A very large proportion of young Danish women have polycystic ovaries: is a revision of the Rotterdam criteria needed?

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BACKGROUND: According to the Rotterdam 2003 criteria, an ovary is defined as polycystic if 12 or more follicles of 2–9 mm are present, when evaluating the ovary by ultrasonography on Days 3–5 of the menstrual cycle in women not using hormonal contraceptives. The aim of this population-based study was to estimate the prevalence of polycystic ovaries (PCO) in a representative sample of young Danish women according to the Rotterdam criteria.

METHODS: From a Danish pregnancy cohort established in 1988–1989, 267 (61%) young adult daughters agreed to participate in a clinical examination and 174 (40%) consented to vaginal ultrasound. Sufficient image quality in at least one ovary was obtained from 154 women. Both users and non-users of hormonal contraceptives were included and the examination was not restricted to a particular time of the menstrual cycle.

RESULTS: The median (range) age was 20.1 (19.5–21.0) years. The median follicle number per ovary was 14 (6–30) and 12 or more follicles were counted in 104 of the 154 women. Thus, the prevalence was estimated to 68% [95% confidence interval (CI): 60–74%]. PCO were present in 80% (95% CI: 65–89%) of non-users (n = 44) of hormonal contraceptives. Of the 104 women with PCO, 41% (95% CI: 32–51%) could be defined as having polycystic ovary syndrome.

CONCLUSIONS: A very large proportion of the young women had PCO according to the Rotterdam 2003 criteria. As the number of follicles is higher at a younger age, we believe the Rotterdam criteria should be revised, particularly to avoid misdiagnosis in this age group.

Key words: ovarian follicles / vaginal ultrasound / population-based study / polycystic ovaries / polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is considered to be one of the most common endocrine disorders in women of reproductive age (Franks, 1995; Ehrmann, 2005). It is a condition of ovarian dysfunction and is characterized by hyperandrogenism, anovulation and polycystic ovaries (PCO) resulting in cosmetic problems, menstrual disturbances and infertility. Additionally, PCOS is associated with abdominal obesity, insulin resistance, hypertension and dyslipidemia, and is thereby related to type 2 diabetes and cardiovascular diseases (Ehrmann, 2005).

For several years, there has been debate regarding the diagnostic criteria for PCOS. According to the criteria made by The National Institutes of Health (NIH) in 1990, the diagnosis can be made, when chronic anovulation is present in combination with clinical and/or biochemical signs of hyperandrogenism, and when related disorders have been excluded (Zawadzki and Dunaif, 1992). In 2003, a PCOS consensus workgroup, held in Rotterdam and sponsored by the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), agreed on a revision of the criteria (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Compared with the NIH 1990 criteria, PCO were added as one of the diagnostic features of PCOS. According to the Rotterdam 2003 criteria, an ovary is defined as polycystic when 12 or more
folicles measuring 2–9 mm in diameter are present, and/or there is an increased ovarian volume of more than 10 ml. Only one ovary meeting the criterion is sufficient to define the woman as having PCO. Since hormonal contraceptives modify the ovarian morphology (Falsetti et al., 2001), including a reduction of the number of follicles (Somunkiran et al., 2007), the definition is only applicable for women who are not using contraceptives. Furthermore, no dominant follicle or corpus luteum of more than 10 mm should be present and the ovary should be evaluated in the early follicular phase (Days 3–5) of the menstrual cycle, when the ovary is relatively quiescent (Ballen et al., 2003; The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

Prior to the consensus on the Rotterdam criteria in 2003, Polson et al. investigated the prevalence of PCO in the general British population using the Adam’s criterion on PCO (10 or more cysts measuring 2–8 mm associated with an increase in ovarian stroma). PCO was found in 22% of 257 healthy volunteers at 18–36 years of age (Polson et al., 1988). As seen in the study by Polson et al., the prevalence of PCO often is estimated in a wide age group, not taking into account the decrease in the number of follicles with age (Reuss et al., 1996; Scheffer et al., 1999; Haadsma et al., 2007). Since the definition of a polycystic ovary is partly based on the number of ovarian follicles, it would be expected that the proportion of women with PCO will decrease with increasing age concurrently with a reduction in the number of follicles. Koivunen et al. (1999) found that the prevalence of PCO was 21.6% in women younger than 36 years and only 7.8% in women at 36 years or older.

To our knowledge, only one study has used the Rotterdam 2003 criteria to assess the age-dependent prevalence of PCO: Duijkers and Klipping (2010) estimated the prevalence in 171 healthy volunteers of different age groups in a spontaneous menstrual cycle and found that the prevalence of PCO decreased with age. Among 42 young women aged 18–22 years, 83–84% had PCO compared with 19–33% of 21 women in the age group of 33–37 years. Hence, the prevalence of PCO among young women seemed to be extremely high.

The aim of our study was to evaluate the Rotterdam criterion on follicle number (12 or more follicles measuring 2–9 mm) in young women aged 19–20 years. To the best of our knowledge, this population-based study is the first of its size to focus on this age group.

Materials and Methods

Subjects and protocol

The participants were female offspring from a Danish Pregnancy cohort established in 1988–1989 which included pregnant women scheduled to attend the routine 30th week antenatal visit at a midwifery practice in the city of Aarhus (Olsen et al., 1995). Among eligible pregnant women, 965 (80%) were enrolled. In the present study, a total of 436 young adult daughters were asked to participate (Fig. 1) and 267 (61%) agreed to attend the clinical examinations which were conducted in the time period from August 2008 to August 2009. The daughters provided information on menstrual pattern, menstrual cycle day at the time of examination and current use of hormonal contraceptives, and they were asked to report if they had problems with acne on a daily basis or with male hair growth. Additionally, the presence of acne and hirsutism was evaluated by a single investigator (S.L.K.). The severity of these findings was not graded. Blood samples were obtained to analyze total testosterone and sex hormone-binding globulin (SHBG).

There were 174 young women (40%) who consented to a transvaginal ultrasound (Fig. 1). Because of insufficient image quality, it was not possible to count follicles in at least one ovary in 19 subjects, and due to pregnancy, follicles were not counted in one subject, resulting in the study group to 154 women.

Both users and non-users of hormonal contraceptives were included in the study and for practical reasons, the examination was not limited to a particular time of the menstrual cycle.

The study was approved by the local scientific ethical committee and written informed consent was obtained from all subjects before examination.

Ultrasonographic examination

Two-dimensional ultrasound scans were performed using three different VOLUSON e (GE Healthcare, Zipf, Austria) devices equipped with 3.7–9.3 MHz transvaginal transducers. Prior to the examination, each woman was asked to empty her bladder. The examination was performed with the woman lying on her back with her legs bent on a standard examination couch. The ovary was visualized in whatever plane gave the best image quality, which was optimized by use of high magnification, appropriate frequency and by activating the device feature of automatic optimization. Follicle size was determined from a mean of two perpendicular measurements. Special focus was brought to follicles of size close to the diagnostic limit of 2–9 mm, in which case measurements were performed carefully. Follicles between 2 and 9 mm were counted by scanning from one margin of the ovary to the other. The counting process was performed twice in each ovary. In case of discrepancy between the two counts, evaluation of follicle number was repeated until two equal results were obtained and this value was used for further analysis. Ovarian volume was estimated based on measurements in three perpendicular planes. All examinations were performed by the same investigator (S.L.K.). The counts were performed in cooperation with one of two technicians, who were monitoring the counts in an attempt to ensure that follicles were not missed or counted twice.
Hormonal analysis

Total testosterone was measured in serum by immunoassay (cobas 6000 e 601, Roche Diagnostics, Mannheim, Germany) and SHBG was measured by chemiluminescent immunoassay assay (IMMUNULITE 2000, Siemens Healthcare, Gwynedd, UK). Measurements of total testosterone below the detection limit of 0.2 nmol/l were set to 0.1 nmol/l before statistics were performed. Free testosterone was estimated by calculating the ratio of total testosterone/SHBG.

Defining clinical and biochemical features of PCOS

PCOS was defined by the presence of a polycystic ovary according to the Rotterdam criterion on 12 or more follicles measuring 2–9 mm and (i) oligo-/amenorrhea and/or (ii) clinical hyperandrogenism (acne, hirsutism) and/or hyperandrogenemia.

Oligo-/amenorrhea was diagnosed by menstrual cycle length of more than 35 days. For users of hormonal contraception, menstrual cycle length was based on information on menstrual pattern prior to use of these contraceptives. Acne was defined as present if the woman claimed that acne was a constant problem concurrently with an objective finding of acne at the clinical examination, or if the woman was on prescription medication for acne. Hirsutism was defined as present when the woman claimed to have dark, thick hair on upper lip, chest, abdomen, back or thighs. Hyperandrogenemia was defined as total testosterone and/or testosterone/SHBG-ratio above the 95th percentile (3.4 nmol/l and 0.11, respectively) of those women (n = 17) from the study population of 267 subjects, who had normal menstrual cycle length (21–35 days), no signs of acne or hirsutism, were non-users of hormonal contraceptives and without PCO on vaginal ultrasound.

Statistics

Statistical analyses were performed using Stata software version 11.0 (StataCorp, College Station, TX, USA). The follicle-count from whichever ovary contained most follicles per individual was used for statistical analysis, since only one ovary meeting the criteria is needed to define PCO. The follicle numbers between groups (hormonal contraceptives: yes/no; menstrual cycle Days 3–5: yes/no) were compared using the non-parametric Mann–Whitney U-test. The proportion of women having PCO between these groups was compared using the χ² test. Prevalence and confidence intervals (CIs) were estimated by logistic regression. When testing for possible selection bias between the two groups of women who accepted or declined vaginal ultrasound, either unpaired two-sample t-test or Mann–Whitney U-test was used for continuous data; for discontinuous variables χ² test was performed. A P-value of <0.05 was considered statistically significant.

Results

The clinical and biochemical characteristics of the 154 women are shown in Table I. More than or equal to 12 follicles were counted in 104 of the 154 women and the prevalence was estimated at 68% (95% CI: 60–74%).

Women who did not use hormonal contraceptives at the time of examination (n = 44), had a median (range) follicle number of 17 (8–30), which was significantly higher than the follicle number of the 110 users (P < 0.001; Fig. 2A). There were 12 or more follicles present in 80% (95% CI: 65–89%) of non-users of hormonal contraceptives and in 63% (95% CI: 53–71%) of users of hormonal contraceptives. The proportion of women having PCO in the two groups was statistically significantly different (P = 0.04).

Among the 147 women, who were able to provide information on menstrual cycle day, 16 participants were examined on cycle Days 3–5 as recommended by the Rotterdam PCOS workgroup. The median (range) follicle number for these 16 women was 11.5 (7–21) (Fig. 2B). There was no statistically significant difference between the follicle number for the women who were examined on Days 3–5 and the 131 women who were scanned at any other time of the menstrual cycle (P = 0.15). More than or equal to 12 follicles were found in 50% (95% CI: 27–73%) of the women who were examined on cycle Days 3–5, and in 71% (95% CI: 63–78%) of the women who were examined on any other day (P = 0.09).

The estimated prevalence of PCO among women who were both non-users of hormonal contraceptives and examined on cycle Days 3–5 was 73% (95% CI: 42–91%).

Measuring ovarian volume did not add any subjects with PCO. Of the ovaries that did not meet the follicle criterion, only three subjects had an ovarian volume of more than 10 ml, but since the ovaries of these subjects contained a dominant follicle of more than 10 mm or a large simple cyst, the ovarian volume measurements were not reliable.

When other features of PCOS were evaluated for the 104 women who had a polycystic ovary according to the Rotterdam 2003 criteria, it showed that 41% (95% CI: 32–51%) had PCOS (Table II). The median (range) follicle number for these 43 women was 17 (12–30).

Discussion

To the best of our knowledge, this is the first population-based study of its size to focus particularly on young women in evaluating the proportion of women with PCO according to the Rotterdam follicle criterion.

We found that a very large proportion of these young women had PCO. The overall prevalence of PCO was 68%, and this may even be

<table>
<thead>
<tr>
<th>Table I Clinical and biochemical data on the 154 women who had follicles counted in a least one ovary by use of vaginal ultrasound.</th>
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<tbody>
<tr>
<td>Age (years), median (range)</td>
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<tr>
<td>BMI (kg/m²), median (range)</td>
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<tr>
<td>Follicle number, median (range)</td>
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<tr>
<td>Cycle length (days)², median (range)</td>
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<tr>
<td>Cycle day at time of examination³, median (range)</td>
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<tr>
<td>Users of hormonal contraception, n (%)</td>
</tr>
<tr>
<td>Acne, n (%)</td>
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<tr>
<td>Hirsutism, n (%)</td>
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<tr>
<td>Testosterone (nmol/l), median (range)</td>
</tr>
<tr>
<td>SHBG (nmol/l), median (range)</td>
</tr>
<tr>
<td>Testosterone/SHBG-ratio, median (range)</td>
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</table>

Data only available for 149 and 147 women, respectively. BMI, body mass index; SHBG, sex hormone-binding globulin.
an underestimation, since the majority of women were users of hormonal contraception with fewer follicles than non-users. According to the Rotterdam workgroup, women should be examined in a spontaneous menstrual cycle, when the ovaries are not affected by hormonal contraceptives. In our study, a very high proportion (80%) of non-users of hormonal contraceptives had 12 or more follicles of 2–9 mm and thus PCO, and the median (range) follicle number was 17 (8–30). In line with our results, Duijkers and Klipping (2010) showed that 83–84% of 42 non-users aged 18–22 years had PCO, and the median (range) follicle number was 17 (6–39). This agreement is present even though Duijkers and Klipping counted all visible follicles and not just follicles measuring 2–9 mm as recommended by the Rotterdam workgroup.

It is striking that we find this large proportion of women having PCO compared with results from previous studies. Michelmore et al. (1999) studied 224 young volunteers aged 18–25 years from two universities and two general practice surgeries in Oxford. PCO were only identified in 33%. The low prevalence of PCO compared with our results can most likely primarily be explained by the transabdominal approach used by Michelmore et al., which may have underestimated the number of follicles. Recent advances in ultrasonographic technology and the development of high-resolution transvaginal scanners provide a more accurate view of the ovary (Porter, 2008) enabling us to identify more and more follicles when examining the ovary. Undoubtedly, the evolution of high-resolution ultrasound devices is an extremely important explanation for the discrepancy in reported prevalence of PCO between earlier and recent studies. Variability in prevalence of PCO can, however, not solely be explained by technical advances and use of different ultrasound devices. The effect of age on follicle number is, in our opinion, also a highly important reason for differences in reported prevalence of PCO. This is illustrated in studies evaluating groups of women at different ages using the same ultrasound machine on all subjects (Koivunen et al., 1999; Duijkers and Klipping, 2010).

Our study has several limitations. In order to evaluate a large and representative sample, we included users of hormonal contraception, although this use significantly reduced the number of follicles. If we had excluded users, we would have limited our study group to 44 participants. We would probably also have introduced bias, since features of PCOS such as menstrual disturbances and hyperandrogenism may

### Table II Features of PCOS in the 104 women with PCO.

<table>
<thead>
<tr>
<th>Number of women</th>
<th>Percentage</th>
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<tr>
<td>Oligo-/amenorrhoea&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>Clinical hyperandrogenism and/or hyperandrogenemia</td>
<td>33</td>
</tr>
<tr>
<td>Acne</td>
<td>16</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>17</td>
</tr>
<tr>
<td>Elevated total testosterone&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>Elevated testosterone/SHBG-ratio&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>PCOS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43</td>
</tr>
</tbody>
</table>

<sup>a</sup>Information on oligo-/amenorrhoea is based on n = 103 since one woman was unable to recall menstrual cycle length.

<sup>b</sup>Level above the calculated 95th percentile (3.4 nmol/l and 0.11 for total testosterone and testosterone/SHBG-ratio, respectively) of eumenorrheic women, who were non-users of hormonal contraceptives and had no signs of acne, hirsutism or PCO.
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tend to use of hormonal contraceptives. Also, for practical reasons, we did not limit the time of the examination to menstrual cycle days 3–5 as recommended by the Rotterdam consensus group. It is likely that the proportion of women with PCO in our study may be overestimated compared with that if we had restricted the time of examination to menstrual cycle days 3–5.

We did not exclude participants with a dominant follicle of more than 10 mm. Duijkers and Klipping (2010) evaluated both subjects with and without these dominant follicles. In the age group of 18–22 years, 84.0% of the women had PCO when subjects with dominant follicles were excluded, and 83.3% had PCO when subjects with dominant follicles were included. This could indicate that a dominant follicle does not considerably influence the result in this age group.

Of the 174 women who consented to have vaginal ultrasound performed in our study, it was not possible to visualize the ovaries sufficiently to count the follicles in at least one ovary in 19 subjects. This could possibly be because the participants were not examined in a gynecologic position, which might have prevented free manipulation of the transvaginal transducer. Another explanation could be that the participation rate of 40% compared with a similar study (Michelmore et al., 2007). The number of follicles decreases with age (Reuss et al., 1996; Scheffer et al., 1999; Haadsma et al., 2007) and consequently it would be natural to assume, that the Rotterdam criterion for follicle number cannot be applied to all age groups. The ovaries of a young woman, who has many follicles solely as a consequence of her age, is more likely to meet the Rotterdam criterion than the ovaries of another woman some years older.

It is probably going to be very difficult to establish a new ultrasonographic diagnostic threshold on follicle number that is simple and applicable in clinical practice. In addition to the need for revising the diagnostic criterion on follicle number, a revision of the criterion on ovarian volume has also been suggested (Jonard et al., 2005). If new criteria are defined, the high variability in resolution of the ultrasonographic devices should be considered, since this inevitably leads to differences between clinics in the ability to identify and evaluate the appearance of the ovary. The challenges in defining new ultrasonographic criteria for PCO make it desirable to find an alternative diagnostic tool. Anti-Müllerian hormone (AMH) has been suggested as such a marker (Pigny et al., 2006). AMH is primarily produced in the granulosa cells of the pre-antral and small antral follicles (Weenen et al., 2004). The small follicles are of particular interest in PCOS patients since the number is higher in PCO than in normal ovaries (Jonard et al., 2003), and the number has been shown to be associated with the severity of follicular arrest seen in women with PCOS (Dewally et al., 2007). The level of AMH correlates with the number of follicles (Pigny et al., 2003; Laven et al., 2004). It is presumably more replicable to obtain an AMH value from a blood sample than to count the follicles by vaginal ultrasound. Furthermore, AMH seems to be constant during the menstrual cycle (La Marca et al., 2006; Tsepelidis et al., 2007) and it has high inter-cycle reproducibility (Fanchin et al., 2005). Because of these advantages, this marker could be a valuable tool in diagnosing PCO in the future.

In conclusion, a very large proportion of the young women had PCO according to the Rotterdam 2003 criteria. It is known that the number of follicles is higher at a young age than later in life. We find it necessary to modify the Rotterdam criteria in order to avoid young women being incorrectly diagnosed with polycystic ovary and in particular the PCOS, since establishment of the diagnosis has substantial consequences for the woman giving rise to worries about future health aspects and additionally leading to unnecessary socio-economic expenditures due to medical examination and treatment.
Authors’ roles
S.L.K. contributed to design, acquisition of data, analysis and interpretation of data and drafting the article. C.H.R.-H. contributed to analysis and interpretation of data. E.E. contributed to conception and design. S.F.O. initiated the pregnancy cohort study in 1988 and contributed to conception and design of the present study. J.P.B. contributed to conception and design, and interpretation of data. A.V. contributed to recruitment of participants and interpretation of data. G.T. contributed to conception, design and analysis and interpretation of data. All authors have revised the article critically and approved the final version.

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