fertility center were assessed. In a small subgroup of women early morning cortisol levels were also assessed.

MAIN RESULTS AND THE ROLE OF CHANCE: DHEAS marginally varied between the three groups (P = 0.04), with older women with DOR actually demonstrating higher levels than controls (P = 0.03). TT differed between the three groups more profoundly (P = 0.005), with women with POA/OPOI demonstrating significantly lower levels than controls (P = 0.009). Adjustment for body mass index, age and race in principle maintained observed differences in TT between groups, while adjustment for FMR1 (fragile X mental retardation 1) genotypes/sub-genotypes eliminated all differences. All three patient groups demonstrated low morning cortisol levels.

LIMITATIONS, REASONS FOR CAUTION: While results support lower androgen levels in women with DOR, and even more so in women with POA/OPOI, presented data should be viewed as preliminary, considering the known variability of androgen levels and the small number of women in whom morning cortisol levels were available.

WIDER IMPLICATIONS OF THE FINDINGS: Especially at young ages DFOR appears associated with significant hypoandrogenism (low T) in comparison with young control patients with normal FOR, raising the question whether this hypoandrogenism originates in adrenals or ovaries. POA/OPOI, thus, phenotypically mimics the polycystic ovary syndrome, where similar questions arise, though in regard to hyperandrogenism.

STUDY FUNDING/COMPETING INTEREST(S): This research was supported by the Foundation for Reproductive Medicine, a not-for-profit medical research foundation and intramural funds from the Center for Human Reproduction. N.G. and D.H.B are members of the Board of the Foundation for Reproductive Medicine. N.G., A.W. and D.H.B. received research support, lecture fees and travel support from a variety of pharmaceutical and medical device companies, none in any way related to the issues discussed in this manuscript. N.G. and D.H.B. are listed as co-inventors on two, already granted US user patents, which claim therapeutic benefits from DHEA supplementation in women with DFOR and DOR; both authors are also listed on additional pending patents in regard to DHEA supplementation and on pending patents, claiming diagnostic and therapeutic benefits from the determination of CGG repeats on the FMR1 gene. N.G. is the owner of the Center for Human Reproduction, where this research was performed.
**Introduction**

Ovarian reserve (OR) is generally perceived as the sum of all still available follicles/oocytes within ovaries (Ledger, 2010; Gleicher et al., 2011a). It is made up of resting follicles at primordial stages of maturation and of growing follicles after recruitment. The latter enter an ~3–5 month long journey of maturation, during which most follicles degenerate and undergo apoptosis, while the remaining follicles are aligned in waves of approximately monthly menstrual cycle patterns. Ultimately, only one follicle is ovulated spontaneously in each cycle (Gleicher et al., 2011a).

Various methods have been proposed to assess OR. The most utilized are FSH, anti-Müllerian hormone (AMH) and the antral follicle count (AFC), the latter assessed by ultrasound (Ledger, 2010). All three of these methods, however, assess somewhat different components of the growing follicle pool, with the much larger resting follicle pool being inaccessible to direct measurements (Ledger, 2010; Gleicher et al., 2011a). Because the number of recruited follicles is believed to correlate with the size of the resting follicle pool, AMH, which, together with AFC, best represents small growing follicles, is now by many considered to also best represent total OR (Kevenaar et al., 2006; Ledger, 2010). AMH is, however, less specific than FSH at older ages, and, in general, does not as well reflect the pre-ovulatory, gonadotrophin-sensitive follicle pool (Gleicher et al., 2010a). Uniformly accepted methods to reliably assess total OR or even only a woman’s ‘functional’ OR (FOR), the total growing follicle pool alone, are, therefore, still lacking.

FOR (i.e. the number of small, growing follicles) is, of course, of great importance since they represent the follicles that within a few short months will produce pre-ovulatory follicles responsive to gonadotrophin stimulation during fertility treatments (Gleicher et al., 2011a). Practically, all attention over the last 50 years of fertility therapy has been directed at only the last 2 weeks of follicle maturation, the gonadotrophin-sensitive stage. Earlier phases of follicle maturation, and especially the stages of small growing follicles between primary and small pre-antral stages, have largely remained unattended. These early stages of follicle maturation, however, appear crucial to normal folliculogenesis since they appear to regulate follicle recruitment rates, developing egg numbers and egg quality (Gleicher et al., 2011a). By the time follicles reach the gonadotrophin-sensitive stage of maturation, their quantitative as well as the qualitative fates have already been largely determined.

Recent experiments in androgen receptor knock out (ARKO) mice have demonstrated the importance of androgens during these early growth stages of follicles (Sen and Hammes, 2010). Human clinical experiences appear to support the importance of proper androgen levels to female fertility (Gleicher et al., 2011b).

Our center for a number of years has been utilizing dehydroepiandrosterone (DHEA) supplementation in women with objectively low functional OR (FOR) (Gleicher and Barad, 2011). Recently, we attempted to determine whether the effectiveness of DHEA in women with low FOR related to the conversion of DHEA to other androgens (Weghofer et al., 2012; Gleicher et al., 2013). In doing so, we discovered that pregnancy chances directly appear to relate to the improvement of testosterone levels: the lower testosterone was at initiation of supplementation, and the higher the increase in testosterone levels with supplementation via DHEA, the better the patient’s pregnancy chances (Gleicher et al., 2013).

This observation led to the question to what degree low FOR actually represents an androgen deficient state? Here this presented study was designed to answer this question, and will demonstrate that low FOR, indeed, appears to represent an androgen deficient state.

**Methods**

**Patient population**

This study investigated 359 consecutive infertility patients and oocyte donor candidates, who presented to our center between 2008 and 2012. Four oocyte donor candidates were removed from consideration because they, at the time of hormonal baseline evaluation, were on oral contraceptives. The final study population, thus, involved 355 infertile women. They subdivided into two study and one control group: 140 women had a diagnosis of premature ovarian aging (POA), by some also called occult primary ovarian insufficiency (OPOI) ( Nelson, 2009), and generally considered a precursor stage of premature ovarian failure (POF), also often given the acronym primary ovarian insufficiency (POI) (Gleicher et al., 2009). Patients in this study group were age <38, and objectively demonstrated, based on age-specific 95% CI, premature low FOR, characterized by abnormally high FSH levels (Barad et al., 2007) and/or abnormally low AMH (Barad et al., 2011). Such age-specific parameters of FOR define patients objectively, while criteria of poor ovarian response, as for example in the recently established Bologna criteria byESHRE (Ferraretti et al., 2011) are still subject to confounders, like, for example, gonadotrophin dosage administered for ovarian stimulation.

A second study group of 166 women comprised females age >40, considered to represent physiologic diminished ovarian reserved (DOR) due to physiologic aging. At advice of our statistical consultant, we on purpose eliminated women between ages 38 and 40 from this study. This was done to distinctively differentiate between ‘younger’ and ‘older’ women with abnormally low OR, since ‘older’ age in many fertility studies is defined as either age >38 or 40 years.

A control group was made up of 49 infertile women aged <38 years but with normal age-specific ovarian function values, based on FSH and AMH levels (Barad et al., 2007, 2011). These control patients, therefore, served as relatively age-appropriate controls to POA/OPOI patients and as relative functional controls to DOR patients, who by definition were older women though, as Table 1 demonstrates, the three groups differed significantly in patient characteristics, like age and body mass index (BMI).

**Laboratory evaluations**

Women in all three groups were evaluated for baseline FSH and estradiol (E2) levels on Days 2 or 3 of the menstrual cycle, AMH at random and also at random for the androgens dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS), total testosterone (TT) and free testosterone (FT). All hormonal assessments were performed via routine commercial
The guidelines for the study were followed, and there were no women in our study groups of Native American or Asian (NOT-OD-010053, 2012). Because many female fertility parameters differ between races (Butts and Seifer, 2010), we initially classified women self-identified as such were, if possible, assigned to above noted three races and, if that was not possible, were excluded from participation. There were no women in our study groups of Native American or Pacific Islander races, the two other races recognized by NIH guidelines.

**Table I Patient characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>POA/OPOI</th>
<th>Physiologic DOR</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>140</td>
<td>166</td>
<td>49</td>
<td>355</td>
</tr>
<tr>
<td>Age (years) (range)</td>
<td>34.5 ± 3.3a (26–38)</td>
<td>43.3 ± 2.4a (40–54)</td>
<td>30.5 ± 5.0a (22–38)</td>
<td>38.0 ± 6.0 (22–54)</td>
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<tr>
<td>BMI (kg/m²) (range)</td>
<td>24.0 ± 4.8b (17–40)</td>
<td>24.3 ± 5.0b (18–43)</td>
<td>21.4 ± 4.6bc (15–34)</td>
<td>23.8 ± 5.0 (15–43)</td>
</tr>
<tr>
<td>FSH (mIU/ml) (range)</td>
<td>13.4 ± 14.1id (8–35)</td>
<td>17.8 ± 13.1id (10–40)</td>
<td>6.3 ± 2.5id (2–8)</td>
<td>14.8 ± 13.4 (2–40)</td>
</tr>
<tr>
<td>AMH (ng/ml) (range)</td>
<td>0.8 ± 1.2 (0.1–2.0)</td>
<td>0.3 ± 0.3 (0.1–0.6)</td>
<td>3.6 ± 2.1 (2.1–10.0)</td>
<td>1.0 ± 1.5 (0.1–10.0)</td>
</tr>
<tr>
<td>Gravidity (range)</td>
<td>1.0 ± 1.5 (0–8)</td>
<td>1.6 ± 1.90 (0–9)</td>
<td>0.9 ± 1.4 (0–5)</td>
<td>1.3 ± 1.7 (0.9)</td>
</tr>
<tr>
<td>Parity (range)</td>
<td>0.2 ± 0.7 (0–4)</td>
<td>0.4 ± 1.00 (0–6)</td>
<td>0.2 ± 0.80 (0–3)</td>
<td>0.3 ± 0.8 (0–6)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>93 (66.4)</td>
<td>127 (76.5)</td>
<td>30 (61.2)</td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>16 (11.4)</td>
<td>19 (11.4)</td>
<td>5 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>31 (22.1)</td>
<td>20 (12.0)</td>
<td>14 (28.6)</td>
<td></td>
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<tr>
<td>FMR1, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norm</td>
<td>78 (57.8)</td>
<td>93 (60.0)</td>
<td>31 (64.6)</td>
<td></td>
</tr>
<tr>
<td>Het-norm/high</td>
<td>23 (17.0)</td>
<td>25 (16.1)</td>
<td>8 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Het-norm/low</td>
<td>23 (17.0)</td>
<td>31 (20.0)</td>
<td>6 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Hom-low/low</td>
<td>7 (5.2)</td>
<td>4 (2.6)</td>
<td>0 (0.0)</td>
<td></td>
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<tr>
<td>Hom-low/high</td>
<td>3 (2.2)</td>
<td>1 (0.6)</td>
<td>2 (4.2)</td>
<td></td>
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<tr>
<td>Hom-high/high</td>
<td>1 (0.7)</td>
<td>1 (0.6)</td>
<td>1 (2.1)</td>
<td></td>
</tr>
</tbody>
</table>

The table compares patient demographics and functional ovarian OR parameters between POA/OPOI, physiologic DOR and control groups. The same comparisons are made within different racial and or ethnic groups. Values are presented as means ± SD and as ranges (minimum to maximum).χ² analysis shows an almost significant relationship between racial categories and the three patient groups (P = 0.053), with Asian women less likely to fall into the physiologic DOR group than the control or the POA/OPOI groups by 10–17%.

**FMR1 genotypes and sub-genotypes were determined as previously reported (Gleicher et al., 2010b, c), utilizing PCR and Southern blot (Laboratory Corporation of America).**

Data were further analyzed by adjusting for race and FMR1 genotype and sub-genotype, with the latter obtained as previously reported (Gleicher et al., 2010b, c). In brief, FMR1 genotypes are based on a normal range of CGGn = 26–34 (mean CGGn = 30). Women with both alleles in normal range are considered to have a norm genotype; those with one allele outside normal range are considered heterozygous (het) and those with both outside normal range as homozygous (hom) genotypes. Het and hom genotypes of FMR1 are further subdivided into sub-genotypes based on whether a het or hom allele is above (high) or below (low) normal range.

In a small subgroup of women baseline cortisol levels were available, uniformly drawn before noon. Cortisol was assessed on morning bloods before 10:00 a.m. by routine commercial assay via electrochemiluminescence immunoassay (ECLI; Laboratory Corporation of America).

This included 15 women in the POA/OPOI group, 15 patients with physiologic DOR and 3 among controls. They are here presented as a possible indication of adrenal function levels.

**Statistical analyses**

Mann–Whitney U tests and a series of ANOVA tests were used to test for mean differences in continuous variables of patient demographics and OR parameters. The primary analysis consisted of a series of analyses of covariance, corrected for age, which were used to calculate differences in mean androgen levels between groups. Post hoc analyses were performed
using the Sidak test. Distributions of categorical data were evaluated using $\chi^2$ tests. Subgroup analyses of categorical data were performed using binomial tests. All tests performed were two-tailed, with a $P < 0.05$ considered statistically significant.

**Human subject protection**

This study only involved data from the center’s anonymized electronic data bank. All of the center’s patients at initial consultation sign an informed consent, which allows for the assessment of their medical records for research purposes, as long as the patients’ anonymity is maintained and the content of their medical record remains confidential. Both of these conditions were met, and the study, therefore, qualified for expedited review by the center’s Institutional Review Board.

**Results**

The mean age for the whole study population was 38.0 ± 6.0 years. Table I demonstrates that mean age in the POA/OPOI study group was 34.5 ± 3.3, in the physiologic DOR group 43.3 ± 2.4 and in controls 30.5 ± 5 years (Sidak test: POA/OPOI versus DOR, POA/OPOI versus controls, DOR versus controls, all $P < 0.001$).

The mean BMI in POA/OPOI women was 24.0 ± 4.8, in DOR women 24.3 ± 5.0 and in controls 21.4 ± 4.6 (Sidak test: POA/OPOI versus controls, $P = 0.004$; DOR versus controls, $P = 0.001$).

Table I also demonstrates that the OR in all three groups varied: FSH was 13.4 ± 14.1 in POA/OPOI patients, 17.8 ± 13.1 in physiologic DOR and 6.3 ± 2.5 mIU/ml in control patients (Sidak test: POA/OPOI versus DOR, $P = 0.01$; POA/OPOI versus controls, $P = 0.02$; DOR versus controls, $P < 0.001$). AMH values were 0.8 ± 1.2 in POA/OPOI, 0.31 ± 0.3 in DOR and 3.6 ± 2.1 ng/ml in controls (Sidak test: POA/OPOI versus DOR, POA/OPOI versus controls, DOR versus controls, all $P < 0.001$). Women with physiologic DOR, thus, had lower OR than women with POA/OPOI, and both study groups had distinctively lower OR than control patients.

Table I also summarizes the racial background of all three groups and their respective $FMR1$ genotype and sub-genotype distributions. $\chi^2$ analysis shows an almost significant relationship between racial categories and the three patient groups ($P = 0.053$), with Asian women less likely falling into the physiologic DOR than the POA/OPOI group, with up to a 17% difference.

Among 338 women with $FMR1$ data, 202 (59.8%) demonstrated a norm $FMR1$ genotype, 116 (34.3%) a het and 20 (5.9%) a hom genotype. Controls had the most women with norm $FMR1$ (binomial test, 64.7 versus 57.8% in POA/OPOI, $P = 0.51$ and versus 60.0% in DOR patients, $P = 0.67$). No significant differences in distribution were noted for $het-norm$/low $FMR1$ sub-genotypes, where DOR patients presented with highest (20.0%) and controls with lowest (12.5%) prevalence, with POA/OPOI in the middle at 17.0% (binomial test, controls versus POA/OPOI versus DOR, $P = 0.43$; controls versus DOR, $P = 0.19$; and POA/OPOI versus DOR, $P = 0.52$). These results suggest a non-significant directional tendency, indicating that $het-norm$/low women, with ~8% difference, are less likely to fall into the control group than POA/OPOI and DOR groups. Individual hom sub-genotypes were too small in numbers to allow for valid statistical analyses.

Figure 1a demonstrates androgen values in all three groups: as can be seen, DHEA values did not differ between the three groups; DHEAS levels between the three groups, however, varied mildly [$F (2, 327) = 3.24, P = 0.04$]. Post hoc comparison suggested a significant difference in DHEAS between controls and women with physiologic DOR (Sidak test, $P = 0.03$).

FT was not, but TT was significantly varied between the three groups [$F (2, 273) = 5.51, P = 0.005$]. Post hoc comparison demonstrated significantly higher TT concentrations in controls than in women with POA/OPOI (Sidak test, $P = 0.009$).

Adjustment of results for race did not affect previously observed differences in androgen concentrations between the three groups with TT remaining significantly higher [$F (2, 267) = 4.12, P = 0.017$].

Adjustment for $FMR1$ genotypes and sub-genotypes, however, eliminated all androgen differences between the three groups. Morning cortisol data were only available for a total of 33 women. As Fig. 1b demonstrates, in this limited number of patients, no statistical difference in levels was visible between the three groups, as even three control patients demonstrated low morning cortisol levels. These preliminary data, best demonstrated by the TT/cortisol relationship (Fig. 1c), however, do support the possibility that morning cortisol levels in patients with low functional OR are uniformly low. Figure 1c summarizes in all three groups the relationship between TT and morning cortisol. While the data set is too small to reach definite conclusions, the figure points out highest TT/cortisol levels in controls and lowest in POA/OPOI patients.

The Supplementary Information, of this manuscript also demonstrates that a series of ANOVA adjustments for BMI, age and BMI and age, BMI plus race in the model did not change the results reported herein.

**Discussion**

Here presented data for the first time demonstrate that low FOR, in principle, represents an androgen deficiency condition, characterized by low TT. This androgen deficiency is most pronounced among young women with POA/OPOI, now widely considered a precursor condition of POF/POI (Gleicher et al., 2009), and appears less so in older women with physiologic DOR, who demonstrate elevated DHEAS levels, suggestive of a conversion problem from DHEA to testosterone (Fig. 1a). Low androgen levels, therefore, may also be a characteristic of POF/POI, though this remains to be confirmed.

On first glance these findings may surprise but on further consideration they appear logical: testosterone levels are known to decline with advancing female age (Barbieri et al., 2005), as does the conversion efficiency from DHEA to testosterone (Gleicher et al., 2013). It, therefore, should not surprise that older women with physiologic DOR accumulate DHEAS, as here demonstrated by demonstrating increased levels in comparison with controls (Fig. 1a).

This study, unfortunately, was unable to answer the question whether the observed androgen deficiency is primarily an adrenal, ovarian or a combined defect. Universally, low morning cortisol levels may point towards adrenal insufficiency as the primary defect but such a conclusion requires further study and confirmation.

In the descending order of concentration, the following androgens are found in sera of premenopausal women: DHEAS, DHEA androstenedione (AN), testosterone (T) and dihydrotestosterone (DHT) (Burger, 2002). DHEAS is exclusively produced by the adrenals (normal range 75–375 μg/dl); DHEA ca. 50% by the adrenal, 20%
Figure 1  Androgens, cortisol and androgen/cortisol ratios. Box and whisker plots of serum androgen levels, serum cortisol levels and the ratio of total testosterone concentration over serum cortisol levels for controls, POA/OPOI and physiologic DOR. The mean is depicted with a dashed line, the median with a solid line and the normal range for serum androgens with a grey background.  

(a) Comparison of serum androgen levels.  

(b) Shows the distribution of serum cortisol levels.  

(c) Shows the ratio of TT over cortisol levels. DOR, diminished ovarian reserve; OPOI, occult primary ovarian insufficiency; POA, premature ovarian ageing; TT, total testosterone.
by the ovary and 30% by peripheral conversion from DHEAS (normal range 0.2–0.9 µg/dl) (Longcope, 1986); AN half and half by adrenals and ovaries (normal range 160–200 ng/dl) (Horton and Tait, 1966); and T, ~25% by adrenals and ovaries, each, with the other half coming from peripheral conversion of AN (normal range 20–70 ng/dl) (Longcope, 1986). DHT is primarily an intracellular androgen (Abraham, 1974).

Like the association of hyperandrogenism with increased adrenal as well as ovarian androgen production (Puurunen et al., 2009), it, therefore, appears possible that the androgen insufficiency in women with DOR may also represent a combined adrenal/ovarian pathophysiology.

Here the presented results are supportive of such a conclusion, as TT levels in young women with POA/OPOI were lower than in older women with physiological DOR, while the latter demonstrated significantly higher DHEAS levels, suggestive of an androgen conversion problem, we previously described to a significant degree more severe in older than younger women (Gleicher et al., 2013). Combined, these data, therefore, point towards differences between POA/OPOI and DOR patients, with the former representing a more profound androgen deficiency which, likely also explains why they were found to be more responsive to androgen supplementation with DHEA (Gleicher and Barad, 2011).

This interpretation of here reported data derives further support from adjustments: such adjustments for race maintained all observed associations with androgens, while the adjustments for FMR1 genotypes/sub-genotypes eliminated all significant findings. Considering the relatively small number of study subjects, especially conclusions in regard to genetic effects require caution. The FMR1 gene has, however, been associated with distinct ovarian aging patterns (Gleicher et al., 2010b, c) and variability in androgen conversion (Weghofer et al., 2012; Gleicher et al., 2013).

For example, the het-norm/low sub-genotype of FMR1, characterized by a low (CGGn < 26) allele, has been demonstrated to convert DHEA to TT poorer than other FMR1 genotypes/sub-genotypes (Gleicher et al., 2013). As Table I demonstrates, this sub-genotype is the lowest in controls and the highest in older infertile women with physiologic DOR, with POA/OPOI patients falling in-between. Older women with DOR, overall, also demonstrated the most severe low FOR, based on AMH and FSH levels (Table I).

Interestingly, a very specific ovarian PCO-like phenotype has also been associated with the het-norm/low FMR1 sub-genotype, and is characterized by high FOR at young ages but a quickly depleting follicle pool, thus leading to DFOR at relative young ages (Gleicher et al., 2010b).

One can, therefore, hypothesize that POA/OPOI in infertile women may represent more of a primary androgen deficiency condition, while physiologic DOR in older infertility patients represents relatively more of an androgen conversion insufficiency. If correct, this would also suggest that POA/OPOI might be more a condition of adrenal etiology, while physiologic DOR may represent more of an ovarian theca cell exhaustion and incompetence.

Independent of underlying pathophysiology, both conditions, as this study demonstrates, led to hyperandrogenism, characterized by low TT. That they also are associated with low FOR, characterized by small oocyte numbers and poor egg quality (Ledger, 2010; Gleicher et al., 2011a, b), should not surprise: androgens in ARKO mice have been demonstrated to be essential for normal growth and development of small growing follicles between primary and small pre-antral stages (Sen and Hammes, 2010). Here reported findings, therefore, also offer indirectly further support for observations, which have associated higher androgen levels (Gleicher et al., 2011b) and androgen supplementation with DHEA (Gleicher and Barad, 2011) with better fertility treatment outcomes.

This study raises a number of important new questions, among them why do POA/OPOI patients at such young ages already demonstrate such profound hyperandrogenism? If this hyperandrogenism in young women should indeed prove to be primarily adrenal in origin, then the next question becomes what causes such profound suppression of androgen activity in the zona reticularis?

The association of the het-norm/low FMR1 sub-genotype with a PCO-like ovarian phenotype was shown to further strengthen in the presence of autoimmune laboratory findings (Gleicher et al., 2010b).

One, therefore, could hypothesize about a regulatory adrenal autoimmune network controlling the zona reticularis (Gleicher et al., 2007). Such autoantibody-driven functional controls,_of course, exist in the thyroid gland (Lyton and Kahaly, 2010), which in regard to autoimmunity demonstrates considerable cross-reactivity with both adrenals and ovaries (Kelkar et al., 2005). Moreover, such autoimmune-driven processes have also recently been reported in association with at least one classical autoimmune disease (Baroni et al., 2006), and may represent a wider prevalent concept within autoimmunity (Lyton and Kahaly, 2010).

Aside from iatrogenic causes (Fleischer et al., 2011), POA/OPOI is primarily associated with genetic and autoimmune causes (Gleicher et al., 2009). It remains to be determined whether the hyperandrogenism in association with POA/OPOI is specifically associated with an autoimmune etiology, a question currently under investigation at our center.

Another important question raised by this study is whether the treatment of hyperandrogenism in POA/OPOI and age-associated DOR should remain the same. Currently, we supplement both of these patient groups with identical DHEA protocols (Gleicher et al., 2007). Based on here reported and earlier data (Weghofer et al., 2012; Gleicher et al., 2013), one could conclude that older women with DOR may be more successfully directly supplemented with testosterone than DHEA. A clinical trial to test this hypothesis has been recently initiated at our center.

The here presented study investigated only selective androgens. Demonstrating significantly decreased TT especially in POA/OPOI patients, we failed to demonstrate an association with FT. This should, however, not surprise since patients in this study were not assessed for sex hormone-binding globulin (SHBG), which determines FT levels. Over 90% of circulating T is usually believed bound to SHBG (Speroff et al., 1994). SHBG is the main transport-binding protein for sex steroid hormone and regulates their access to target cells. It, in itself, is under the control of hormonal and genetic factors (Hammond, 2011).

Finally, the beneficial effects of androgens on follicle maturation appear mediated through the AR on granulosa cells in small growing follicles (Sen and Hammes, 2010). Different androgens demonstrate different binding affinities to the AR, with DHT demonstrating the highest affinity (Gleicher et al., 2010b). Further clinical studies, involving different androgenic compound appear, therefore, indicated in

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women with diminished functional OR, whatever the underlying pathophysiology.

**Authors’ roles**

N.G. and D.H.B. conceived the project, and developed the study design with participation of A.W. and A.K., who also performed data accumulation and statistical analyses. N.G. and D.H.B. interpreted the data and N.G. wrote the manuscript, with all other authors participating in the editing and revision process. All authors approved of the final manuscript. Portions of these data were presented at the Annual Meeting of the American Society for Reproductive Medicine (ASRM), October 20–24, 2012, San Diego, CA.

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**Conflict of interest**

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**References**


Gleicher N, Weghofer A, Barad DH. Discordance between follicle stimulating hormone (FSH) and anti-Müllerian hormone (AMH) in female infertility. *Reprod Biol Endocrinol* 2010a; 8: 64.


Hypoandrogenism and low ovarian reserve


