Anti-Müllerian hormone confirms the novel classification of female functional androgenization including polycystic ovary syndrome

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Abstract

Objective: Functional androgenization (FA) can be divided into five groups corresponding to the predominant organ pathology as recently shown by our group: functional cutaneous androgenization (FCA, skin) and FA syndrome (FAS) I (ovary, lean individual), II (adrenal gland), III (ovary, fat tissue, pancreas, and hyperinsulinemia), and IV (residual FA dysfunctions). Group-specific clusters are based on primary variables such as LH, testosterone, DHEAS, sex hormone-binding globulin (SHBG), body mass index (BMI), glucose, insulin, and enlarged polyfollicular ovaries. Because anti-Müllerian hormone (AMH) positively correlates with the antral follicle count, its relevance as an additional primary variable for classifying FA was investigated.

Design: In this study, 178 patients with FA were consecutively enrolled and classified into the five FA groups as described earlier and 30 women with regular menstrual cycles served as control.

Methods: Primary variables and serum AMH were analyzed in the early follicular phase.

Results: FA patients showed significantly elevated AMH levels (11.1 ± 6.7 ng/ml) versus control (3.0 ± 2.0 ng/ml; P < .0001). AMH was significantly increased in groups FAS I (15.6 ± 5.8 ng/ml) and FAS III (11.6 ± 6.6 ng/ml) compared with groups FCA (7.0 ± 3.8 ng/ml), FAS II (5.05 ± 3.0 ng/ml), and FAS IV (6.9 ± 4.6 ng/ml) and correlated positively (P < .0001) with LH (r = 0.538) and testosterone (r = 0.368). In regression and multivariate analyses, AMH was not dependent on SHBG, DHEAS, BMI, glucose, or insulin. In receiver operating characteristic analysis, 9.21 ng/ml AMH showed 90% specificity with 71.2% sensitivity for the diagnosis of the two ovarian FA groups, FAS I and III.

Conclusion: AMH confirms the novel stratification system and constitutes a useful primary variable in the algorithm of FA classification.

European Journal of Endocrinology 165 323–330

Introduction

The term ‘female androgenization’ defines a wide spectrum of heterogeneous dysfunctions and disorders comprising one or several organs or organ systems and touching on pediatric and adult endocrinology, gynecology, reproductive medicine, and dermatology as well as molecular genetics. It can be differentiated into three major areas: ‘tumorous’, ‘pharmacological’, and ‘functional androgenization (FA)’ (1–3) resembling the classification proposed by Buggs & Rosenfield (4) in a basic setup. The most important area in clinical practice, FA, is algorithmically divided into five groups in order to describe in detail the entire spectrum of FA phenotypes and associated morbidities. The FA groups are defined by anthropometric, endocrine, ultrasonographic, metabolic, and molecular genetic criteria and according to therapeutically projected aspects including dermatology and gynecology, as well as internal medicine-related questions (5).

Detailed scientific descriptions of the FA groups were recently published (2, 3, 6–8). Briefly, the first group is termed ‘functional cutaneous androgenization (FCA)’ because the androgenization is predominantly focused on the skin (hirsutism, acne vulgaris, and androgenetic alopecia), and none or only discrete other systemic symptoms being present. e.g. patients with ‘idiopathic hirsutism’ (9) would fit into this group. This cutaneous dysfunction markedly differs from the oligo- to multisymptomatic/organic ‘FA syndrome (FAS) I–IV’. The FAS I group (predominantly focused on the ovary) comprises lean androgenized patients with enlarged polyfollicular ovaries (EPO; for details, see Materials and methods section) without significant metabolic alterations.
Thus, these individuals correspond in principle to patients with ‘polycystic ovary syndrome (PCOS)’ who do not show any variables of the metabolic syndrome (MetS) as defined by the Rotterdam Consensus (RC, (10)). In the following, the term ‘PCOS’ will be cited merely regarding references that used the RC criteria, (10). The FAS II group predominantly focusing on the adrenal gland consists of patients with elevated testosterone and/or DHEAS levels without the variable clusters specific for the FAS I and III groups; furthermore, females presenting a symptomatology of the so-called ‘non-classical congenital adrenal hyperplasia’ (NC-CAH) or ‘late onset adenogenital syndrome’ (Joint LWPE/ESPE CAH Working Group, (11)) belong to the FAS II group. Subjects diagnosed as FAS III (fat tissue, pancreas, insulin target organs, and ovary) are in part equivalent to ‘PCOS’ patients associated with a MetS according to the RC criteria (10). The classification of FCA and FAS I–III is based on different, strictly determined, group-specific variable clusters consisting of specifically defined and exactly reproducible primary variables; moreover, the patients are characterized by several secondary and additional variables, resulting in the final overall diagnosis. Patients in the FAS IV group cannot be classified into any of the four FA groups described above. The dysfunctions experienced by these patients do not fit into any currently known pathogenic concept and reflect the heterogeneity of female FA (2, 3, 6); an equivalent term might be ‘non-PCO-PCOS’ (12). A further sub-classification of FCA and FAS I–III was regarded as useful, with subset ‘a’ showing a classic full-blown feature, whereas subset ‘b’ reflecting a non-classic minimum standard core or a miscellaneous constellation (e.g. ovarian–adrenal or adrenal–metabolic constellation; for detailed description, see Geisthövel et al. (2)). Therefore, subset ‘a’ constitutes an ideal group for scientific application. The entire stratification described allows a complete diagnosis, including both classification and an individualized characterization, resulting in an individually tailored therapy.

Anti-Müllerian hormone (AMH), a member of the transforming growth factor β superfamily, is primarily responsible for the regression of the Müllerian duct in the male fetus during early testis differentiation. In females, AMH is produced by ovarian granulosa cells (13) with maximum expression occurring in pre-antral and small antral follicles, whereas AMH expression declines as antral follicles increase in size (14). Thus, AMH is produced in secondary follicles that have undergone recruitment from the primordial follicle pool but have not been selected for dominance. Serum AMH levels do not show any major fluctuations across the menstrual cycle (reviewed in (15)) consistent with AMH’s role representing the continuous, non-cyclic growth of small follicles in the ovary. Furthermore, AMH correlated more strongly with the antral follicle count (AFC) than with other hormonal markers (16). In patients diagnosed with ‘PCO’, AMH is significantly elevated in serum (17, 18) as well as in the follicular fluid (19). In addition, AMH seems to be related to body mass index (BMI) (20), fasting insulin levels (21), and DHEAS (22).

One of the primary variables of FA classification proposed by our team (2, 3) is the ultrasonographic visualization of EPOs. Their presence differs among the five FA groups. Consequently, the granulosa cell mass, reflected indirectly by ovarian size and AFC, and, therefore, the amount of granulosa cell-derived circulating AMH are supposed to be different among the FA groups. Thus, AMH appears to be a promising parameter for the classification of FA. In this study, the role of AMH in the FA classification and the possible impact on FA pathogenesis (ovary, adrenal gland, and glucose metabolism) were analyzed.

Materials and methods

Patients and control group

After exclusion of other pathologies like hyperprolactinemia, 197 premenopausal women who were examined at the Centre for Gynaecological Endocrinology and Reproductive Medicine Freiburg (CERF) were consecutively selected for the study and evaluated retrospectively. The reasons for the patients’ clinical visit were cutaneous androgenizing symptoms (CAS: acne vulgaris, hirsutism, and androgenetic alopecia), menstrual cycle irregularities (oligo-, amenorrhea), infertility, hyperandrogenemia, decreased circulating sex hormone-binding globulin (SHBG) levels, hyperinsulinemia, and/or EPOs (see Transvaginal ultrasonography section). Of the total population, 19 patients had to be excluded because of missing data leaving 178 women for the study. Patients were grouped into FCA, FAS I–IV and subgroups ‘a’ and ‘b’ according to Geisthövel et al. (2, 3).

The control group consisted of 30 healthy premenopausal women with no signs of FA. These women had a history of ≥3 regular menstrual cycles in the last 6 months (≥21 to ≤35 days); mean circulating progesterone values obtained around 7 days prior to the expected start of menstrual bleeding were > 30 nmol/l, suggesting a sufficient luteal phase (2).

No individual had taken any hormonal or metabolic medication during the preceding 3 months aside from treatment with iodine and/or thyroxine (T4). The study was approved by the ethical committee of the University of Freiburg, Freiburg im Breisgau, Germany, and all patients and volunteers gave written informed consent.

Endocrine and metabolic variables

Venous blood sampling was performed between days 3 and 7 of regular menstrual cycles and at random in oligomenorrhea, oligo-amenorrhea, and amenorrhea. The early- to mid-follicular phase was hormonally
confirmed by serum levels of estradiol (E2) < 440 pmol/l and progesterone ≤ 6 nmol/l, as described previously (2). After an overnight (12 h) fast, venous blood samples were obtained between 0800 and 0900 h at 20 min intervals (baseline value, 0 h). Blood samples were centrifuged and the sera pooled in equal volumes, processed immediately, and an aliquot was stored at −20 °C until assayed. Serum LH, FSH, testosterone, SHBG, DHEAS, 17-hydroxy-progesterone (17-OH-P), and insulin levels were determined using commercially available immunoassay kits. Serum glucose was analyzed using standard methods. E2, progesterone, prolactin, cortisol, TSH, and free T4 were determined as internal controls to confirm the early follicular phase and to exclude other pathologies. In addition, the free androgen index (FAI = testosterone/SHBG x 100) was calculated. Serum AMH was analyzed in kryo-preserved sera using the highly specific DSL-ELISA kit (Sinsheim, Germany). For all assays, the intra- and inter-assay variations were < 6% and < 10% respectively.

In addition to determining baseline serum levels at 0 h, the following endocrine test procedure was performed in all patients and in 25 of the 30 volunteers (for details, see Geisthövel et al. (2)). Briefly, an oral administration of 75 g glucose (Dextro O.G-T.; Roche) was administered after the last baseline blood sampling, and further blood samples were collected 1 and 2 h afterward to determine serum glucose and insulin (oral glucose loading test) levels. The evaluation was performed according to Moltz (23). In addition, the homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated (fasting insulin (mU/ml) x fasting glucose (mmol/l))/22.5). Thereafter, 250 μg ACTH (Synacthen; Novartis Pharma) was i.v. administered over 3 min, and a last blood sample was collected 1 h later to determine serum 17-OH-P (ACTH test) as an endocrine marker of cytochrome peroxidase 21A2 deficiency.

**Transvaginal ultrasonography**

Ultrasonography of the ovaries using a vaginal ultrasound probe (Sonoline SI 200, Siemens, München, Germany; Logiq 200 Pro Series, General Electrics, Solingen, Germany; Sonoace SA 8000, Marl, Germany) was performed at the time of blood sampling. Based on the current literature regarding ultrasonographic ovarian findings (24, 25) and our own studies (26), an EPO was defined if the maximum (max) ovarian diameter was ≥ 31 mm, and the AFC (4–10 mm follicles/max ovarian area) was ≥ 8; a term that corresponds to the so-called ‘PCO’ (1). The AFC fits in with reports on an excessive number of pre-antral and antral follicles found in ovaries of the so-called ‘PCO’ (27). A further but not obligate condition of the EPO/‘PCO’ was a stroma/total area ratio > 0.33 (28). The presence of EPOs was evaluated on a binary basis whereby the unilateral visualization/individual was considered positive.

**Statistical analysis**

The data analysis was performed using SAS V8 for Windows (SAS Institute, Cary, NC, USA). For the

**Table 1** AMH and primary variables (2, 3) for all patients with functional androgenization (FA) compared with the control group. Data are shown as the mean±s.d. Upper/lower cut-off levels for FA diagnosis were reviewed in Geisthövel et al. (2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cut-off value</th>
<th>FA</th>
<th>Control</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td></td>
<td>178</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>25.5±5.4</td>
<td>30.3±5.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>TBD</td>
<td>11.1±6.7</td>
<td>3.0±2.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LH (U/l)</td>
<td>&gt; 8.4</td>
<td>10.6±5.4</td>
<td>5.4±2.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>&gt; 1.3</td>
<td>2.19±1.12</td>
<td>0.96±0.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>&gt; 2.1</td>
<td>2.58±1.0</td>
<td>1.37±0.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>&lt; 25</td>
<td>30.4±18</td>
<td>51.1±14.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FAI (testosterone/SHBG x 100)</td>
<td>&gt; 5</td>
<td>11.2±7.9</td>
<td>2.9±1.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DHEAS (μmol/l)</td>
<td>&gt; 9.6</td>
<td>6.71±3.2</td>
<td>3.95±1.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>17-OH-P (nmol/l)</td>
<td>&gt; 310</td>
<td>5.63±14.2</td>
<td>2.67±1.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>0 h</td>
<td></td>
<td>10.6±25.1</td>
<td>5.67±1.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1 h</td>
<td></td>
<td>–</td>
<td>69</td>
<td>20</td>
</tr>
<tr>
<td>EPO (n %)</td>
<td></td>
<td>4.8±0.6</td>
<td>4.5±0.3</td>
<td>0.0085</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>&gt; 5.5</td>
<td>6.5±2.2</td>
<td>3.5±0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1 h</td>
<td>&gt; 7.7</td>
<td>5.2±1.6</td>
<td>4.7±1.2</td>
<td>0.0005</td>
</tr>
<tr>
<td>2 h</td>
<td>&lt; 14.4</td>
<td>11±12</td>
<td>5±2.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>&gt; 9.7</td>
<td>98±89</td>
<td>31.2±18.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>0 h</td>
<td>&gt; 2.2</td>
<td>81±115</td>
<td>26.7±15.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>&gt; 24</td>
<td>27.0±6.5</td>
<td>21.1±1.8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

AMH, anti-Müllerian hormone; SHBG, sex hormone-binding globulin; FAI, free androgen index; 17-OH-P, 17-hydroxy-progesterone; BMI, body mass index; TBD, to be determined. *The P value was derived from Wilcoxon rank sum test. To compare the presence of enlarged polyfollicular ovaries (EPO), Fisher’s two-sided exact test was used.
variable ‘age’; the unpaired t-test was performed. All other numeric variables could not be assumed to be normally distributed; therefore, the Wilcoxon rank-sum test was used. Fisher’s two-sided exact test was applied to compare categorical variables. A value of $P < 0.05$ was considered statistically significant. To predict the variables of interest, linear and multiple linear regression analyses were carried out. In the multiple regression analysis, the backward selection procedure at the level 0.1 was used. Receiver operating characteristic (ROC) curves were determined to estimate the predictive value of AMH regarding the diagnosis ‘FA’ or ‘FAS I or FAS III’.

**Results**

The analysis of AMH and primary variables determined in FA patients and the control group are presented in Table 1 showing significant differences in all variables. Levels of AMH were increased 3.7-fold in FA patients versus controls.

Detailed analysis of all variables in the five FA groups is shown in Table 2. In the FAS I group, AMH was significantly elevated compared with other groups ($P < 0.0001$). Patients with FAS III had significantly higher AMH levels than those in the FAS II ($P < 0.0001$), IV ($P = 0.0025$) and FCA ($P = 0.014$) groups and controls ($P < 0.0001$). The distribution pattern of AMH concentration among the FA groups was similar to that of LH, testosterone, and EPO with the exception of testosterone in FAS II patients (Fig. 1).

Circulating AMH significantly and positively correlated ($P < 0.0001$) with LH and testosterone (Fig. 2), furthermore with the LH/FSH ratio ($R = 0.527, n = 209$). In multivariate regression analysis, LH and testosterone were related to AMH ($P < 0.0001$ and $P = 0.0056$ respectively), whereby the variability of AMH is 30% explained by LH and 15% by testosterone. However, in linear regression and multivariate analyses, AMH was not dependent on SHBG, DHEAS, BMI, glucose, insulin, or the HOMA index respectively. In addition, there was no correlation between LH and BMI ($r = 0.05, P = 0.5$).

The ROC analysis, an AMH level $> 9.21$ ng/ml predicts the diagnosis ‘FAS I or FAS III’ with a specificity of 90% and a sensitivity of 71.2% (Fig. 3). The approximate area under the curve was 0.886. Patients were significantly younger than controls (Table 1), but as shown in Fig. 4, there was no correlation between AMH and age in patients with FA ($r = 0.09$). However, a significant negative correlation among these variables was observed in the control group ($r = 0.44$). Age-adjusted ROC analysis did not show relevant differences (data not shown); therefore, we present ROC analysis without age adjustment.

The comparison of AMH levels in the subgroups a and b from FAS I, II, III, and FCA showed no significant differences among the sub-groups for FAS I

### Table 2 Variables with significant differences between the FA groups and controls. All data are shown as the mean±S.D.

<table>
<thead>
<tr>
<th>Variable</th>
<th>FAS I</th>
<th>FAS II</th>
<th>FAS III</th>
<th>FAS IV</th>
<th>FCA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>51</td>
<td>27</td>
<td>74</td>
<td>16</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.6±3.9</td>
<td>25.5±7.0</td>
<td>26.2±5.7</td>
<td>22.1±4.4</td>
<td>24.5±5.7</td>
<td>30.3±5.2</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>15.6±5.8</td>
<td>5.05±3.01</td>
<td>11.6±6.6</td>
<td>6.9±4.6</td>
<td>7.0±3.8</td>
<td>3.0±2.0</td>
</tr>
<tr>
<td>LH (U/l)</td>
<td>14.0±4.9</td>
<td>5.7±3.4</td>
<td>11.3±4.6</td>
<td>8.7±5.1</td>
<td>4.7±1.6</td>
<td>5.4±2.1</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>2.7±1.0</td>
<td>1.1±0.65</td>
<td>2.5±1.1</td>
<td>1.8±0.82</td>
<td>1.1±0.33</td>
<td>0.96±0.39</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>2.85±0.8</td>
<td>2.4±1.2</td>
<td>2.76±1.0</td>
<td>1.99±0.89</td>
<td>1.37±0.46</td>
<td>1.37±0.4</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>45.2±21.7</td>
<td>26.9±10.1</td>
<td>22.5±11.4</td>
<td>19.8±10.6</td>
<td>38±13.6</td>
<td>51±14.4</td>
</tr>
<tr>
<td>FAI</td>
<td>8.04±1.7</td>
<td>9.3±3.9</td>
<td>15.2±9.8</td>
<td>11.46±4.5</td>
<td>4.0±1.7</td>
<td>2.9±1.3</td>
</tr>
<tr>
<td>DHEAS(μmol/l)</td>
<td>5.5±2.4</td>
<td>10.3±3.2</td>
<td>6.6±3.0</td>
<td>6.8±3.3</td>
<td>5.1±1.5</td>
<td>3.95±1.61</td>
</tr>
<tr>
<td>17-OH-Prog (nmol/l)</td>
<td>4.5±2.17</td>
<td>26.9±10.1</td>
<td>22.5±11.4</td>
<td>19.8±10.6</td>
<td>38±13.6</td>
<td>51±14.4</td>
</tr>
<tr>
<td>Glucose (nmol/l)</td>
<td>5.5±1.8</td>
<td>10.9±36</td>
<td>4.7±1.8</td>
<td>3.9±2.1</td>
<td>2.7±0.9</td>
<td>2.7±1.1</td>
</tr>
<tr>
<td>1 h</td>
<td>8.2±2.7</td>
<td>22±63</td>
<td>9.4±4.8</td>
<td>7.9±2.4</td>
<td>6.7±2.1</td>
<td>5.7±1.8</td>
</tr>
<tr>
<td>EPO (%)</td>
<td>100 (1a,c)</td>
<td>22.2 (2e)</td>
<td>100 (3c–e)</td>
<td>18.75 (4e)</td>
<td>54.6</td>
<td>20</td>
</tr>
<tr>
<td>0 h</td>
<td>4.7±0.4</td>
<td>4.6±0.6</td>
<td>4.9±0.8</td>
<td>4.7±0.3</td>
<td>4.7±0.4</td>
<td>4.5±0.3</td>
</tr>
<tr>
<td>1 h</td>
<td>5.6±1.6</td>
<td>6.5±2.1</td>
<td>7.2±2.5</td>
<td>6.5±1.7</td>
<td>4.9±1.9</td>
<td>3.5±0.7</td>
</tr>
<tr>
<td>2 h</td>
<td>4.8±1.2</td>
<td>4.9±1.8</td>
<td>5.6±1.8</td>
<td>5.7±0.9</td>
<td>4.9±1.9</td>
<td>4.7±1.2</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>0 h</td>
<td>5.7±2.1</td>
<td>9.7±7.79</td>
<td>16±16</td>
<td>13.2±12</td>
<td>6.5±3.1</td>
</tr>
<tr>
<td>h</td>
<td>50.4±20</td>
<td>89±84</td>
<td>137±104</td>
<td>117±96</td>
<td>48.3±23</td>
<td>31.2±18</td>
</tr>
<tr>
<td>2 h</td>
<td>30±18</td>
<td>65±92</td>
<td>124±155</td>
<td>92±61</td>
<td>42±0.18</td>
<td>26.7±15</td>
</tr>
<tr>
<td>HOMA-IR index*</td>
<td>1.2±0.5</td>
<td>2.1±1.8</td>
<td>3.6±3.9</td>
<td>2.7±2.3</td>
<td>1.4±0.8</td>
<td>1.0±0.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8±2.0</td>
<td>26.7±7.0</td>
<td>30.7±6.2</td>
<td>28.7±5.7</td>
<td>23.8±3.1</td>
<td>21.1±1.8</td>
</tr>
</tbody>
</table>

$P$ values (given in brackets) ranged from $< 0.05$ to $< 0.0001$ and were derived from Wilcoxon rank sum test and Fisher’s two-sided exact test (EPO). FCA, functional cutaneous androgenization; FAS, functional androgenization syndrome; AMH, anti-Müllerian hormone; SHBG, sex hormone-binding globulin; FAI, free androgen index calculated as: testosterone/SHBG x 100; 17-OH-Prog, 17-hydroxy-progesterone; EPO, enlarged polyfollicular ovaries; HOMA-IR, homeostasis model assessment of insulin resistance; BMI, body mass index. *(Insulin 0 h) x (Glucose 0 h)/22.5. Description of significant differences between groups: 1: FAS I vs other groups (1a: FAS II; 1b: FAS III; 1c: control; 1d: FAS IV; 1e: FCA); 2: FAS II vs other groups (2b: FAS III; 2c: control; 2d: FAS IV; 2e: FCA); 3: FAS III vs other groups (3c: control; 3d: FAS IV; 3e: FCA); 4: FAS IV vs other groups (4c: control; 4e: FCA); 5: FCA vs control.

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The analysis of AMH confirms the logistic stratification of the functional androgenization groups and the controls. Detailed statistical analysis is shown in Table 2. C, control; FCA, functional cutaneous androgenization; FAS, functional androgenization syndrome I, II, III, and IV.

(a \(n=42\)); 15.4 ± 4.9 ng/ml; b \(n=9\): 16.2 ± 9.1 ng/ml), FAS III \(a \(n=22\): 11.4 ± 6.5 ng/ml; b \(n=52\): 11.8 ± 6.7 ng/ml), and FCA \(a \(n=6\): 6.1 ± 2.4 ng/ml; b \(n=5\): 8.1 ± 5.1 ng/ml), whereas subgroup FAS IIa \(n=13\): 3.7 ± 3.0 ng/ml) was significantly different \((P=.02)\) from FAS IIb \(n=14\): 6.3 ± 2.5 ng/ml).

Discussion

The analysis of AMH confirms the logistic stratification of the novel FA classification \((2, 3, 5–8)\). Groups FAS I and III where EPOs were present by definition revealed the highest AMH levels (Table 2), and the distribution of AMH levels among the FA groups was similar to the distribution of EPOs and LH (Fig. 1) and the LH/FSH ratio (Table 2). The strong positive correlation among AMH and LH, LH/FSH ratio, and testosterone in the linear regression analysis as well as the results of the multivariate analysis provide further evidence that AMH is a specific marker of ovarian pathology in women diagnosed as FAS I and III. An EPO, with its increased number of pre-antral and antral follicles \((27)\), has a higher granulosa cell mass. In vitro, these granulosa cells produce more AMH than cells from controls \((13)\), increasing the AMH concentration in the follicular fluid \((19)\). Consequently, the perception ‘EPO’ resembling the term ‘PCO’ \((10)\) does not reflects, in fact, polycystic but polyfollicular, endocrinologically highly active ovaries \((1)\).

Groups FCA and FAS II showed elevated AMH levels compared with the control as well; although by definition, the basic pathology is either cutaneous or adrenal respectively. However, the FCAb subgroup (described in detail in \((2)\)) may be characterized through a non-classical feature, namely the presence of EPO and a normo-androgenemic status. In the FAS II group, subgroup b had significantly higher AMH levels and may present with EPO constituting an adrenal-ovarian miscellaneous group \((2)\). In addition, patients from groups FCA and FAS II were significantly younger than the control (Table 2, Fig. 4), which explains in part higher AMH levels \((29)\).

The FAS II group had elevated testosterone concentrations too, which were derived from an increased adrenal \(C_{19}\) sex steroid secretion, but normal LH levels. This constellation explains the much stronger correlation between AMH and LH versus AMH and testosterone, and the higher prediction rate of LH versus testosterone for AMH variability. These observations further underline the FA classification concept, which distinguishes between predominantly ovarian (FAS I and III), adrenal (FAS II), and cutaneous (FCA) pathologies \((2, 3, 5, 7)\).

According to the ROC analysis, AMH is a specific variable for diagnosing FAS I as well as FAS III (Fig. 3). It will, therefore, be included as a further primary variable in the FA classification system. Comparable AMH threshold values were obtained for the diagnosis ‘PCOS’ \((30)\), with AMH being analyzed by Immunotech ELISA (Beckman Coulter, Marseille, France). Especially, in virgins (e.g. peri- or postpubertal girls) or religious women in whom a vaginal sonographic examination is often impossible or in obese women in whom the resolution in the cul-de-sac may be limited, AMH has become an additional diagnostic tool as previously suggested for ‘PCOS’ \((30)\). Further studies and clinical observation will prove its usefulness. Currently, the main problem with its application consists of the use of different immunoassays (Immunotech, DSL/Gen II) with individual standard curves that provide AMH concentrations that vary by factors of 0.75–1.3 (our unpublished results, data from DSL). Consequently, it will be impossible to uniformly apply the results of one ROC analysis. Hopefully, one ELISA will be available in the near future, making the standardization and comparison of results possible.

![Figure 1](https://via.placeholder.com/150)

**Figure 1** Concentration of anti-Müllerian hormone (AMH), LH, and testosterone (shown as the median) and the presence of enlarged polyfollicular ovaries (EPO as a percentage of the total) in the functional androgenization groups and the controls. Detail statistical analysis is shown in Table 2. C, control; FCA, functional cutaneous androgenization; FAS, functional androgenization syndrome I, II, III, and IV.

![Figure 2](https://via.placeholder.com/150)

**Figure 2** Linear regression analysis of anti-Müllerian hormone (AMH) with LH (left panel) and testosterone (right panel). AMH correlated significantly \((P<.0001)\) and positively with LH \((r=0.538, n=209)\) and T \((r=0.368, n=209)\) respectively.
A functional androgenization syndrome.

FA, functional androgenization; FAS, functional androgenization syndrome.

An important role of AMH most probably is the regulation of growth and selection of early follicles in an inter- and intra-follicular manner (reviewed in (15)) like other well-known growth factors. Elevated AMH levels in FA patients appear to derive not only from the increased granulosa cell mass but also from the increased AMH secretion by these cells. In vitro, cells from 'PCOS' women secreted 7.5 times more AMH than cells from controls, and the addition of LH further augmented AMH production in these cells (13). High systemic LH levels are a primary variable in FAS I and III, and very probably a secondary phenomenon (2), which might consequently further augment local AMH synthesis by granulosa cell stimulation, possibly resulting into a vicious cycle of disturbed follicular recruitment. Accordingly, LH was found to be a strong determinant of AMH in our study, and in four subgroups of patients with 'PCOS' (31). In clinical practice, 'PCOS' patients with LH levels $>10$ U/l show little or no response to clomiphene stimulation, and AMH may predict the response to clomiphene citrate stimulation in these women (32). Androgen priming in a specific stimulation protocol increased the AMH concentration in follicular fluid (22), fitting in with the positive correlation between AMH and testosterone in this and earlier studies (17, 31, 33). However, to date, there is insufficient knowledge about the specific role of AMH in human ovaries and its relationship to LH and androgens, as most in vitro studies used animal models or granulosa cells from IVF cycles, which are not ideal models for the study of native granulosa cell function.

According to the results presented here, AMH seems to be specifically related to ovarian pathology, because there was no correlation found in the linear regression or multivariate analysis with adrenal (e.g. DHEAS) or metabolic parameters (e.g. BMI, glucose, or insulin). Previous studies described various and partly contradictory correlations between AMH and these variables in uni- or multivariate analyses, most probably resulting from different study populations (age, race) and classification systems (RC, two and/or three variables present) (17, 20, 21, 31, 34, 35). The FAS Ia patients who are – per definition – lean and predominantly show an ovarian dysfunction presented with significantly higher AMH levels than FAS IIa patients who are characterized by ovarian as well as anthropometric and metabolic alterations. A recent study analyzing four 'PCOS' subgroups observed highest AMH levels in a subgroup 'severe PCOS' that was defined by the presence of oligo-/anovulation, hyperandrogenemia, and EPO, whereby AMH was significantly lower in obese patients than in normal-weight women (31). Although applying different classification systems, these data show in principle the same results as we have shown here. Further studies with exact definitions of study groups are necessary to elucidate these important aspects. Particularly, pure subgroups such as FAS Ia, FAS IIa, or FAS IIa are ideal models for further research in etiology and pathogenesis of FA.

Because of the presence of EPOs, women belonging to either FAS I or FAS III need to be considered fertile with regard to their ovarian reserve (33–35). This is further underlined by a recent study calculating an age-related nomogram for AFC (36): Women with an AFC $>50$th percentile seem to have a slower decline in the AFC until the age of 35 years compared with women belonging to the lower percentiles. In women with regular menstrual cycles, there is a strong negative correlation between age and AMH levels (37). We observed this correlation in the control group despite the relatively small sample size (Fig. 4, right panel), but no correlation was found in the much larger group of women with FA (Fig. 4, left panel) in accordance with an earlier study in 'PCOS' women with a mean age of 27.4 years (17). However, in a group of 'PCOS' patients with a mean age of 31 years, a negative correlation between AMH and age was found (33). This discrepancy might be explained by delayed ovarian aging in these patients (36, 38), which is a very important aspect of treatment and counseling in
reproductive medicine. Patients with FAS I and FAS III probably have a very high basic ovarian reserve that lasts for a longer time in a woman’s lifetime (36, 38, 39). However, the chance of pregnancy seems to be more closely related to a woman’s age than ovarian reserve in ‘PCOS’ patients (40). In women presenting with FAS II, FAS IV, or FCA, it can be assumed that the ovarian response fits their age and medical history.

In conclusion, AMH was shown to be a useful parameter for FA classification and will, therefore, be added as a primary variable in this stratification system. Our results suggest that AMH is primarily a marker of ovarian function and not associated with other organ pathologies such as adrenal gland dysfunction or metabolic disturbances.

Declaration of interest

B Wetzk and F Geisthovel received honoraria for lectures and travel grants from MSD/Essex Pharma, Munich, Germany.

Funding

This study was supported by MSD/Essex Pharma who did not influence the design, evaluation, or publication of the study.

Acknowledgements

We are thankful to Marina Tetyusheva (Dr Köhler GmbH Freiburg, Germany) for her great support regarding the statistical analyses. Parts of the study were presented at the ESHRE Annual Meeting in Rome, June 2010.

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Received 21 April 2011
Accepted 19 May 2011