Predictive markers for the FSH sensitivity of women with polycystic ovarian syndrome

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STUDY QUESTION: Do parameters which are involved in the pathogenesis of polycystic ovarian syndrome (PCOS) predict the dosage of recombinant FSH required to achieve monofollicular development for ovulation induction?

SUMMARY ANSWER: Anti-Müllerian hormone (AMH) appeared to be an independent predictor of the required dosage of FSH to achieve monofollicular development for ovulation induction in a study sample of clomiphene-resistant PCOS patients.

WHAT IS KNOWN ALREADY: AMH plays a key role in the pathogenesis of PCOS. This is the first study that has evaluated the association between AMH and the required FSH dosage to achieve the development of a large follicle of at least 18 mm, in the presence of additional predictors of ovarian responsiveness. In the few studies to date which have evaluated predictors of ovarian responsiveness in PCOS patients, fasting insulin has been shown to be a significant predictor.

STUDY DESIGN, SIZE, DURATION: A total of 48 infertile PCOS patients aged 18–43 years were enrolled in this prospective, observational study between 2009 and 2013. Study participants received between one and six cycles of ovarian stimulation with recombinant FSH using a step-up protocol. The mean total FSH dosage per cycle for reaching a monofollicular development for ovulation induction was evaluated to investigate its association with AMH, LH, FSH, LH/FSH-ratio, sex hormone-binding globulin (SHBG), androstenedione, testosterone, free testosterone index, antral follicle count, ovarian volume, body mass index (BMI) and the age of patients.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We used AMH-Gen-II ELISA (Beckman Coulter, Immunotech, Webster, TX, USA) for the assessment of AMH levels. Crude and multiple linear regression models were fitted to explore potential predictors of the required FSH dosage.

MAIN RESULTS AND THE ROLE OF CHANCE: An interquartile range (IQR) increase in AMH was associated with a 51.4% [95% confidence interval (CI): 24.7–79.0%; \( P = 0.0003 \)] increase in the mean total FSH dosage per cycle (in IU) in a crude regression model, corresponding to a 7.2% increase in the mean total FSH dosage per cycle per ng/ml AMH. Adjustment for BMI augmented the effect of AMH, with a 58.3% (95% CI: 33.2–84.2%; \( P = 1.8 \times 10^{-5} \)) increase in FSH dosage per IQR AMH (corresponding to an 8.2% increase per ng/ml AMH) and a 46.2% (95% CI: 16.5–76.6%; \( P = 0.003 \)) increase per IQR BMI (corresponding to a 3.7% increase per kg/m²). AMH was the only independent variable for which the effect on FSH dosage was statistically significant in the crude regression model as well as after adjustment for other promising predictors. The association of BMI with FSH dosage was statistically significant while adjusted for AMH, but not in the crude model.

LIMITATIONS, REASONS FOR CAUTION: The impact of metabolic parameters such as insulin resistance on the reported association between AMH and FSH dosage was not assessed.

WIDER IMPLICATIONS OF THE FINDINGS: Knowledge about the predictors of ovarian sensitivity to FSH can facilitate a physician’s decision-making in providing the optimal infertility therapy for PCOS patients.

STUDY FUNDING/COMPETING INTERESTS: Funding was provided by the University Hospital of Essen.

Key words: polycystic ovarian syndrome / recombinant FSH / ovulation induction / anti-Müllerian hormone

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Introduction

The polycystic ovary syndrome (PCOS) affects up to 8% of women of reproductive age and is the most frequent endocrine disorder observed in this population (Michelmore et al., 1999; Azziz et al., 2004). PCOS is defined by the presence of two of the three following diagnostic criteria: hyperandrogenism, polycystic ovaries with ≥12 sonographically measured small follicles between 2 and 9 mm and/or an ovarian volume >10 ml and oligo-/anovulation (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

Anti-Müllerian hormone (AMH) is a highly reliable parameter of PCOS diagnosis with high sensitivity and specificity as recently shown in a meta-analysis by Iliodromiti et al. (2013). It is produced by the granulosa cells of small antral and pre-antral follicles and reflects the pool of these follicles. It correlates better than other hormonal factors with the severity of PCOS and the arrested follicle pool (Fanchin et al., 2003; Pigny et al., 2003, 2006; Dewailly et al., 2011). AMH inhibits the initiation of follicle growth and the FSH-dependent selection process (Durlinger et al., 2001, 2002; Knight and Glicer, 2006). Additionally, it acts as an FSH-antagonizing parameter and seems to induce the follicular arrest in an ‘auto-inhibiting effect’ (Jaron and Dewailly, 2004).

Anovulation leads to sterility. The first-line therapy consists of anti-estrogens such as clomiphene citrate. In the case of clomiphene resistance, recombinant FSH or human menopausal gonadotrophin (HMG) is recommended in daily injections until sufficient follicle development is reached for ovulation induction (Homburg and Howles, 1999).

The individual response to a standardized stimulation dosage pattern varies strongly. There are only a few published studies that describe predictive factors of ovarian sensitivity of PCOS patients to gonadotrophins. In a study by Homburg et al., fasting insulin was concluded to be a predictor for the number of HMG ampoules required for ovulation induction (Homburg et al., 1996). Laven et al. examined the relationship between AMH and the FSH ampoules needed for ovulation induction as well as the duration of stimulation in WHO 2 anovulatory patients and found no association (Laven et al., 2004). Some further studies evaluated predictive factors for ovarian sensitivity with different study designs. Mulders et al. showed that androstendione and the antral follicle count (AFC) predict mono- or multifollicular development in WHO 2 anovulatory patients with and without PCOS (Mulders et al., 2003). Some further studies evaluated predictive factors of the ovarian FSH-responsive dose, defined as the daily dose of exogenous FSH to reach follicle development of 10 mm diameter, in WHO 2 anovulatory patients and observed correlations with body mass index (BMI) and β-cell-function parameters (Imani et al., 2002).

The purpose of our study was to examine whether parameters such as AMH, age, BMI, LH, LH/FSH ratio, androgens and sonographic aspects predict the FSH dosage required to achieve monofollicular development for ovulation induction in women with PCOS. It is hypothesized that these factors could act as surrogate markers of the ovarian sensitivity in PCOS patients and thus may give new insights into the disturbed follicle maturation related to PCOS. To our knowledge, this is the first study that examines the prediction of FSH dosage by AMH in the presence of additional diagnostic features such as androgens, endogenous gonadotrophins and sonographic aspects in PCOS patients.

Materials and Methods

Study population

A total of 48 women aged 18–43 years and diagnosed with PCOS-related sterility were included consecutively. All patients were treated in the University Hospital of Essen, Germany, Department of Gynecology and Obstetrics, between 2009 and 2013. Informed written consent was obtained from all women. The study was approved by the Local Research Ethics Committee (no. 11-4688). Diagnosis of PCOS was performed according to the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004). Oligomenorrhea was defined as cycles lasting longer than 35 days. Amenorrhea was defined as cycles lasting longer than 3 months. Clinical or biochemical signs of hyperandrogenism were confirmed in case of a Ferriman–Gallway score >7 (Ferriman and Gallway, 1961) or obvious acne or alopecia (Ludwig, 1977) or an elevated total testosterone (normal range 0.5–2.6 nmol/l) and/or DHEAS (normal range 6–123 μg/dl) and/or androstendione (normal range 0.3–3.3 ng/ml). Endocrine variables were determined between the second and fifth day of menstrual cycle or after artificial bleeding induction in cases of amenorrhea. Free testosterone index (FTI) was calculated as [(total testosterone/SHBG) × 100]. BMI was calculated as [weight/(height)²]. 21-Hydroxylase deficiency was excluded with an ACTH test and genetic testing. In all patients, an oral glucose tolerance test with 75 g glucose was performed before starting metformin therapy. Patients with pituitary, adrenal, other ovarian diseases or confirmed adenogenital syndrome were excluded. None of the participants had taken hormonal contraceptives at least 3 months before entering in the study. The patients in our study failed to ovulate or conceive after six cycles with clomiphene therapy with a maximal dosage of 150 mg daily. Thus, exogenous FSH therapy was indicated. To exclude other causes of sterility, sperm analysis and sonographic hysterosalpingography or laparoscopy were performed.

Transvaginal scan

All patients were examined by the same experienced physician to avoid inter-observer variance. Real-time ultrasound measurements were taken using a 7-MHz transducer (Sonoline Elegra, Siemens Ultrasound Division; Voluson E8, General Electric Systems; IU22, Philips Healthcare). Ovarian volume was obtained by measuring the greatest diameter in every plane. The algorithm for a prolate ellipsoid \( V = \frac{4}{3} \pi x y z \) was used for this purpose (Balen et al., 2003). For the determination of the AFC, we calculated small follicles with a diameter between 2 and 9 mm, following the recommendations of Balen et al. (2003). Women with follicles >10 mm or any kind of ovarian masses were excluded.

Study design

In this prospective, observational single-centre study, all features of PCOS used in the analyses, including AMH, LH, FSH, LH/FSH-ratio, SHBG, androstendione, total testosterone, FTI, AFC, ovarian volume, BMI and age of patient, were determined before the beginning of the FSH therapy. A low-dose step-up protocol starting with 25 IU recombinant FSH (Puregon®, MSD Sharp and Dohme GmbH, Haar, Germany) was given to every woman. Every woman had a transvaginal scan between Day 2 and Day 5 of menstrual bleeding before starting the FSH therapy. The therapy was initiated only if there were no cysts >10 mm and if the endometrium was smaller than 5 mm in diameter. Follicular development was controlled after 7 days of FSH therapy (Homburg and Howles, 1999). The daily dosage was elevated every 7–10 days with steps of 25 IU until reaching a follicle of at least 18 mm in diameter and an endometrium diameter of at least 7 mm according to White et al. (1996). We used the sonographic parameters to...
assure sufficient follicular development and estrogen production. Subsequently, 5000 units HCG (Predalon® MSD Sharp and Dohme GmbH) or Choriongonadotropin alpha 250 μg (Ovitrelle® MecRichGermo GmbH) was given to induce ovulation. Luteal support with micronized progesterone or dydrogesterone was administered. In patients who received a starting FSH dosage of 25 IU and failed to have adequate follicular development within 21 treatment days, subsequent cycles were started with a daily dosage of 50 IU. Therapy cycles without adequate follicular development were excluded from the analyses. To ensure the examination of comparable ovarian response patterns, therapy cycles leading to bi- or trifollicular development were also excluded from the study. The study outcome is given by the sum of daily FSH dosages per cycle in IU. In patients who were treated for more than one therapy cycle leading to adequate follicular development, the mean of the total FSH dosage was calculated per cycle. For instance, if a patient received 450 IU (e.g. 8 days with 25 IU per day + 5 days with 50 IU per day) in the first cycle and 550 IU (e.g. 8 days with 25 IU per day + 7 days with 50 IU per day) in the second cycle, a mean total FSH dosage per cycle of 500 IU was used for analysis. The maximal treatment duration was six cycles of adequate follicular development (White et al., 1996). Patients who did not become pregnant after six cycles were eligible for further investigations, e.g. laparoscopy. To avoid a negative impact of high insulin levels on the ovarian response, all patients received metformin (body weight ≤60 kg: 850 mg twice daily; body weight >60 kg: 1000 mg twice daily) before starting the stimulation therapy irrespective of insulin resistance (Legro et al., 2007; Tan et al., 2007). All features of PCOS used in the analyses were assessed before starting metformin therapy.

Sampling of serum
A 27 ml volume of blood was collected with the blood collection system S-Monovette® (Sarstedt AG & Co.) from each woman (18 ml was used for hormonal analysis and 9 ml was stored at 4°C and processed within 4 h to avoid blood cell lysis for AMH analysis). Blood fractionation was carried out by centrifugation for 10 min at 2500 × g. Subsequently, 3–4 ml of the upper phase, constituting blood serum, was removed and subjected to biochemical determination.

Biochemical analysis
Automated chemiluminescence immunoassay systems were used for the determination of LH, FSH, testosterone (ADVIA Centaur, Siemens Healthcare Diagnostics, Eschborn, Germany), androstendione and SHBG (Immulite 2000 XPi, Siemens Healthcare Diagnostics, Eschborn, Germany). Intra-assay variation was <5% and inter-assay variation was <8% for all parameters. For AMH determination, the enzymatically amplified two-site AMH-Gen-II ELISA was applied in serum samples (Beckman Coulter, Immunotech, Webster, TX, USA). AMH concentrations <0.08 ng/ml were considered as undetectable.

Statistical analysis
Characteristics of the study population are given as medians and interquartile ranges (IQR). First, crude linear regression models were fitted to examine the association between all potential predictors assessed in the study population and the FSH dosage needed to achieve monofollicular development for ovulation induction by calculating effect size estimators, their corresponding 95% confidence intervals (95% CI) and the respective P-values separately for each potential predictor. To address the skewed distribution of the response variable, it was loge-transformed prior to analysis. The results were presented as percent change in mean total FSH dosage per cycle per IQR increase of the respective variable to standardize the effect size estimators. Second, a multiple linear regression model was fitted including only promising predictors to assess the significance of their association with FSH dosage adjusted for each other. To avoid overfitting of the regression model, the selection of the variables included was based on avoiding multicolinearity (r ≥ 0.4), favoring those variables with prior evidence for associations from by comparable studies or those which showed the highest standardized effect size estimators revealed by the crude regression analyses.

Third, based on the selection of variables included in the multiple linear regression model, stepwise model selection was performed to identify the most useful subset of predictors for the study outcome by using Akaike’s information criterion as an indicator of model fit.

In addition, all analyses were conducted using the mean duration of stimulation across cycles (in days; median: 12.0; inter quartile range: 9.0–17.4) as the study outcome. As both study outcomes were highly correlated (r = 0.93; P < 0.0001), the results for duration of stimulation differed only slightly from those obtained for FSH dosage and thus were not shown in this report.

All analyses were performed using the R statistical package version 2.15.2 (R Core Team, 2012).

Results
Patient characteristics are shown in Table I. The median age of the study population was 30 years. As expected for women with PCOS, the median BMI was rather high (26.6 kg/m²).

At the beginning of the study, 26 patients were nulligravida, 12 were primigravida and 10 had two or more prior pregnancies. Out of the 22 patients with at least one previous pregnancy, 13 had produced a life birth in the past. Study participants received between one and six cycles of FSH therapy. Overall, 23 patients became pregnant as a consequence of treatment, including 22 patients with a singleton pregnancy and one patient pregnant with twins. There were 7 pregnancies which resulted in miscarriage, one was an extrauterine pregnancy and 15 resulted in a full term life birth. There were no cases of multifollicular development, one case with bifollicular development, and no patients showed ovarian hyperstimulation syndrome. In five patients, no follicular development was seen after 21 days of therapy with a starting dose of 25 IU. All of them showed follicular development with a starting dose of 50 IU in subsequent cycles, suitable for analysis.

Table I Characteristics of the study population (n = 48) given as medians and interquartile ranges.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.0 (25.0–34.3)</td>
</tr>
<tr>
<td>Average FSH dosage per cycle (IU)</td>
<td>450.0 (281.2–781.9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 (22.8–35.3)</td>
</tr>
<tr>
<td>Ovarian volume (ml)</td>
<td>13.0 (10.3–16.1)</td>
</tr>
<tr>
<td>AFC (n)</td>
<td>12.0 (9.0–14.0)</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>1.9 (1.5–2.5)</td>
</tr>
<tr>
<td>FTI (%)</td>
<td>4.7 (3.2–7.3)</td>
</tr>
<tr>
<td>Androstendione (ng/ml)</td>
<td>2.6 (1.8–3.5)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>41.9 (29.5–69.9)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>7.4 (5.1–10.4)</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>5.7 (4.7–7.2)</td>
</tr>
<tr>
<td>Ratio between LH and FSH</td>
<td>1.3 (0.9–2.0)</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>4.7 (2.9–10.0)</td>
</tr>
</tbody>
</table>

AFC, antral follicle count; FTI, free testosterone index; SHBG, sex hormone-binding globulin.
The results of the crude linear regression analyses are shown in Table II. Statistically significant effect size estimators indicating an association with the required mean total dosage of FSH per cycle were observed for the following parameters: AFC, total testosterone and AMH. The mean total FSH requirement per cycle showed a 30.5% increase per IQR increase in AFC (P = 0.04), a 33.4% increase per IQR increase in testosterone (P = 0.04) and a 51.4% increase per IQR increase in AMH (P = 0.0003), respectively. As these predictors showed the highest standardized effect size estimators, they were included in the multiple regression model as well as BMI, which was also based on effect size in addition to prior evidence (Homburg et al., 1996), and age. As for ovarian volume, FTI, androstendione, SHBG, LH and LH/FSH-ratio, either no meaningful effects on FSH dosage were shown in the crude regression models or substantial correlations (r ≥ 0.4) to at least one of the variables selected for the multiple regression model were observed; therefore they were excluded to avoid multicollinearity and overfitting of the regression model. After adjusting for the selected variables, the effect size estimators for the association of total testosterone and AFC with FSH dosage appeared considerably diminished without showing statistical significance (Table III). However, the effect size estimator for the association of AMH with FSH dosage was elevated compared with the crude regression model, showing a 58.2% increase in the mean total FSH dosage per cycle per IQR increase in AMH (P = 0.002), corresponding to an 8.2% increase in the mean total FSH dosage per cycle per ng/ml AMH. In addition, a statistically significant increase in FSH dosage of 46.6% was observed per IQR increase in BMI (P = 0.006), corresponding to a 3.7% increase per kg/m². Thus, AMH and BMI were the only independent variables in the multiple regression analysis for which the effect on FSH dosage was statistically significant after adjustment for other potential predictors.

Stepwise model selection revealed that BMI and AMH mostly contributed to the model fit. Based on this final model, a 46.2% (95% CI: 16.5 – 76.6%; P = 0.003) increase in the mean total FSH dosage per cycle was observed per IQR increase in BMI, corresponding to a 3.7% increase per kg/m². The effect size estimator for AMH still showed a 58.3% (95% CI: 33.2 – 84.2%; P = 1.8 × 10⁻⁵) increase in the mean total FSH dosage per cycle per IQR increase in AMH, corresponding to an 8.2% increase per ng/ml AMH. The total variance explained by BMI and AMH was 38.8%, of which 31.2% was attributed to AMH and 7.6% to BMI.

### Table II Effect size estimators [percent change per interquartile range (IQR) of the respective variable], 95% confidence intervals (95% CI) and P-values of the crude linear regression models for potential predictors reporting the association with FSH dosage.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimated percent change per IQR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>–21.9</td>
<td>(–57.2 to 14.9)</td>
<td>0.23</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.9</td>
<td>(–1.2 to 70.0)</td>
<td>0.06</td>
</tr>
<tr>
<td>Ovarian volume (ml)</td>
<td>4.6</td>
<td>(–18.8 to 28.9)</td>
<td>0.70</td>
</tr>
<tr>
<td>AFC (n)</td>
<td>30.5</td>
<td>(0.8 to 61.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>33.4</td>
<td>(1.4 to 75.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>FTI (%)</td>
<td>22.3</td>
<td>(–1.1 to 47.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>Androstendione (ng/ml)</td>
<td>5.3</td>
<td>(–22.7 to 38.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>–28.6</td>
<td>(–59.6 to 2.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>17.7</td>
<td>(–3.7 to 40.0)</td>
<td>0.10</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>25.5</td>
<td>(–2.7 to 56.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Ratio between LH and FSH</td>
<td>–8.9</td>
<td>(–25.3 to 10.5)</td>
<td>0.34</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>51.4</td>
<td>(24.7 to 79.0)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

AFC, antral follicle count; FTI, free testosterone index; SHBG, sex hormone-binding globulin.

### Table III Effect size estimators [percent change per interquartile range (IQR) of the respective variable], 95% confidence intervals (95% CI) and P-values of the multiple linear regression model adjusted for all promising predictors reporting the association with FSH dosage.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimated percent change per IQR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>–13.9</td>
<td>(–45.3 to 18.6)</td>
<td>0.39</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>46.6</td>
<td>(14.1 to 80.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>AFC (n)</td>
<td>–6.3</td>
<td>(–36.3 to 25.6)</td>
<td>0.68</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>3.6</td>
<td>(–20.9 to 35.7)</td>
<td>0.79</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>58.2</td>
<td>(22.7 to 95.5)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

AFC, antral follicle count.

### Discussion

In a prospective cohort of clomiphene-resistant PCOS patients, we examined potential predictors of the required mean total FSH dosage, i.e. the average sum of daily injected FSH IU per cycle, necessary to obtain monofollicular development sufficient for induction of ovulation. To our knowledge, this is the first study examining predictive factors for ovarian sensitivity to FSH, such as AMH, androstenedione, FTI and sonographic aspects in PCOS patients. The median level of AMH in the study population was 4.7 ng/ml (IQR 2.9 – 10.0), which is comparable with the results obtained for clomiphene citrate nonresponders in a recently
published study (Mahran et al., 2013). The main finding of our study is the association of AMH with FSH dosage, confirming the importance of AMH sensitivity in the diagnosis of PCOS (Pigny et al., 2006; Iliodromiti et al., 2013). In linear regression analyses, AMH was the only independent variable that reached a statistically significant association with the mean total FSH dosage per cycle in the crude regression model as well as after adjustment for other potential predictors under investigation. After applying stepwise regression, BMI and AMH were selected as the most useful subset of predictors. While adjusted for each other, both predictors showed elevated effect size estimators compared with those obtained by crude regression models. The results of the multiple regression analysis suggest that the association of AFC and total testosterone with FSH dosage observed in the crude regression models could be largely explained by correlated variables additionally included in the model, such as AMH. Out of the variables under investigation, AMH seems to be the most significant independent predictor for the FSH dosage needed to reach monofollicular development for ovulation, showing on average an 8.2% increase in the mean total FSH dosage per cycle for each additional ng/ml of AMH as assessed before FSH therapy and explaining about one-third of the outcome’s variance while controlling for BMI.

A comparable study by Laven et al. examined the association of AMH with FSH dosage and duration of therapy to reach follicular development in a cohort of 79 clomiphene-resistant, anovulatory women classified as World Health Organization (WHO) class 2 patients (Laven et al., 2004). In this study population, no association between AMH and the FSH response dose, the number of needed ampoules or therapy duration was reported regardless of PCOS status. One important difference is that Laven et al. did not examine the relationship between AMH and FSH dosage in PCOS patients exclusively. Our results suggest that AMH is more predictive for ovarian sensitivity in PCOS patients than in the more heterogeneous group of WHO 2 patients.

AMH seems to play a key role in follicle maturation failure in PCOS patients (Pigny et al., 2003). It is elevated in PCOS patients and the AMH receptor is overexpressed in granulosa cells of polycystic ovaries (Catteau-Jonard et al., 2008). Granulosa cells of polycystic ovaries produce much more AMH than those in normal ovaries, suggesting that follicle maturation and ovulation in PCOS patients might be inhibited. Some authors have concluded that in PCOS the arrested follicles in themselves produce more AMH as a consequence of the disturbed maturation process (Cook et al., 2002). Furthermore, aromatase activity has been found to be inhibited by AMH, leading to higher androgen production (di Clemente et al., 1992; Pellatt et al., 2011). AMH correlates with testosterone (Pigny et al., 2006; Wetzka et al., 2011), androstenedione (Pigny et al., 2003) and LH (Wetzka et al., 2011; Nardo et al., 2009, 2009). As shown by Pellatt et al., AMH reduces FSH receptor expression in vitro, suggesting an inhibited FSH action on granulosa cells (Pellatt et al., 2011). A recent study of Grynberg et al. showed that FSH action on AMH expression in human granulosa cells seems to be an estradiol-mediated procedure (Grynberg et al., 2012), as an FSH-induced estradiol increase during follicular development led to AMH down-regulation through estrogen receptor (ER)β. However, in PCOS patients reduced expression of ER-β is supposed (Jakimik et al., 2002), possibly leading to increased AMH levels. Hence, it is not surprising that AMH is a strong predictive factor not only for the severity of PCOS, but also for ovarian sensitivity.

In another comparable study, Homburg et al. showed a significant correlation of fasting insulin and BMI with the required ampoules of HMG to achieve a follicular development to at least 17 mm for ovulation induction (Homburg et al., 1996). After adjusting for additional parameters, only fasting insulin showed a significant association with HMG. No association of the number of ampoules needed with SHBG, LH, FSH and testosterone was observed, as shown in our study. Based on these results, the authors concluded that insulin is responsible for intraovarian disturbance, reflected by its determination as an independent parameter of ovarian resistance.

Our results indicate the importance of BMI for ovarian sensitivity, even if the total explained variance of FSH dosage attributed to BMI was much smaller than the explained variance attributed to AMH. However, our results for BMI are in accordance with other studies that have shown a negative impact of high BMI levels on ovulation and conception rates in PCOS patients (Rausch et al., 2009; Legro, 2012). As shown by Thomson et al., reproductive function parameters in PCOS patients improved after weight loss in an interventional study. AMH levels measured before starting the intervention and weight loss were independent predictors of the improvement of reproductive function in PCOS patients, without showing associations with insulin resistance parameters (Thomson et al., 2009). Our results are consistent with this finding, although the impact of AMH and BMI seemed to be partly overlapping in our study.

Finally, our finding should be discussed with regard to a model previously used for the prediction of the FSH-responsive dose in anovulatory and clomiphene-resistant women, including BMI, FSH, Insulin-like growth factor 1 and clomiphene resistance (Imani et al., 2002). The application of the model in PCOS patients did not correspond to estimated and observed FSH response doses (Van Wely et al., 2006), but the studies did not investigate the impact of AMH. As our results indicate a higher effect of AMH in the prediction of ovarian sensitivity than BMI and other potential predictors, AMH may offer a more powerful prediction model for PCOS patients.

Although our study covered a broad range of variables, we did not examine metabolic parameters such as fasting insulin and insulin resistance. As all of our patients received metformin, likely influencing the impact of β-cell-function parameters on ovarian sensitivity, it was not possible to assess the effect of β-cell-function parameters on FSH dosage for follicular development in a meaningful way. Additionally, since an impact of metformin on ovarian sensitivity cannot be excluded (Tan et al., 2007), our study results may be relevant to patients taking metformin only.

It should also be mentioned that our study used a starting dosage of 25 IU FSH daily, whereas The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group recommended starting with 37.5–50.0 IU daily. We used the lowest applicable starting dosage of 25 IU daily to avoid overstimulation. The aim of our study, however, was not to evaluate the best daily dosage to achieve monofollicular development, but to find predictors of the ovarian sensitivity. For this reason, a standardized stimulation protocol seemed to be most important for the analysis of associations between total exogenous FSH dosage per cycle and potential predictors.

Because the follicular development appropriate for ovulation induction with HCG was the primary end-point of our study, we did not assure the ovulation, e.g. by progesterin measurements in luteal phase. However, before beginning a cycle of FSH therapy, we consequently ruled out ovarian cysts of > 10 mm diameter with a vaginal scan. Thus, we were able to assure that the growing follicle was a newly recruited...
dominant follicle. The high pregnancy rate (48%) achieved in our study population indicates that ovulation occurred, and is similar to a comparable study of White et al. with a conception rate of 45% (White et al., 1996).

AMH may serve as an independent predictor of ovarian sensitivity to FSH, thus having the potential to facilitate a physician’s decisions regarding the optimal infertility therapy for PCOS patients. Our results suggest that women with high AMH levels at the beginning of the therapy will need a higher FSH dosage. The therapy might be extended when compared with women presenting with lower AMH levels, reflecting the severity of the underlying PCOS. This could mean higher costs and longer therapy duration to achieve monofollicular development. The ladder may have an impact on the frequency of the physicians’ visits. Furthermore, in cases of high AMH values, a higher starting dose should be considered.

Despite the limitations, our study shows that AMH appears to be an independent predictor of the required dosage of FSH to achieve monofollicular development for ovulation induction in a study sample of clomiphene-resistant PCOS patients. Further investigations in larger cohorts, including controls, are recommended to consider all established diagnostic features of PCOS and AMH as well as other potential predictors such as metabolic parameters. This would be required to provide supporting evidence that AMH is an independent predictor for the FSH sensitivity of patients with polycystic ovarian syndrome.

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Authors’ roles

A.K. gave substantial contributions to the study conception and design, acquisition of data and interpretation of data, and drafting of the article, and gave final approval of the version to be published. L.S., P.E. and R.K. gave substantial contributions to acquisition of data and revising of the article critically for important intellectual content and gave final approval of the version to be published. S.K.-B. gave substantial contributions to the study conception and design, drafting of the article and revising of the article critically for important intellectual content, and gave final approval of the version to be published. T.S. and B.S. gave substantial contributions to the analysis and interpretation of data and revising of the article critically for important intellectual content, and gave final approval of the version to be published.

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Conflict of interest

None declared.

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