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The relationship of serum anti-Mullerian hormone with polycystic ovarian morphology and polycystic ovary syndrome: a prospective cohort study

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STUDY QUESTION: What is the relationship of serum anti-Mullerian hormone (AMH) with polycystic ovarian morphology (PCOM) and polycystic ovary syndrome (PCOS)?

SUMMARY ANSWER: Serum AMH concentrations are capable of differentiating between normal ovaries, PCOM and PCOS.

WHAT IS KNOWN ALREADY: Serum AMH levels are high in PCOS reflecting the number of small antral follicles and an intrinsic defect of individual granulosa cells.

STUDY DESIGN, SIZE, DURATION: Data were collected prospectively and analysed from three groups of women: those with PCOS according to Rotterdam criteria, those with PCOM but no symptoms and those with normal ovaries.

PARTICIPANTS/MATERIAL, SETTING, METHODS: Women with PCOS ($n = 90$), with PCOM ($n = 35$) and with normal ovaries (controls, $n = 90$), matched for age and body mass index, were all being treated for infertility at Homerton University Hospital, a tertiary referral centre.

MAIN RESULTS AND THE ROLE OF CHANCE: Using adequate numbers and statistical methods for demographically similar groups, there were significant differences in the mean serum AMH concentrations between women with PCOS [77.6 pmol/l (95% CI 64.8–90.3)], those with PCOM [52.2 pmol/l (95% CI 40.1–64.2)] and controls [23.6 pmol/l (95% CI 20.5–26.7)] ($P < 0.001$). The combination of AMH >48 pmol/l and LH > 6 IU/l diagnosed 82.6% of women with PCOS. The mean serum FSH was lower in both PCOS and PCOM compared with controls, whereas LH was higher in PCOS compared with PCOM and controls and correlated positively with AMH ($r = 0.321$, $P < 0.01$).

LIMITATIONS, REASONS FOR CAUTION: Further research is needed to determine the relationship of AMH, PCOS and PCOM. The study was restricted to women who sought out treatment for infertility.

WIDER IMPLICATIONS OF THE FINDINGS: The study suggests that the severity of symptoms of PCOS is positively related to the number of small follicles and that AMH may play an important part in the pathophysiology of anovulation.

STUDY FUNDING/COMPETING INTEREST: None.

Key words: polycystic ovary syndrome / anti-Mullerian hormone / gonadotrophins

Introduction

The polycystic ovary syndrome (PCOS) is a prevalent condition affecting up to 7% of women of fertile age and is associated with 75% of the causes of anovulatory infertility (Kousta *et al.*, 1997). The diagnosis, source and pathophysiology of PCOS are still in dispute.

It is now well established that serum anti-Mullerian hormone (AMH) concentrations reflect the number of pre-antral and small antral follicles in the ovary (Fanchin *et al.*, 2003; Laven *et al.*, 2004; Weenen *et al.*, 2004; Hendriks *et al.*, 2005; Nelson *et al.*, 2007; La Marca *et al.*, 2010). The emergence of a single unified assay for the measurement of AMH has added a new facet to the armamentarium of those investigating PCOS (Wallace *et al.*, 2011). The number of follicles of 2–9 mm in the polycystic ovary seems to be key in determining the severity of the syndrome (Jonard and Dewailly, 2004; Pigny *et al.*, 2006; Piouka *et al.*, 2009) and the AMH produced by these follicles could be hypothesized to reflect this. However, up to 20% of the female population of fertile age have been reported to have polycystic ovaries on ultrasound examination but only 5–8% actually suffer from symptoms such as oligo/amenorrhoea or hyperandrogenism (clinical or biochemical), including hirsutism, persistent acne or hyperandrogenemia (Balen *et al.*, 2009). The question has been raised whether polycystic ovarian morphology (PCOM) alone is simply a normal variant of ovarian morphology or is a precursor of the syndrome.

As FSH is responsible for the follicular development leading to ovulation, the oligo/anovulation, characteristic of PCOS, could be due to a dysfunction of FSH which may be quantitative, or qualitative or both. The reported property of AMH to counteract the actions of FSH imply that the high production of AMH by the polycystic ovary may have an important role in the pathophysiology of the syndrome (Pellatt *et al.*, 2010, 2011). In addition, a positive correlation between AMH and both LH and testosterone serum concentrations in PCOS has been reported (Fleming *et al.*, 2005; Carlsen *et al.*, 2009; Piouka *et al.*, 2009; Lin *et al.*, 2011; Rosenfield *et al.*, 2012).

The aims of this study, utilizing the measurement of serum AMH concentrations, were firstly, to define the place of PCOM in the hierarchy of the syndrome; secondly, to investigate the relationship of AMH with gonadotrophin concentrations; and thirdly, to clarify a role for AMH as a diagnostic aid.

Materials and Methods

This was a single-centre, prospective observational study for which permission was received from the National Research Ethics Service (10/H0703/84). A total of 234 women referred to the fertility service of the Homerton Fertility Unit from January 2011 to December 2011 were recruited. Of these 90 were diagnosed with PCOS according to the European Society of Human Reproduction and Embrology (ESHRE)/American Society for Reproductive Medicine (ASRM) (Rotterdam) criteria (2004), 35 had polycystic ovaries on ultrasound examination (>12 follicles of 2–9 mm in each ovary) but no symptoms of PCOS (PCOM) and 90 had normal ovaries on ultrasound examination (controls). The control group was made up of women, matched for age and body mass index (BMI) means and ranges to the other two groups.

All women had serum AMH, FSH and LH concentrations performed on Day 3 of a spontaneous or progestin-induced menstruation as part of our routine infertility work-up during which BMI was recorded.

Of the 215 women recruited, 171 eventually underwent treatment with IVF: 70 with PCOS, 35 with PCOM and 66 controls. The stimulation protocol was individualized to aim for the optimal number of oocytes for each patient. The protocol and starting dose of FSH were decided based on age, AMH levels, ultrasound scan findings and response in previous treatment cycles if any. Ovarian response was monitored by ultrasound scans and serum estradiol concentrations. Oocyte retrieval was done 35 h after hCG administration and embryos were transferred 2, 3 or 5 days later.

Assays

The AMH assay used was initially the ELISA by DSL (DSL-10-14 400 Active MIS/AMH ELISA (Diagnostic Systems Laboratories, Webster, TX) till March 2011, then replaced by the Beckman Coulter generation II assay in April 2011 (AMH Gen II A79765 ELISA kit Diagnostic Systems Laboratories, Webster, TX). Initial results (till March 2011) were converted to pmol/l (pmol/l = micrograms/l × 7.143) and multiplied by 2.857 to compensate for the 40% positive bias of the Beckman Coulter generation II assays in comparison with the generation I assays (Wallace *et al.*, 2011). All values were finally equivalent to Beckman Coulter generation II assays (Beckman Coulter, Nyon, Switzerland) and presented as pmol/l. Inter-assay coefficients of variation for a low and high control for the DSL assay were 10.9 and 11.1, respectively. Inter-assay coefficients of variation for a low and high control for the Beckman Coulter assay were 12.0 and 11.2, respectively.

Statistics

The data were analysed using SPSS for Windows Version 19 (SPSS, Inc., Chicago, IL). Statistical tests used include Oneway Analysis of Variance with *post hoc* Bonferroni analysis to identify differences between the three groups: for data with non-parametric distributions the Kruskal–Wallis test was used instead. Correlations were determined using Spearman's correlation coefficients. Alpha was set at 0.05. In an attempt to determine a value of AMH for the prediction or additional diagnostic criterion for PCOS a receiver operating characteristic (ROC) curve was constructed.

Results

The mean age, BMI and hormonal values of the PCOS, PCOM and control groups are shown in Table I. By design, age and BMI were similar in all three groups.

The main result of interest is the distinction made by serum AMH concentrations between the three groups. This is graphically illustrated in Fig. 1 which demonstrates this distinction by employing means and 95% confidence intervals (CI). The mean AMH levels in women with PCOS were 77.6 pmol/l (95% CI 64.8–90.3), with PCOM were 52.2 pmol/l (95% CI 40.1–64.2) and in controls were 23.6 pmol/l, (95% CI 20.5–26.7). A Kruskal–Wallis test revealed a significant difference between the groups ($P < 0.001$). *Post hoc* tests (Tamhane's T2 test) showed that all groups were significantly different from each other; controls versus PCOS and controls versus PCOM ($P < 0.001$) and PCOS versus PCOM ($P < 0.05$).

On Day 3 of a spontaneous or progestin-induced menstrual cycle, serum LH concentrations were similar in the control and PCOM groups but significantly lower than in women with PCOS ($P < 0.001$) (Table I and Fig. 2A). Day 3 serum FSH concentrations were similar in the PCOM and PCOS groups but significantly lower than

Table I Details of the 215 participants expressed as means ($\pm$ SD). Between-group differences calculated by one way ANOVA or Kruskal–Wallis with a *post hoc* test, Bonferroni or Tamhane's T2, depending on the distribution of the data.

	n	Age	BMI	FSH (IU/l)	LH (IU/l)	AMH (pmol/l)
Controls	90	32.5 (3.3)	24.8 (2.6)	6.3 (2.0)	4.9 (3.0)	23.6 (15.0)
PCOM	35	32.1 (4.2)	24.7 (2.6)	5.6 (1.4)	5.3 (3.0)	52.2* (35.0)
PCOS	90	31.6 (4.4)	24.9 (2.4)	5.1* (1.4)	8.8* (5.2)	77.6*,** (61.0)

AMH, anti-Mullerian hormone; PCOM, polycystic ovary morphology; PCOS, polycystic ovary syndrome.
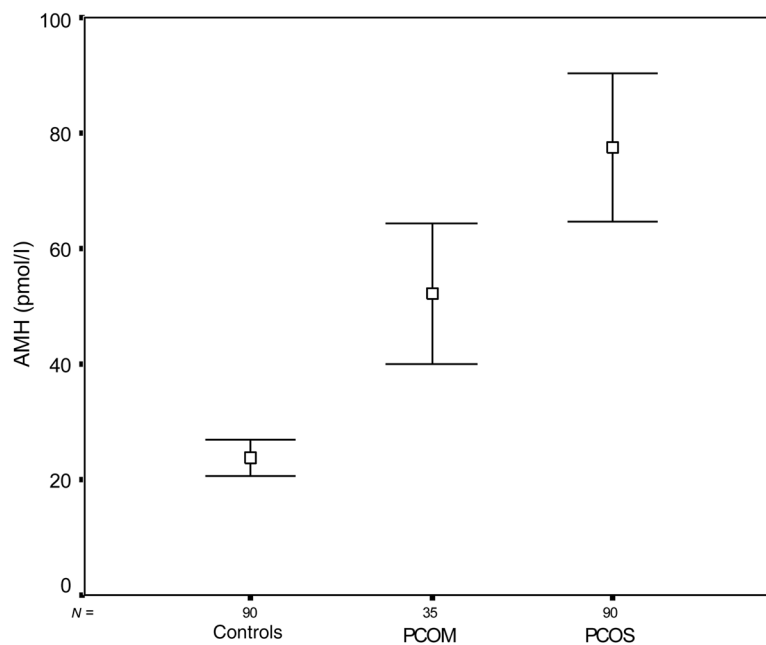*$P < 0.001$ versus control.
**$P < 0.05$ versus PCOM.

Figure I Mean values and 95% confidence intervals for AMH (pmol/l) in the group of controls, PCOM and PCOS.

in controls ($P < 0.001$) (Table I and Fig. 2B). For all participants in the study, AMH correlated positively with LH ($r_s = 0.321$, $P = 0.01$).

Of those who underwent IVF, the total dose of FSH required was similar in women with PCOM and PCOS, but significantly higher in the control group ($P < 0.001$) (Table II). The number of eggs retrieved in the PCOM and PCOS groups was similar but significantly higher than that in controls (controls versus PCOS, $P < 0.001$; controls versus PCOM, $P < 0.05$) (Table II). AMH correlated negatively with the total dose of FSH required in all groups ($r_s = -0.606$, $P < 0.001$). AMH correlated positively with the number of eggs retrieved ($r_s = 0.525$, $P < 0.001$).

In an attempt to determine a value of AMH for the prediction or additional diagnostic criterion for PCOS a receiver operating characteristic (ROC) curve was constructed (Fig. 3). The area under the curve for AMH was 0.81. Using an AMH concentration of 48 pmol/l, the sensitivity would be 60% and the specificity 98.2%. However, if

an LH of >6 IU/l is added, this would predict 82.6% of the women with PCOS.

Discussion

The main finding of this study is the ability of serum AMH concentrations to differentiate between PCOM and PCOS. There has been much debate as to whether PCOM is merely a normal variant of the morphological appearance of the ovary with no clinical significance or whether it is a possible precursor in the progression to the syndrome itself. Our findings based on AMH concentrations suggest that PCOM is a distinct entity located midway between normal ovaries and PCOS; and that PCOM is an abnormal condition with a granulosa cell abnormality similar, but not as severe, to that in PCOS. These findings are in agreement with those recently reported by Catteau-Jonard *et al*. (2012). The clinical significance of this
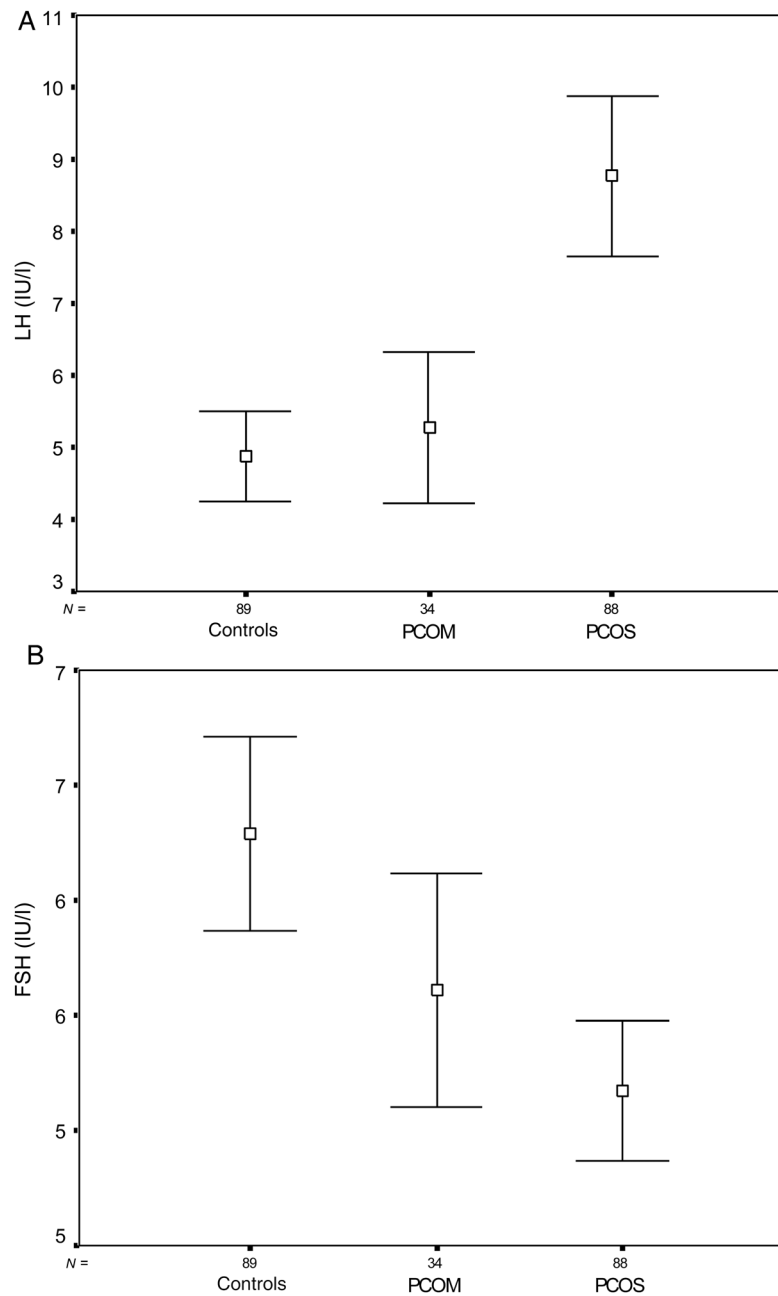
Figure 2 Mean values and 95% confidence intervals for LH (IU/L) (**A**) and FSH (IU/L) (**B**) in the groups of controls, PCOM and PCOS.

observation is 2-fold. First, it strengthens the hypothesis that the basic lesion of PCOS is situated in the ovary but that the clinical symptoms of the syndrome are influenced by factors outside the ovary, such as LH and/or insulin concentrations which when elevated can convert women with PCOM to PCOS. Secondly, when facing an IVF procedure, our results concerning the total dose of FSH required and number of eggs retrieved suggest that not only should women with PCOS be administered a starting dose of FSH lower than that of women with normal ovaries to avoid overstimulation and, potentially, ovarian hyperstimulation syndrome, but that the same principle be applied to women with PCOM alone. The same holds true for the choice

of GnRH analogue to be used, largely recommended to be a GnRH antagonist for these women and, possibly, with the use of a GnRH agonist rather than hCG for the triggering of ovulation (Kol *et al.*, 2012).

It is now well established that serum AMH concentrations reflect the number of pre-antral and small antral follicles in the ovary (Fanchin *et al.*, 2003; Laven *et al.*, 2004; Weenen *et al.*, 2004; Hendriks *et al.*, 2005; Nelson *et al.*, 2007; La Marca *et al.*, 2010). This would account for the raised AMH levels found in both PCOM and PCOS. However, it has been shown, in addition, that each individual follicle in a polycystic ovary in women with PCOS produces significantly

more AMH than its sized-matched counterpart from a normal ovary (Pellatt et al., 2007). Whether this is a result of the influence of androgens, LH or insulin or whether it is an intrinsic property of the follicles of a polycystic ovary is not known.

There is strong evidence that the severity of the symptoms of PCOS is influenced by the number of small antral follicles present in the ovary, reflected by serum AMH concentrations. This was elegantly demonstrated by Pigny et al. (2006), who found significantly higher serum levels of AMH in women with PCOS and amenorrhoea compared with those with oligomenorrhea whose AMH levels were, in turn, higher than in women with PCOS (according to Rotterdam criteria) but with normal cycles. Our finding that AMH concentrations are significantly higher in PCOS compared with PCOM also adds

Table II Total dose of FSH required (IU) and number of eggs retrieved in the 171 women who underwent IVF expressed as means ($\pm$ SD).

	n	Total dose FSH	Eggs retrieved
Controls	66	4215 (2010)	11.2 (6.8)
PCOM	35	2841* (1156)	16.5** (8.4)
PCOS	70	2848* (1144)	18.8* (9.1)

PCOM, polycystic ovary morphology; PCOS, polycystic ovary syndrome.
*$P < 0.001$ versus control.
**$P < 0.05$ versus control.

credence to the statement that the size of the 2–5 mm follicle pool is an independent and important contributor to the follicular arrest in PCOS (Dewailly et al., 2007). Further contributing to this statement is the fact that a reduction in the follicle pool by wedge resection, by laparoscopic ovarian drilling or by the normal aging process (Elting et al., 2003), is capable of restoring ovulation.

The above findings also suggest a possible role of AMH in the pathophysiology of anovulation associated with PCOS. FSH is responsible for follicular growth and although we found that serum FSH concentrations were significantly lower in PCOS compared with women with normal ovaries it is unlikely that this is the whole story. *In vitro* studies have shown that the action of FSH in promoting follicular growth is counteracted by AMH (Durlinger et al, 2001; Pigny et al., 2003; Weenen et al., 2004). It could, therefore, be hypothesized that in anovulatory PCOS, the failure of follicle development is due to an intrinsic inhibition of FSH action and that this inhibition is due to the high concentrations of AMH. This AMH-induced inhibition of FSH action may be effected by its inhibition of FSH-stimulated FSH receptor production or inhibition of FSH-stimulated aromatase mRNA (Pellatt et al., 2011).

A further relationship of AMH and gonadotrophins is suggested by our finding of a positive correlation between AMH and LH concentrations in women with PCOS, a finding previously reported (Piouka et al., 2009; Lin et al., 2011). Whether AMH is driving LH or the opposite is not yet clear.

Regarding the use of serum AMH concentrations in the diagnosis or aid in the diagnosis of PCOS in conjunction with clinical symptoms, the
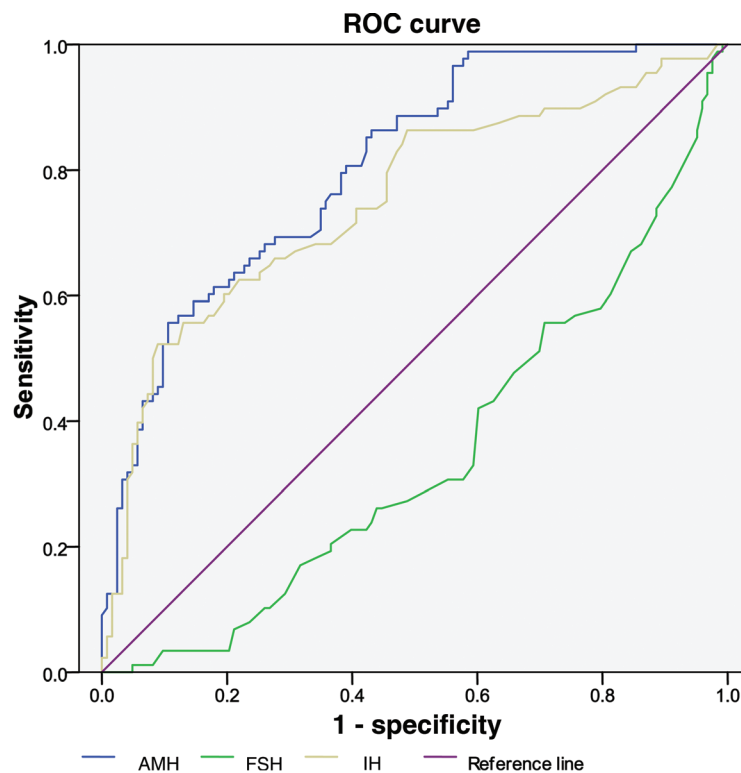

Figure 3 An ROC curve for AMH, FSH and LH. The AUC for AMH is 0.805.

ROC curve indicated that a cut-off value for AMH of 48 pmol/l would give the best compromise between sensitivity (60%) and specificity (98.2%). Pigny et al. (2006) suggested a value for AMH of 60pmol/l to give a sensitivity of 67% and specificity of 92%. In agreement with these and other authors (Rosenfield et al., 2012), we also found that high AMH levels are specific but relatively insensitive for diagnosing PCOS. However, we found that the combination of serum LH >6 IU/l with an AMH >48 pmol/l would predict 82.6% of women with PCOS. It has been suggested (Dewailly et al., 2011) that an AMH of >35 pmol/l was capable of distinguishing between PCOM and normal ovaries and that it could be used as a surrogate for the follicle number on ultrasound when the Rotterdam criteria are applied for the diagnosis of PCOS. Our analysis produced similar results.

In conclusion, the emergence of a unified single assay for the measurement of AMH provides an opportunity for the further investigation of PCOS and its clinical ramifications. Employing AMH values in the present study, we have determined that they can distinguish between PCOM and PCOS as separate entities, adding strength to the notion that PCOM is a precursor to PCOS and suggest that PCOM is not merely a normal variation of ovarian morphology. Whereas the place of AMH as a diagnostic aid for PCOS has yet to be settled, the role of AMH in the pathophysiology of this enigmatic syndrome is a challenge for further research. The inverse relationship between AMH and FSH serum concentrations adds an intriguing facet to the puzzle while the positive correlation of AMH with LH has been confirmed.

Acknowledgement

Authors' roles

R.H. designed the study and wrote the manuscript. A.R. and P.B. recruited the subjects and constructed the database. P.T. measured and advised on all concerning AMH assays. K.G. performed the statistics and A.G. and A.S. critically reviewed the protocol and manuscript.

Funding

Conflict of interest

None declared.

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