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Original research article

Associations between peripheral androgens and cortisol in infertile women

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ABSTRACT

Testosterone has in recent years been proven essential for normal growth and maturation of small growing follicles. Concomitantly, low functional ovarian reserve (LFOR), characterized by a small growing follicle pool, has been associated with low testosterone levels, which can be of ovarian and/or adrenal origin. In this study we, therefore, investigated whether peripheral sex steroid precursors and testosterone levels potentially reflect on adrenal function. In a retrospective cohort study of 355 consecutive infertile women, who presented to an academically affiliated fertility center in New York City, we investigated in a series of statistical models whether low peripheral sex steroid precursors and testosterone are associated with peripheral cortisol (C) levels, reflecting adrenal function. To determine potential correlations, we investigated the dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), androstenedione (AD), total testosterone (TT), free testosterone (FT); sex hormone binding globulin (SHBG), anti-Müllerian hormone (AMH), thyroid stimulating hormone (TSH) and C in a series of multivariate and logistic regression analyses, utilizing C either as a continuous variable or with cut off <5.0 μg/dL, and TT only as a continuous variable. Practically all models demonstrated significant predictability of peripheral sex hormone precursors for C levels, with DHEA demonstrating the strongest and most consistent predictability as an individual parameter and as part of the DHEAS/DHEA ratio. We conclude that in infertile women peripheral sex hormone precursors, especially DHEA, reflect C levels and, therefore, adrenal function. In infertile women, at all ages low levels of sex hormone precursors, therefore, should be considered indications for further adrenal assessments.

1. Introduction

Embryologically, adrenal glands and ovaries arise out of a common adrenogonadal premordium, so-called primordial germ cells [1]. They also share selected steroidogenic production steps for androgens (and estrogens). In association with polycystic ovary syndrome (PCOS) this sharing of androgen production for decades has caused confusion in determining whether the typical hyperandrogenemia of PCOS is of adrenal and/or ovarian origin [2]. Similarly, the more recently described typical low testosterone (T) levels, associated with low functional ovarian reserve (LFOR) [3], can therefore be either consequence of theca cell insufficiency in ovaries and/or of zona reticularis insufficiency in adrenals.

Insufficiency of the zona reticularis is under current definitions not included in the definition of adrenal insufficiency (AI) [4]. This diagnosis is reserved for insufficiencies of either zona fasciculate (glucocorticoids) and/or zona glomerulosa (mineralocorticoids).

Similarly, only autoantibodies to the steroidogenic enzyme 21-hydroxylase are currently considered diagnostic of primary AI

Abbreviations: AD, androstenedione; AI, adrenal insufficiency; AMH, anti-Müllerian hormone; C, cortisol; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; FOR, functional ovarian reserve; FT, free testosterone; PCOS, polycystic ovary syndrome; LFOR, low functional ovarian reserve; TT, total testosterone; SHBG, sex hormone-binding globulin; TSH, thyroid stimulating hormone.

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(Addison's disease) [4]. They, indeed, often precede diagnosis of AI [5], and approximately 30 percent of asymptomatic patients with anti-21 hydroxylase antibodies will develop AI within five years [6]. An AI diagnosis can, however, also be reached in absence of these autoantibodies, though such cases are considered rare, and mostly only occur in children and elderly people [4].

In absence of tuberculosis and trauma, AI in the developed world is almost uniformly considered autoimmune [4], suggesting still undiscovered (steroidogenic) epitopes, capable of affecting adrenal function. Antibodies to a variety of other steroidogenic enzymes have, indeed, been reported, though none have so far been identified as diagnostic of AI [7,8]. Because AI is primarily an autoimmune condition, it is reasonable to assume that insufficiency of the zona reticularis, leading to low T and LFOR, likely is also autoimmune in nature.

With T now widely recognized as essential for normal follicle growth and maturation [9], any cause of low T can potentially contribute to female infertility by reducing small growing follicle numbers (also called functional ovarian reserve, FOR). Fewer growing follicles represent smaller granulosa/cumulus cell mass and, therefore, lower anti-Müllerian hormone (AMH) production [10,11] and estradiol levels. Via negative feedback lower estradiol, in turn, induces higher follicle stimulating hormone (FSH) [12]. The resulting hormonal phenotype of low AMH and high FSH is typical of LFOR. It, therefore, should not surprise that LFOR at all ages is characterized by relatively low T [3].

Whether a woman's low T is primarily adrenal and/or ovarian in origin is clinically relevant. In some cases, low T levels may be caused by adrenal zona reticularis rather than ovarian theca cell insufficiency. Especially if also associated with insufficiency in glucocorticoids and/or mineralocorticoids, hypoandrogenemia could, thus, theoretically be a presenting finding in previously undiagnosed cases of AI.

These functional associations between adrenals and ovaries led us to assess in this study how peripheral sex steroid precursors and T levels relate to adrenal function in infertile women. We in a series of statistical models investigated their relationships with adrenal function, as reflected by C levels. As this study will demonstrate, sex steroid precursor levels in this patient population, indeed, at all ages correlate with C levels, thus suggesting that such a finding may have to be considered a potential symptom of AI. AI can, therefore, affect functional ovarian reserve (FOR; i.e., the small growing follicle pool) via low sex steroid precursor production, likely causing a form of secondary ovarian insufficiency (SOI) in some women, currently considered to suffer from primary ovarian insufficiency (POI).

2. Subjects and methods

This study utilized an anonymized patient cohort ($n = 355$) from our center's electronic research database, which presented to our center with a primary diagnosis of infertility. Table 1 demonstrates that their mean age was 38.66 years (95% CI 38.10–39.22). The table also presents sex steroid precursors, T, sex hormone binding globulin (SHBG), anti-Müllerian hormone (AMH), thyroid stimulating hormone (TSH) and morning cortisol levels for this patient group, all bloods routinely obtained at initial presentation to the center.

These tests were certified by the Centers for Disease Control Hormone Standardization Program in August 2011. At the time this patient cohort was established our center did not yet routinely test adrenocorticotropic hormone (ACTH). To avoid selection biases, this study, therefore, does not report on associations with ACTH levels.

Table 2 summarizes primary infertility diagnoses of 131 from among the 355 patients of this cohort. Diagnoses for the remaining patients were not available at time of this analysis since they had not initiated in vitro fertilization (IVF) cycles, and are not entered into our center's electronic database records until IVF cycle start. Distribution of infertility diagnoses should, however, not differ significantly from the initial 135 patients since they represented consecutive new patients accessing care at our center. Because some patients had more than one primary diagnosis, the sum of diagnoses exceeds 100%.

Fig. 1 summarizes steroidogenesis in the three zone of the adrenal cortex. Dehydroepiandrosterone (DHEA) has a short half-life, and is quickly sulfonized via sulfotransferase into its 3β-sulfate, considered its storage form (DHEAS). The *SULT2A1* gene primarily codes this conversion in the zona reticularis of the adrenal cortex [13–15], and to lesser degrees in liver and small intestines. Orally ingested DHEA is sulfonized primarily in liver and intestines. Steroid sulfatase is widely distributed in tissues, resulting in desulfonization of DHEAS back into DHEA. As the figure also demonstrates, DHEA (and DHEAS) are substrate for T production in the adrenal (and peripheral aromatization to estrogens).

At least 50% of endogenous DHEA is of adrenal origin, ca. 20% ovarian and ca. 30% is derived from peripheral conversion. In contrast, the zona reticularis of the adrenals produces at least 80% of endogenous DHEAS. DHEAS levels, therefore, are widely used to assess adrenal contributions to female androgen production. DHEAS production by the zona reticularis, however, significantly declines with advancing female age, as the thickness of the zona shrinks in parallel [16].

To better understand how peripheral sex steroid precursor and T levels reflect on adrenal function, this study, therefore,

Table 1
Patient characteristics for the study group.

	Mean	95% CI	
		Lower	Upper
Age	38.7 years	38.10	39.22
DHEA	265 ng/dL	251.50	279.62
DHEAS	139 ug/dL	132.58	145.66
Total testosterone	22.96 ng/dL	21.67	24.25
Free testosterone	1.55 pg/mL	1.43	1.67
Androstenedione	73.9 ng/dL	69.98	77.90
SHBG	90.95 nmol/L	85.34	96.57
AMH	1.30 ng/mL	1.08	1.52
TSH	1.91 Iu/mL	1.72	2.09
Cortisol	8.04 ug/dL	7.60	8.48

Table 2
Primary infertility diagnoses for the study group[a].

Dx	*n*	%
Diminished ovarian reserve	106	80.90%
Other[b]	35	26.70%
Male factor	**34**	**26.00%**
Tubal factor	23	17.60%
Tubal ligation	1	0.80%
Hydrosalpinx	2	1.50%
Other tubal	20	15.30%
PGD	10	7.60%
PCO	9	6.90%
Endometriosis	8	6.10%
Uterine	8	6.10%
Non-infertility patients[c]	3	2.30%

[a] Represents only 131 of the patients in the study group but is likely representative of the whole study group. Since many patients carried more than one primary infertility diagnosis, percentages exceed 100%.
[b] By the Society of Assisted reproductive Technology (SART) defined as immunologic, chromosomal, cancer chemotherapy, or other serious systemic diseases.
[c] Patients undergoing IVF for non-infertility related reasons.