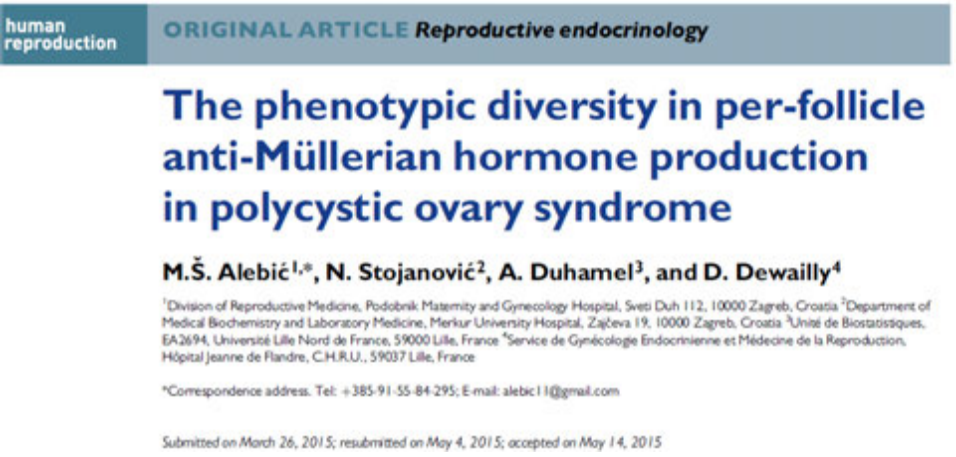

human reproduction ORIGINAL ARTICLE *Reproductive endocrinology*

The phenotypic diversity in per-follicle anti-Müllerian hormone production in polycystic ovary syndrome

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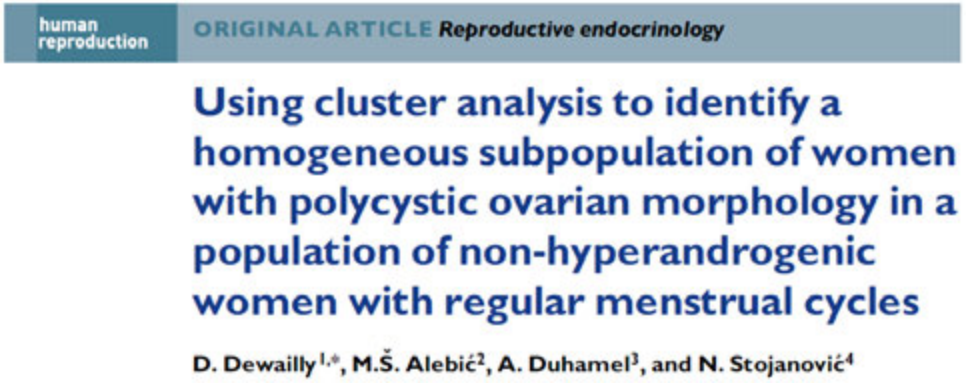


human reproduction ORIGINAL ARTICLE *Reproductive endocrinology*

Using cluster analysis to identify a homogeneous subpopulation of women with polycystic ovarian morphology in a population of non-hyperandrogenic women with regular menstrual cycles

D. Dewailly[1,*], M.Š. Alebić[2], A. Duhamel[3], and N. Stojanović[4]

AMH and PCOS phenotypes

Miro Šimun Alebić, Nataša Stojanović,

Alain Duhamel, Dedier Dewally

Introduction

- anti-Müllerian hormone (AMH) is produced by granulosa cells (GCs) of growing follicles

  Weenen et al., 2004; Jeppesen et al., 2013

- AMH gene expression in GCs increases from the primary follicle stage until the follicles of around 8 mm in diameter and then decreases sharply

  Weenen et al., 2004; Grondahl et al., 2011; Jeppesen et al., 2013

Introduction

- Serum AMH concentration reflects:
  - the number of growing follicles,

    the cohort of AMH producing follicles measuring 2–9 mm (**AFC**) in diameter that mainly contributes to serum AMH concentration can be readily visualized by TV/US

    consequently,

    serum AMH level is tightly correlated with AFC

    Cook et al., 2002; Pigny et al., 2003

  - the wealth of GCs within each follicle and
  - the ability of GCs to produce AMH.

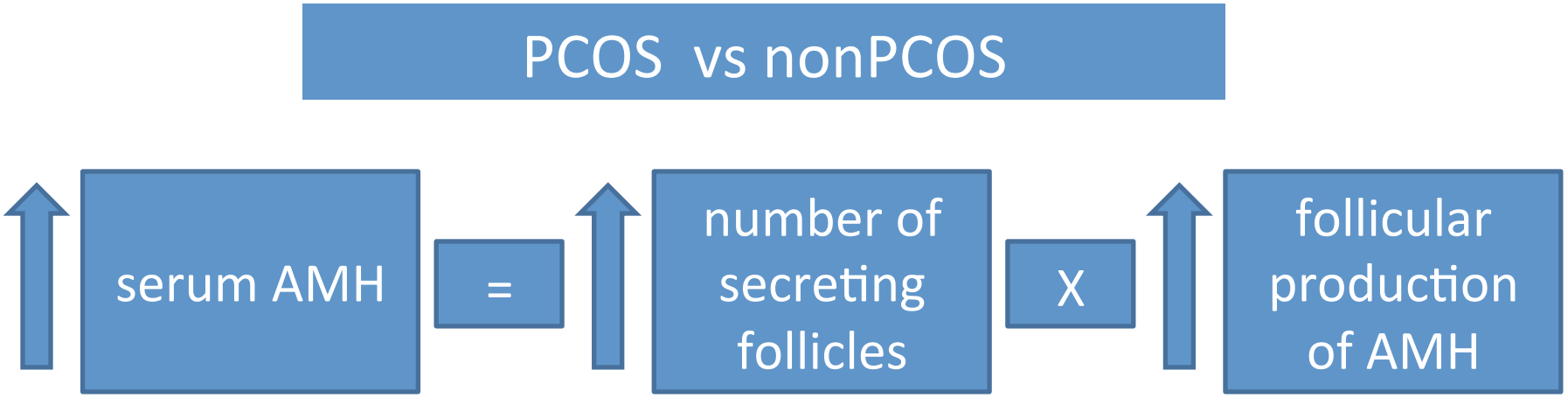
Introduction

Introduction

- In PCOS,
  - excessive accumulation of small antral follicles (2–5 mm) and
  - increased AMH production by each follicle
  - serum AMH levels are higher in women with polycystic ovary syndrome (PCOS) than in women with normal ovarian morphology.

Dewailly et al., 2007; Pellatt et al., 2007; Catteau-Jonard et al., 2008

Introduction



Introduction

- PCOS – Rotterdam criteria
  - Hyperandrogenism (HA)
  - Oligo/anovulation (OA)
  - Polycystic ovarian morphology (PCOM)

HA	OA	PCOM	**Phenotype A**
HA	OA		**Phenotype B**
HA		PCOM	**Phenotype C**
	OA	PCOM	**Phenotype D**

Introduction

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Using cluster analysis to identify a homogeneous subpopulation of women with polycystic ovarian morphology in a population of non-hyperandrogenic women with regular menstrual cycles

D. Dewailly[1,*], M.Š. Alebić[2], A. Duhamel[3], and N. Stojanović[4]

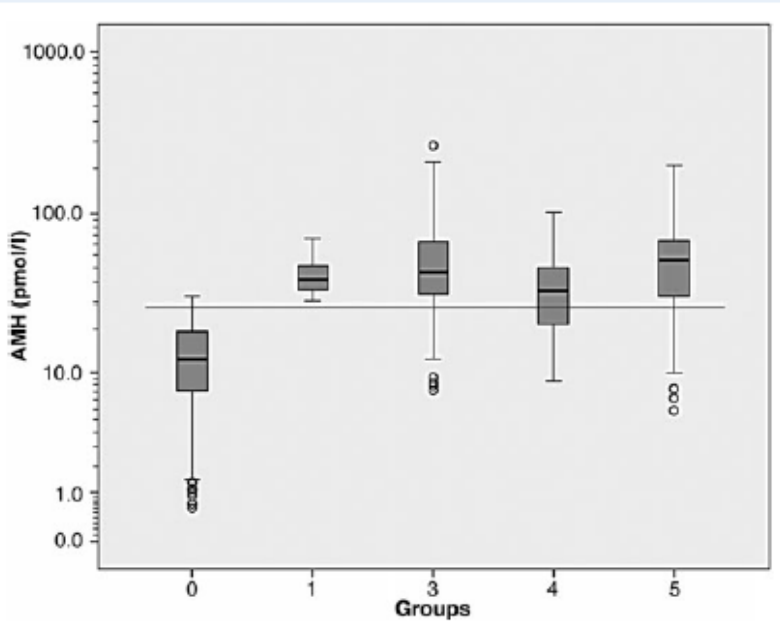


Figure 3 The box-and-whisker plot showing distribution of AMH values in the five study population phenotype groups. Group 1 = 'pure' controls (Cluster 1, $n = 521$); 2 = controls with PCO-like features (Cluster 2, $n = 100$); 3 = OA + PCOM ($n = 110$); 4 = HA + PCOM ($n = 67$); 5 = full-blown PCOS ($n = 95$). The AMH values are presented on a logarithmic y-axis. The dotted line indicates the AMH threshold of 28 pmol/l set by ROC analysis challenging 'pure' controls versus full-blown PCOS (see Results section).

Introduction

- In PCOS,
  - hyperandrogenic normo-ovulatory phenotype had lower median serum AMH level than those with non-hyperandrogenic oligoanovulatory phenotype

    Sahmay et al., 2013

  - some correlation exist between the PCOS phenotypes, as defined by the Rotterdam criteria, and serum AMH level
    - Piouka et al., 2009; Tal et al., 2014

Introduction

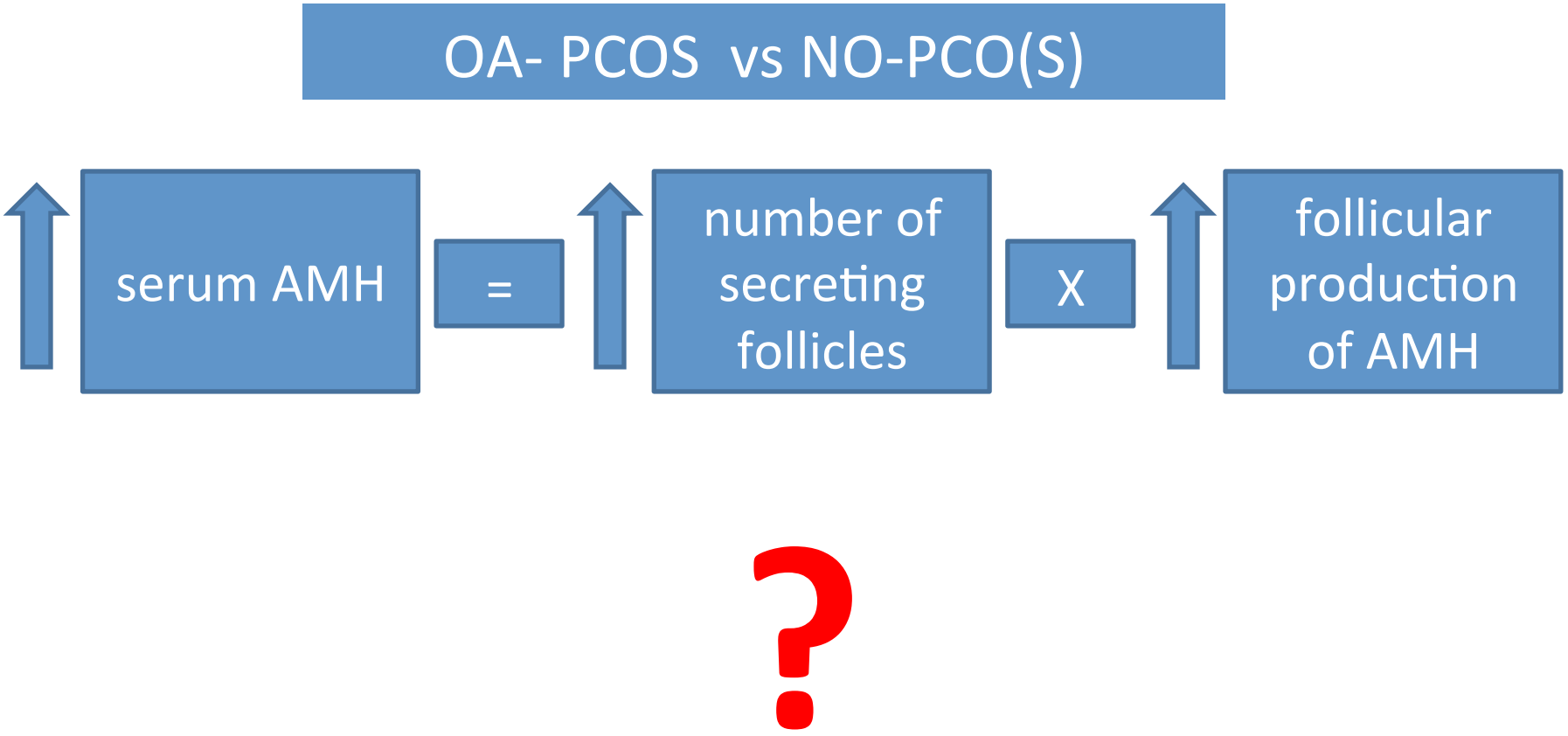
- higher AMH concentration in the follicular fluid of women with anovulatory PCOS than from those with ovulatory PCOS

  (Das et al., 2008).

- AMH production in GCs isolated from anovulatory polycystic ovaries was higher than in GCs from ovulatory polycystic ovaries and normal ovaries, all being significantly different from each other

  (Pellatt et al., 2007).

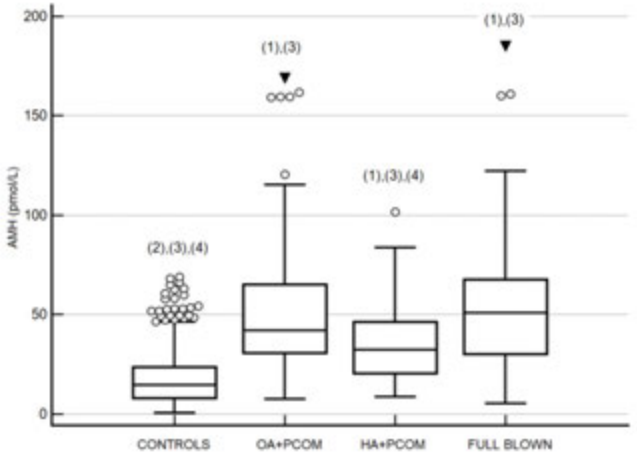
Introduction



Introduction

- hyperandrogenism (HA) is suspected to increase the AMH production by promoting an excess of small growing follicles and GC proliferation

Vendola et al., 1998; Jonard and Dewailly, 2004; Homburg, 2009



Dewailly, 2014

Introduction

- other(s) factor(s), involved in the ovulation disorder is(are) more tightly linked to the AMH excess of PCOS than androgens

and/or

- the increased production of AMH is an intrinsic property of GCs in PCOS?

Pellatt et al., 2010

Aim

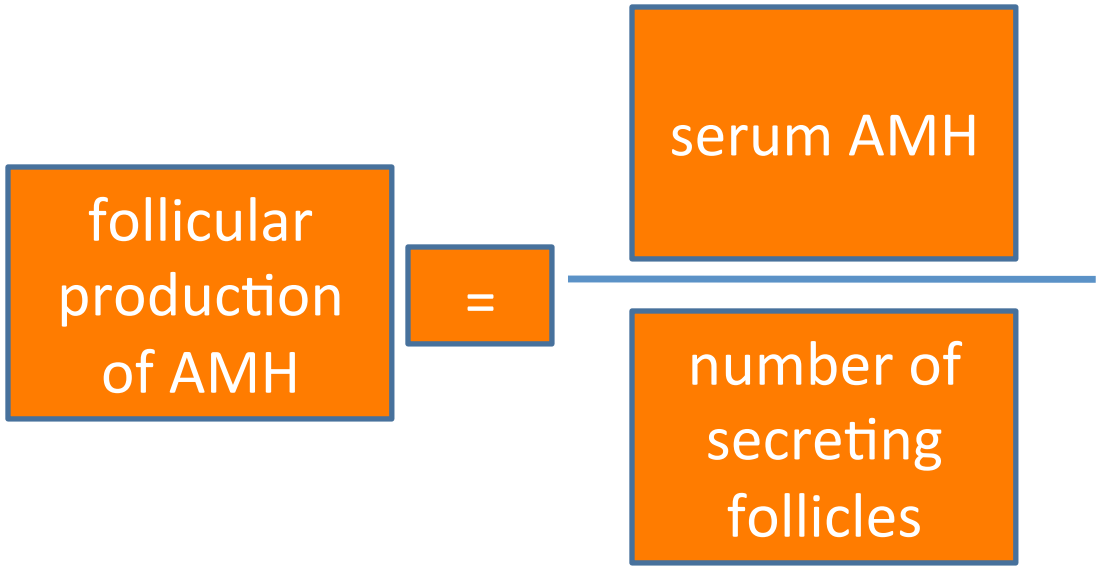
- by analysing the phenotypic diversity in follicular AMH production to investigate the associations of the level of follicular AMH production with other PCOS-associated features; hyperandrogenism, menstrual and/or metabolic dearrangements?
- for that aim, the AMH to AFC ratio (AMH/AFC) served as a surrogate marker for average per-follicle AMH production

Nardo et al., 2009; Bhide et al., 2015

AMH/AFC

- IF

- THEN



Materials and methods

- Study population:
  - (1) controls (n=581)
    - non-hyperandrogenic, eumenorrheic women with normal (non-polycystic) ovarian morphology
  - (2) the PCOM group (n=168)
    - women with PCOM not associated with HA and/or OA,
  - (3) the PCOS group (n=272)
    - consisted of women who were diagnosed as having PCOS according to the Rotterdam criteria
    - subdivided into four subgroups according to PCOS phenotype:
      - PCOS A(PCOM + HA + OA; n=92);
      - ~~PCOS B (HA + OA, n =12);~~
      - PCOS C (PCOM + HA, n=62);
      - PCOS D (PCOM + OA, n =118).

The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004

Three levels of per-follicle AMH production

Table I Comparison of baseline characteristics between study groups.

Characteristic	Controls ($n = 581$)	Polycystic ovary syndrome (PCOS) criteria				*P*-value
		Polycystic ovary morphology only (PCOM; $n = 168$)	Polycystic ovary morphology, and androgens (PCOS C; $n = 62$)	Polycystic ovary morphology, and oligomenorrhea (PCOS D; $n = 118$)	Polycystic ovary morphology, oligomenorrhea and androgens (PCOS A; $n = 92$)	
Age (years)	33.4 (30.5–36.4)	30.5 (28.1–33.2)	30.6 (27.8–33.3)	30.2 (28.1–32.4)	29.5 (26.6–31.8)	<0.001[a,b,c]
BMI (kg/m^2)	23 (21–25)	23 (21–25)	25 (22–29)	23 (21–28)	27 (23–32)	<0.001[a,b,c,d,e]
Waist circumference (cm)	70 (66–78)	71 (66–78)	76 (69–85)	75 (67–86)	84 (70–96)	<0.001[a,b,d,e]
Menstrual cycle length (days)	29 (28–30)	30 (29–32)	30 (30–32)	50 (42–90)	78 (49–182)	<0.001[a,b,c]
Antral follicle count (AFC)	11 (8–15)	26 (24–30)	26 (24–36)	32 (26–40)	36 (30–50)	<0.001[a,b,c,e]
Anti-Müllerian hormone (AMH; pmol/l)	10.3 (5.1–16.7)	28.2 (20.7–39.2)	33.7 (20.5–46.5)	43.1 (30.2–68.2)	53.7 (35.7–69.8)	<0.001[a,b,c,e]
Estradiol (pmol/l)	190 (141–256)	180 (123–244)	158 (130–200)	165 (120–208)	175 (128–226)	<0.001[a,b]
FSH (IU/l)	7.5 (6.2–9.2)	6.8 (5.7–7.9)	6.0 (5.0–7.3)	6.1 (5.2–7.4)	5.4 (4.5–7.2)	<0.001[a,b,c,d]
LH (IU/l)	4.5 (3.5–5.9)	5.2 (3.8–6.9)	4.5 (3.5–5.7)	6.1 (4.5–9.6)	6.4 (5.0–9.0)	<0.001[a,b,c,d]
Testosterone (nmol/l)	1.4 (1.0–1.7)	1.6 (1.3–2.0)	2.4 (1.5–2.9)	1.7 (1.4–2.2)	2.5 (1.8–3.1)	<0.001[a,b,c,d,e]
Modified Ferriman–Gallwey score	1 (1–2)	2 (1–4)	9 (6–10)	3 (2–4)	10 (8–13)	<0.001[a,b,c,d,e]
Glucose (mmol/l)	5.3 (5.0–5.5)	5.3 (4.9–5.6)	5.3 (5.0–5.5)	5.2 (4.9–5.5)	5.2 (4.9–5.6)	NS
Insulin (mIU/l)	7.7 (5.6–10.4)	8.1 (6.0–10.9)	8.4 (6.5–11.8)	9.7 (6.1–14.0)	13.2 (8.5–19.4)	<0.001[a,b,c,e]
Homeostasis model assessment for insulin resistance index	1.8 (1.3–2.5)	1.9 (1.4–2.5)	1.9 (1.5–2.9)	2.3 (1.4–3.3)	3.0 (2.0–4.4)	<0.001[a,b,c,e]
AMH/AFC (pmol/l)	0.9 (0.6–1.4)	1.1 (0.8–1.5)	1.1 (0.8–1.7)	1.4 (1.0–2.0)	1.4 (1.1–1.9)	<0.001[a,b,c]

Values are median (interquartile range).
P-values were derived from the Kruskal–Wallis test comparing oligo- or amenorrheic phenotypes (PCOS A and PCOS D) with eumenorrheic phenotypes (PCOS C and PCOM) and controls. Superscript letters [a,b,c,d,e] indicate a statistically significant difference ($P < 0.05$) found by pairwise comparison of these groups according to Conover:
[a]Controls versus oligo- or amenorrheic phenotypes.
[b]Controls versus eumenorrheic phenotypes.
[c]Oligo- or amenorrheic phenotypes versus eumenorrheic phenotypes.
[d]PCOS C versus PCOM.
[e]PCOS A versus PCOS D.

HA vs nonHA phenotypes

Increased follicular production failed to be demonstrated in

- PCOS A vs PCOS D or in
- PCOS C vs PCOM

despite the presence of hyperandrogenism

Possible confounding effects

Table II Correlation coefficients (Spearman's test) between the main variables tested in the study population including all women with polycystic ovary morphology but no other criteria (PCOM) or at least 2 criteria of polycystic ovary syndrome (PCOS) ($n = 440$).

	AMH/AFC	AGE	BMI	MCL	AFC	AMH	FSH	LH	E2	T	Glucose	Insulin	HOMA	WC
AMH/AFC	1.000	−0.025 *0.596*	**−0.123** *0.010*	**0.279** *<0.001*	**0.098** *0.039*	**0.854** *<0.001*	**−0.218** *<0.001*	**0.150** *0.002*	0.074 *0.124*	**0.189** *<0.001*	−0.055 *0.249*	−0.093 *0.05*	−0.093 *0.05*	**−0.135** *0.005*
AGE		1	−0.020 *0.676*	**−0.098** *0.040*	**−0.143** *0.003*	**−0.096** *0.044*	**0.147** *0.002*	0.088 *0.065*	0.045 *0.353*	**−0.105** *0.028*	0.002 *0.967*	−0.082 *0.087*	−0.086 *0.072*	−0.008 *0.861*
BMI			1	**0.180** *<0.001*	**0.203** *<0.001*	0.015 *0.747*	**−0.142** *0.003*	−0.084 *0.079*	−0.039 *0.420*	**0.280** *<0.001*	**0.123** *0.010*	**0.553** *<0.001*	**0.541** *<0.001*	**0.863** *<0.001*
MCL				1	**0.443** *<0.001*	**0.464** *<0.001*	**−0.240** *<0.001*	**0.242** *<0.001*	−0.031 *0.517*	**0.241** *<0.001*	0.008 *0.862*	**0.243** *<0.001*	**0.236** *<0.001*	**0.227** *<0.001*
AFC					1	**0.562** *<0.001*	**−0.218** *<0.001*	**0.277** *<0.001*	0.058 *0.226*	**0.316** *<0.001*	−0.065 *0.176*	**0.194** *<0.001*	**0.172** *<0.001*	**0.248** *<0.001*
AMH						1	**−0.305** *<0.001*	**0.252** *<0.001*	0.077 *0.108*	**0.327** *<0.001*	−0.066 *0.164*	0.019 *0.696*	0.010 *0.834*	0.024 *0.620*
FSH							1	**0.303** *<0.001*	0.011 *0.815*	**−0.239** *<0.001*	−0.024 *0.615*	−0.087 *0.070*	−0.089 *0.063*	**−0.199** *<0.001*
LH								1	**0.198** *<0.001*	**0.161** *0.001*	−0.046 *0.331*	0.012 *0.800*	0.005 *0.915*	−0.064 *0.181*
E2									1	**0.127** *0.008*	0.068 *0.153*	0.048 *0.312*	0.053 *0.272*	−0.063 *0.187*
T										1	0.031 *0.520*	**0.146** *0.002*	**0.148** *0.002*	**0.263** *<0.001*
Glucose												**0.273** *<0.001*	**0.400** *<0.001*	0.080 *0.096*
Insulin												1	**0.988** *<0.001*	**0.542** *<0.001*
HOMA-IR													1	**0.525** *<0.001*
WC														1

P-values are indicated in italics. Values significant at $P < 0.05$ are in bold type.
AMH, anti-Müllerian hormone; AFC, antral follicle count; BMI, body mass index; MCL, menstrual cycle length; HOMA-IR, homeostasis model assessment for insulin resistance index; WC, waist circumference.

Significant correlations of AMH/AFC and other variables were found, with possible multiple confounding effects.

Follicular production of AMH and menstrual status are related independently of AFC, WC and LH

- AFC, AMH/AFC, WC and LH demonstrated independent significant effects on menstrual status
- Inherent dysregulation of AMH gene expresion could not be excluded
- T and insulin primarily act through the accumulation of antral follicles

Conclusions

Our data suggest the existence of an intrinsic GC dysregulation in PCOS leading to increased per-follicle AMH production that is involved in phenotypic presentation of women with polycystic ovaries, mainly menstrual cycle disorder.

Conclusions

Being independent from androgen and metabolic status, this dysregulation is likely an inherent phenotype specific feature of GCs of polycystic ovaries.