The meteor shower of PCOS: potential role of AMH and AMH isoforms

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- Editor-in-chief of the reproduction section of *Frontiers in Endocrinology*
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Educational objectives

- Describe the pathophysiology of PCOS and discuss the role of AMH isoforms
- Integrate new evidence regarding AMH measurements and diagnosis of PCOS
- Understand the fundamental considerations of how AMH secretion is governed in PCOS and relate novel data to clinical practice

Outline and take-home messages

- Diagnosis of PCOS including AMH as a marker
- Using different AMH assays for characterizing women with PCOS
- High precision and high area under the ROC curve with highly specific AMH assays and biochemical markers qualify as diagnostic criteria
- Basic science studies now indicate that specific effect of LH on the follicle is an important mechanism for development of the PCOS phenotype

AMH, anti-Müllerian hormone; LH, luteinizing hormone;
PCOS, polycystic ovary syndrome; ROC, receiver operating curve.

Rotterdam 2003 criteria for the diagnosis of PCOS

Revised 2003 criteria (2 out of 3):

1. Oligo- and/or anovulation
2. Clinical and/or biochemical signs of hyperandrogenism
3. Polycystic ovaries (> 12 follicles measuring 2–9 mm in diameter, or ovarian volume > 10 mL in ≥ 1 ovary)

In addition, exclusion of other aetiologies:

- Congenital adrenal hyperplasias
- Androgen-secreting tumours
- Cushing's syndrome

Combinations of criteria (phenotype)	Frequency
1. Anovulation–hyperandrogenism–PCO	44%
2. Anovulation–hyperandrogenism	23%
3. Hyperandrogenism–PCO	14%
4. Anovulation–PCO	18%

PCO, polycystic ovary.

Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Fertil Steril. 2004;81:19-25.

Human Reproduction, Vol.33, No.9 pp. 1602–1618, 2018
Advanced Access publication on July 19, 2018 doi:10.1093/humrep/dey256

human reproduction | ESHRE PAGES

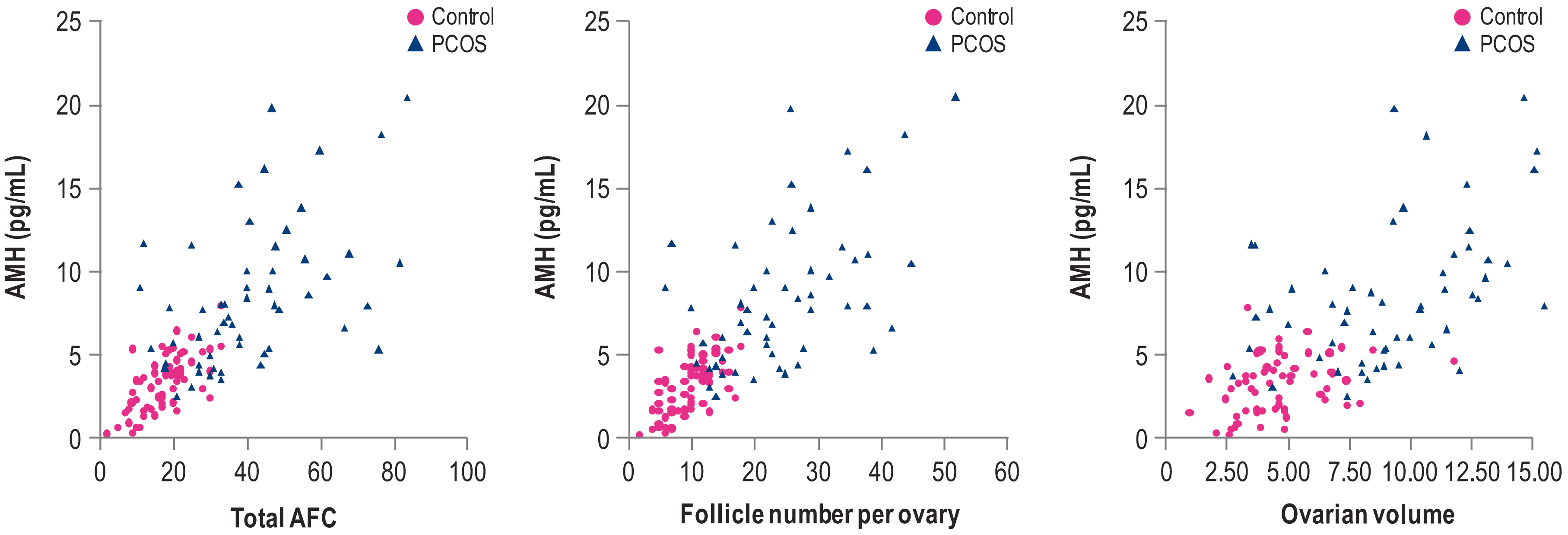
Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome[†,‡]

Helena J. Teede[1,2,3,*], Marie L. Misso[1,2,3], Michael F. Costello[4], Anuja Dokras[5], Joop Laven[6], Lisa Moran[1,2,3], Terhi Piltonen[7], and Robert J. Norman[1,2,8], on behalf of the International PCOS Network[§]

- Endorses Rotterdam criteria
- Evidence-based: development of algorithms to diagnose PCOS
- Indicates that AMH is not yet qualified to be a diagnostic marker

Teede HJ, et al. Hum Reprod. 2018;33:1602-18.

Diagnosing PCOS: accuracy of AMH and total follicle count



The area under the receiver-operating characteristic (ROC) curve of AMH was 0.90

AFC, antral follicle count.

Wongwananuruk, T et al. Taiwan J Obstet Gynecol. 2018;57:499-506.

AMH measurement vs ovarian ultrasound for diagnosing PCOS in different phenotypes

- A retrospective matched controlled study of 113 women with various PCOS phenotypes and 47 matched controls
- Overall, AMH had a low sensitivity of 79%, while follicle number per ovary and ovarian volume were 93% and 68%, respectively
- In classic anovulatory PCOS, AMH exhibited a sensitivity of 91%, while follicle number per ovary and ovarian volume were 92% and 72%, respectively
- In the ovulatory phenotype, AMH sensitivity was only 50%, while follicle number per ovary and ovarian volume were 95% and 50%, respectively
- In the non-hyperandrogenic phenotype, the sensitivity of AMH was 53%, while follicle number per ovary and ovarian volume were 93% and 67%, respectively
- Conclusion: AMH does not appear to be helpful for all subjects with PCOS

Carmina E, et al. Endocr Pract. 2016;22:287-93.

ARTICLE IN PRESS

ORIGINAL ARTICLE: REPRODUCTIVE ENDOCRINOLOGY

Associations of different molecular forms of antimüllerian hormone and biomarkers of polycystic ovary syndrome and normal women

Marie Louise Wissing, Ph.D.,[a] Anne Lis Mikkelsen, DMSc,[a] Ajay Kumar, Ph.D.,[c] Bhanu Kalra, Ph.D.,[c]
Susanne Elisabeth Pors, Ph.D.,[a] Esben Meulengracht Flachs, Ph.D.,[d] and Claus Yding Andersen, D.M.Sc.[b]

- Study involving 88 women with PCOS and 24 healthy regularly cycling women (controls)
- Subjects were characterized based on all PCOS-related parameters
- Serum levels of AMH were measured with assays detecting three different parts of the AMH molecule

Can diagnosis of PCOS be improved by including an AMH measurement?

Wissing ML, et al. Fertil Steril. 2019 May 2.
pii: S0015-0282(19)30245-6. [Epub ahead of print].

Baseline parameters in women with PCOS vs controls

	PCOS	Controls	p value
Women, n	88	24	
Age, years	28.2 ± 0.42	28.4 ± 0.86	NS
BMI	26.8 ± 0.55	24.8 ± 0.82	NS
Number of antral follicles	23.9 ± 0.89	15.8 ± 1.11	< 0.0001
Number of menstrual bleedings/year	4.9 ± 0.45	11.3 ± 0.50	< 0.0001

BMI, body mass index.

Wissing ML, et al. Fertil Steril. 2019 May 2.
pii: S0015-0282(19)30245-6. [Epub ahead of print].

Hormone characteristics of women with PCOS compared with controls

	PCOS	Controls	p value
Total testosterone, nmol/L	2.34 ± 0.11	1.16 ± 0.08	< 0.0001
Free testosterone, nmol/L	0.0382 ± 0.0024	0.0177 ± 0.0018	< 0.0001
SHBG, nmol/L	65.8 ± 4.1	66.7 ± 5.4	NS
Androstenedione, nmol/L	7.4 ± 0.3	4.4 ± 0.3	< 0.0001
DHEA-S, µmol/L	5811 ± 305	4674 ± 465	NS
FSH, IU/L	5.8 ± 0.2	6.2 ± 0.4	NS
LH, IU/L	12.1 ± 1.7	5.8 ± 0.5	0.05
LH/FSH	1.9 ± 0.1	1.1 ± 0.1	0.0006
Estradiol, nmol/L	0.25 ± 0.02	0.21 ± 0.04	NS
Prolactin, IU/L	229 ± 12	244 ± 23	NS
TSH, IU/L	2.0 ± 0.1	2.1 ± 0.2	NS

DHEA-S, dehydroepiandrosterone-sulfate; FSH, follicle-stimulating hormone;
SHGB, sex-hormone-binding globulin; TSH, thyroid-stimulating hormone.

Wissing ML, et al. Fertil Steril. 2019 May 2.
pii: S0015-0282(19)30245-6. [Epub ahead of print].

Biochemical characteristics of women with PCOS vs controls

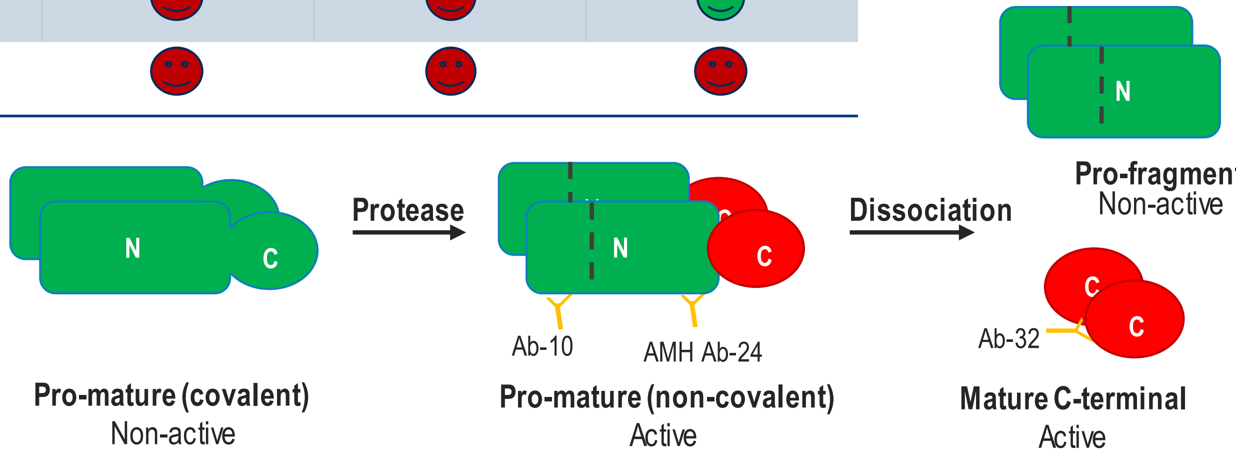
	PCOS	Controls	p value
s-insulin, µU/mL	74.0 ± 6.1	53.5 ± 4.1	NS
Blood glucose, mmol/L	5.2 ± 0.1	5.1 ± 0.1	NS
C-peptide, nmol/L	0.67 ± 0.04	0.58 ± 0.03	NS
Modified HOMA	7.74 ± 0.53	7.37 ± 0.55	NS
HOMA-IR	17.2 ± 1.6	11.7 ± 1.1	NS
ALAT, U/L	25 ± 2	22 ± 5	NS
Total cholesterol, mmol/L	4.7 ± 0.1	4.5 ± 0.2	NS
LDL Cholesterol, mmol/L	2.8 ± 0.1	2.6 ± 0.2	NS
HDL cholesterol, mmol/L	1.5 ± 0.04	1.6 ± 0.1	NS
Triglycerides, mmol/L	1.0 ± 0.07	0.7 ± 0.04	NS

ALAT, alanine transaminase; C-peptide, connecting peptide; HDL, high-density lipoprotein; HOMA, Homeostatic Model Assessment; HOMA-IR HOMA of Insulin Resistances; LDL, low-density lipoprotein; s-insulin, serum insulin.

Wissing ML, et al. Fertil Steril. 2019 May 2. pii: S0015-0282(19)30245-6. [Epub ahead of print].

Epitope localization of AMH-specific monoclonal antibodies

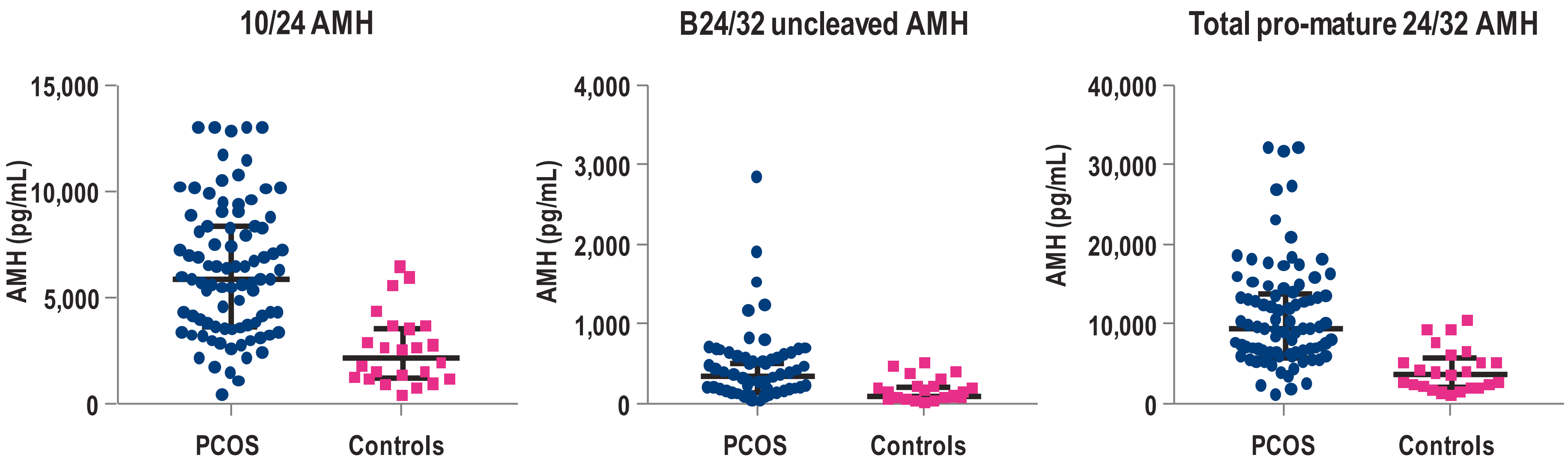
T = SDS-treated	24/32 pico AMH (Cat. no. AL-124)	24/32 T (Cat. no. AL-132)	10/24 (Cat. no. AL-145)
Pro + mature (Associated)	🟢	🔴	🟢
Pro-mature (covalent)	🟢	🟢	🟢
Pro-fragment	🔴	🔴	🟢
Mature fragment	🔴	🔴	🔴



Ab, antibody; C, covalent; SDS, sodium dodecyl sulfate.

Wissing ML, et al. Fertil Steril. 2019 May 2. pii: S0015-0282(19)30245-6. [Epub ahead of print].
Mamsen LS, et al. Mol Hum Reprod. 2015;21:571-82.

Scatter plots of AMH values in women with PCOS and controls: data from 3 different assays



Wissing ML, et al. Fertil Steril. 2019 May 2.
pii: S0015-0282(19)30245-6. [Epub ahead of print].

AMH levels in women with PCOS vs healthy controls: data from different assays

	PCOS	Controls	p value
Total (pro-mature) 24/32 AMH ELISA, pg/mL	10,790	4,056	< 0.0001
10/24 AMH ELISA, pg/mL	6,217	2,479	< 0.0001
24/32 non-cleaved AMH ELISA, pg/mL	417	145	< 0.0001
Ratio 24/32 non-cleaved AMH/10/24 AMH, ×100	6.0	5.11	NS
Ratio 24/32 non-cleaved AMH/24/32 total pro-mature AMH, ×100	3.4	3.0	NS
Ratio 10/24 AMH/24/32 total pro-mature AMH, ×100	60.2	60.8	NS

ELISA, enzyme-linked immunosorbent assay.

Wissing ML, et al. Fertil Steril. 2019 May 2. pii: S0015-0282(19)30245-6. [Epub ahead of print].

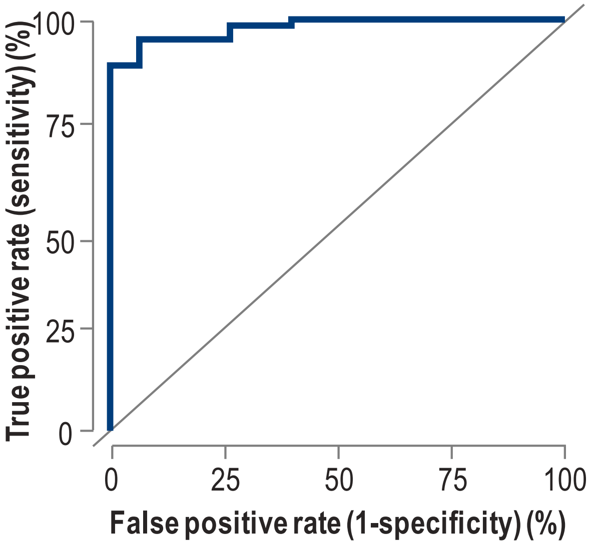
ROC curves for predicting PCOS for different AMH isoforms alone and in combination with baseline parameters

	ROC factor for AMH assays plus various combinations of parameters (95% CI)						
	Only AMH	Androgens	Androgens AFC	Androgens DHEA-S	Androgens DHEA-S AFC	Androgens BMI	Androgens BMI AFC
10/24 AMH	89.9 (82.7–97.2)	94.1 (89.4–98.7)	95.9 (92.3–99.5)	94.3 (89.8–98.9)	96.1 (92.5–99.7)	94.6 (89.8–99.4)	96.7 (93.5–99.9)
24/32 uncleaved AMH	86.9 (78.7–95.2)	92.7 (87.1–98.3)	96.0 (92.5-99.6)	92.6 (86.9–98.4)	96.4 (92.9–99.9)	93.6 (87.6–99.6)	97.0 (93.8–100)
24/32 total pro-mature AMH	91.5 (85.0–98.0)	95.1 (91.0–99.3)	96.0 (92.5–99.6)	95.3 (91.1–99.4)	96.2 (92.8–99.7)	95.3 (90.7–99.9)	96.9 (93.8–100)

Wissing ML, et al. Fertil Steril. 2019 May 2. pii: S0015-0282(19)30245-6. [Epub ahead of print].

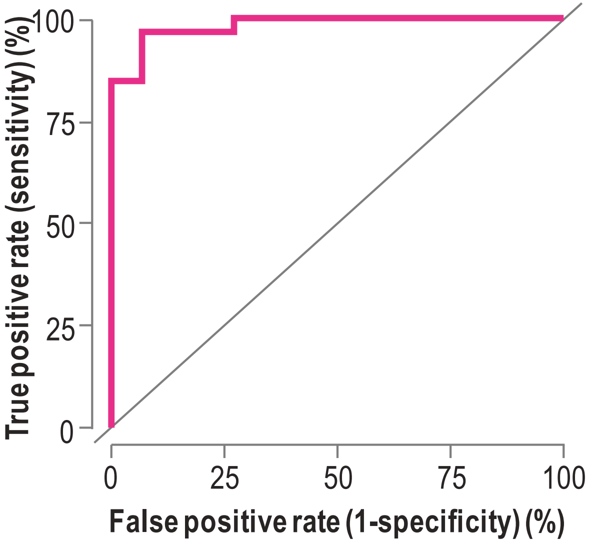
Diagnosis of PCOS using AMH: accurate and precise ROC curves

10/24 AMH



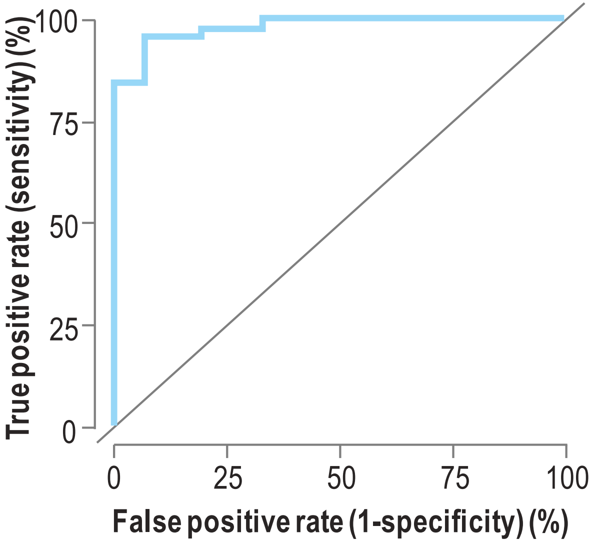
AUC 96.7%
CI 93.5–99.9%

24/32 uncleaved AMH



AUC 97%
CI 93.8–100%

24/32 total pro–mature AMH



AUC 96.9%
CI 93.8–100%

Wissing ML, et al. Fertil Steril. 2019 May 2.
pii: S0015-0282(19)30245-6. [Epub ahead of print].

Human Reproduction Update Advance Access published January 14, 2014
Human Reproduction Update, Vol.0, No.0 pp. 1–16, 2014
doi:10.1093/humupd/dmt062

human reproduction update

The physiology and clinical utility of anti-Müllerian hormone in women

Didier Dewailly[1,*], Claus Yding Andersen[2], Adam Balen[3], Frank Broekmans[4], Nafi Dilaver[5], Renato Fanchin[6], Georg Griesinger[7], Tom W. Kelsey[8], Antonio La Marca[9], Cornelius Lambalk[10], Helen Mason[5], Scott M. Nelson[11], Jenny A. Visser[12], W. Hamish Wallace[13], and Richard A. Anderson[14]

- Current technical difficulties for determining serum AMH may have lessened clinician enthusiasm for this marker of PCOS
- However, data suggest that this assay may be an alternative for AFC in the Rotterdam classification
  - May increase reliability and flexibility, especially where ultrasound is uninformative or not possible (obese women, adolescents)

- More importantly, precise information may be available prior to patient visit

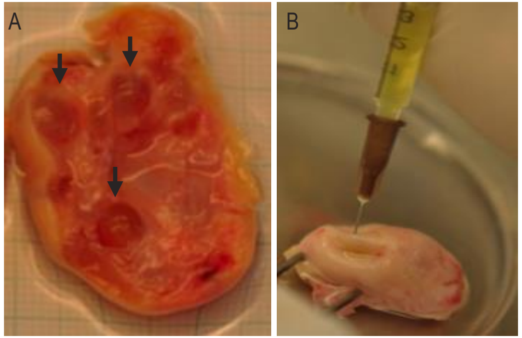
Dewailly D, et al. Hum Reprod Update. 2014;20:370-85.

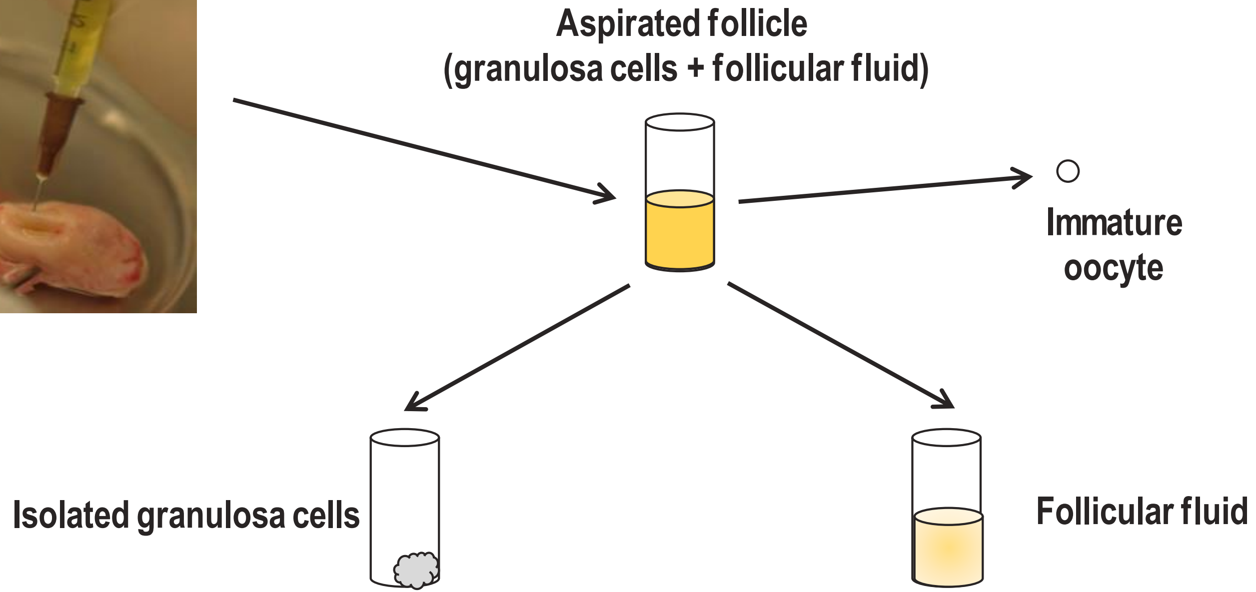
What is the cause of high AMH in women with PCOS?

- Augmented number of small antral follicles
- Compared with normal ovaries, production of AMH per granulosa cell was:
  - 75× higher in anovulatory PCOS
  - 20× higher in ovulatory PCOS
- Increased AMH concentrations were also found in follicular fluid

Das M, et al. Hum Reprod. 2008;23:2122-6.
Pellatt L, et al. J Clin Endocrinol Metab. 2007;92:240-5.

Quantitative differences in concentrations of TGF-β family members measured in fluids from small antral follicles





Isolation of human follicular fluid and granulosa cells from normal human small antral follicles immediately snap-frozen in liquid nitrogen

TGF, transforming growth factor.

Kristensen SG, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-01094.
Owens LA, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-00780.

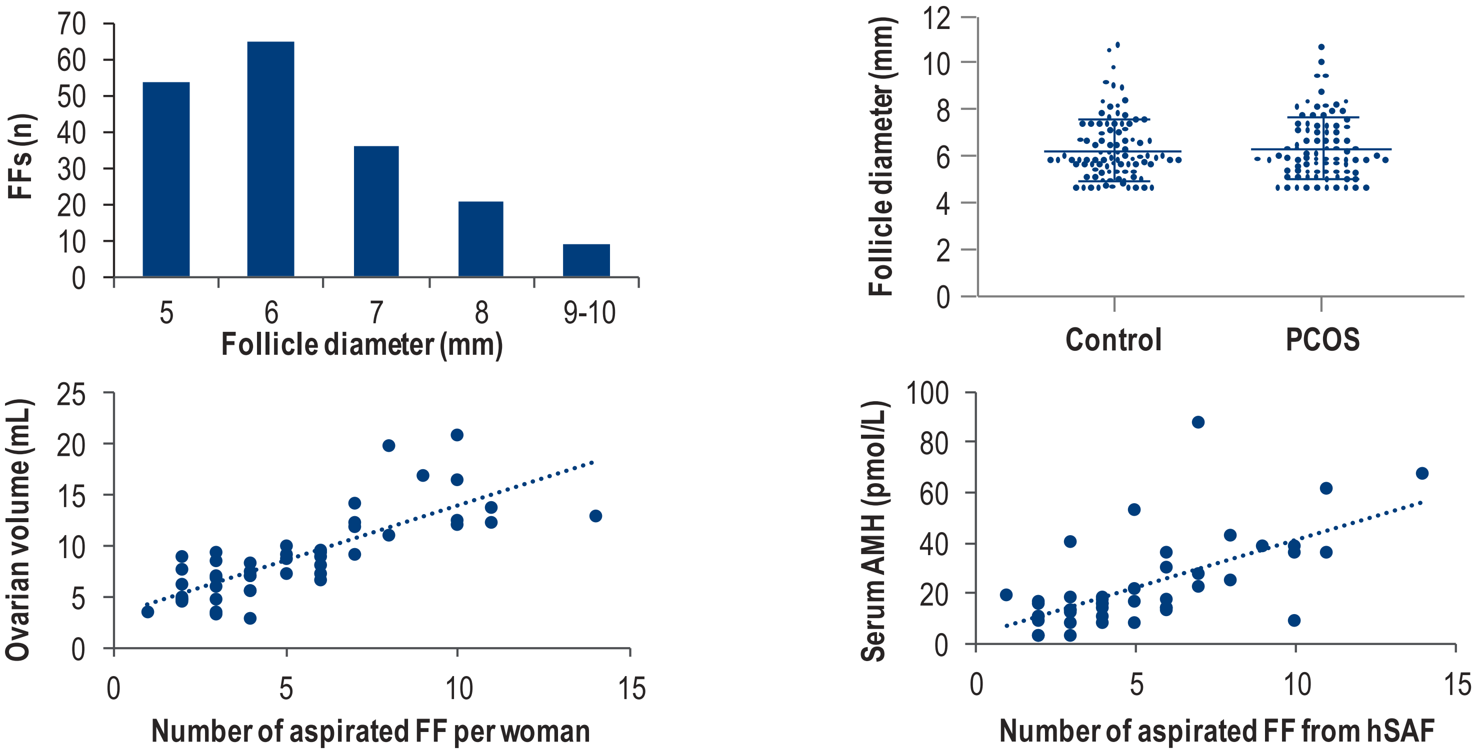
Patient characteristics

	Non-PCO	PCO	p value
Women, N	33	16	
Age, years (mean ± SD) [range]	26.2 ± 5.3 [16–34]	26.0 ± 6.1 [15–34]	NS
Ovarian volume, mL (mean ± SD) [range]	6.8 ± 2.0 [2.9–9.5]	14.4 ± 3.2 [10.0–20.9]	< 0.001
Median number of FF collected per woman [range]	4 [1–7]	10 [7–14]	< 0.001
AMH, pmol/L (mean ± SEM)	15.3 ± 1.5	42.2 ± 5.8	< 0.001
FSH, IU/L (mean ± SEM)	5.7 ± 0.5	5.3 ± 0.5	NS
LH, IU/L (mean ± SEM)	5.6 ± 0.7	10.7 ± 1.8	< 0.01
LH/FSH ratio (mean ± SEM)	1.0 ± 0.1	2.2 ± 0.3	< 0.001
Follicle diameter, mm	4.6–10.6	4.6–10.7	NS
Total number of follicle fluids analysed	92	93	NS
Median number of FF analyzed per woman [range]	2 [1–7]	5 [2–12]	NS

FF, follicle fluid.

Kristensen SG, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-01094.
Owens LA, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-00780.

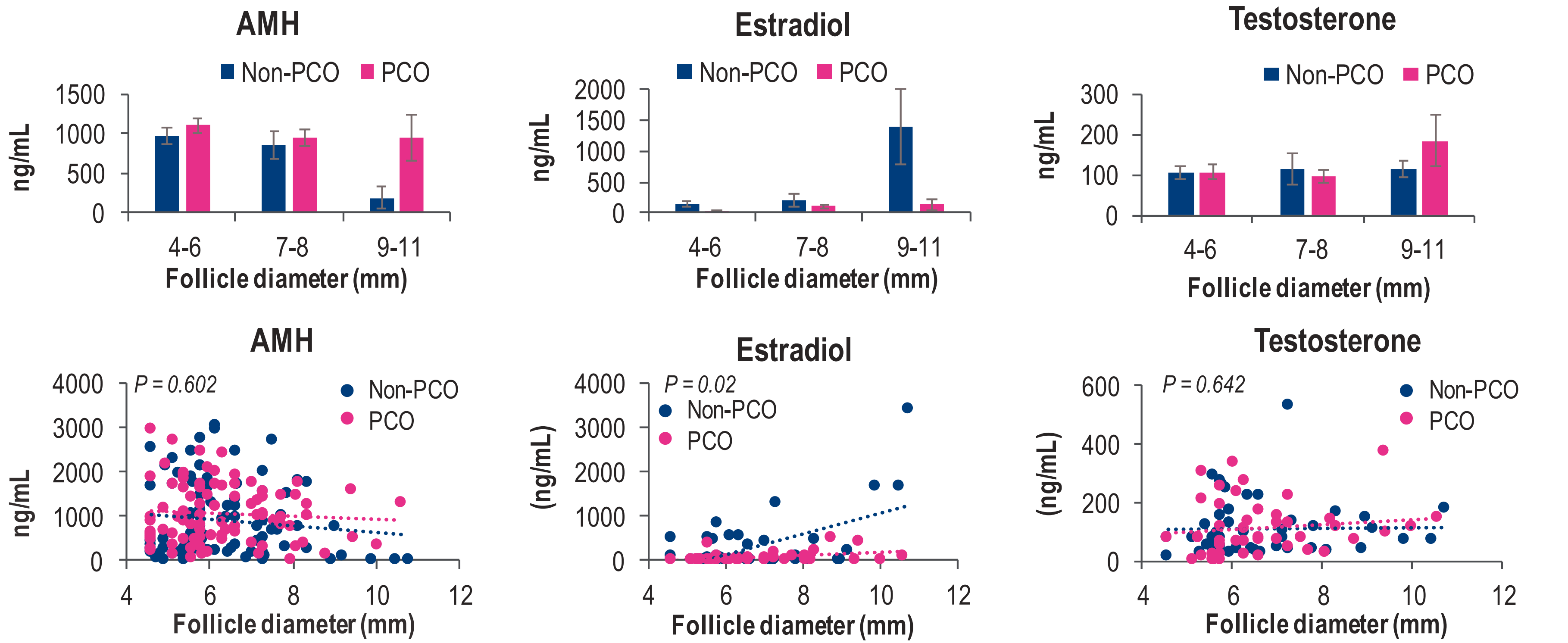
Characteristics: PCOS vs controls



hSAF, human small antral follicle.

Kristensen SG, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-01094.
Owens LA, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-00780.

Intrafollicular concentrations of estradiol and testosterone in small antral follicles grouped by diameter



Kristensen SG, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-01094.
Owens LA, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-00780.

Gene expression in granulosa cells from women with or without PCO

- LHR expression in a fraction of the follicles from PCO follicles is higher in non-PCO
- Levels of LH are twice as high in the circulation of women with PCO as compared with non-PCO
- AR and FSHR are significantly downregulated in granulosa cells from women with PCO as compared with non-PCO
- It appears that LH downregulates AR expression, thereby making the follicle less sensitive to FSH (downregulation of FSHR) – with less aromatase expression – and less E2 synthesis

AR, androgen receptor; E2, estradiol; FSHR, FSH receptor; LHR, LH receptor.

Kristensen SG, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-01094.
Owens LA, et al. J Clin Endocrinol Metab. 2019. pii: jc.2019-00780.

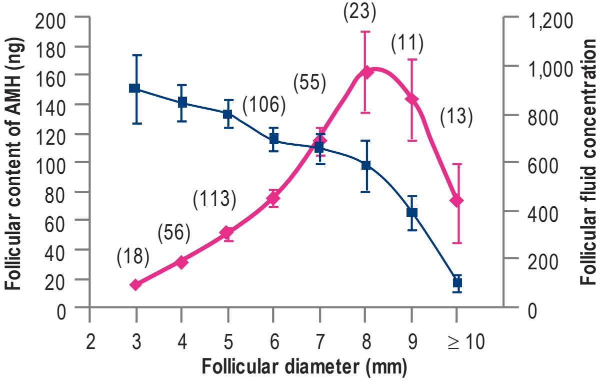
Which follicles produce the most AHM?

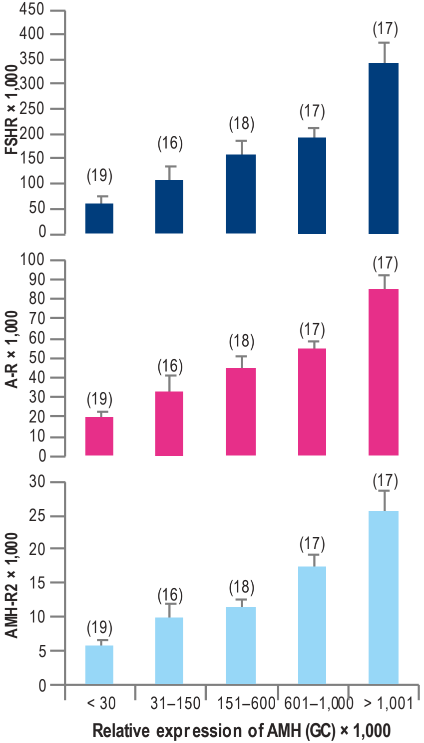

Molecular Human Reproduction, Vol.19, No.8 pp. 519–527, 2013
Advanced Access publication on April 4, 2013 doi:10.1093/molehr/gat024

MHR ORIGINAL RESEARCH

Which follicles make the most anti-Müllerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection

J.V. Jeppesen[1], R.A. Anderson[2], T.W. Kelsey[3], S.L. Christiansen[1], S.G. Kristensen[1], K. Jayaprakasan[4], N. Raine-Fenning[4], B.K. Campbell[4], and C. Yding Andersen[1,*]





AMH gene expression in GC in quintiles

	< 20%	21–40%	41–60%	61-80%	> 81%	Total	Spearman p value
Number	19	16	18	17	17	87	
FSHR	51 ± 16	104 ± 27	155 ± 31	190 ± 24	337 ± 43	163 ± 16	< 0.00001
AR	18 ± 25	32 ± 6	44 ± 5	54 ± 4	84 ± 7	46 ± 3	< 0.00001

GC, granulosa cell.

Jepesen JV, et al. Mol Hum Reprod. 2013;19:519-27.

JCEM ONLINE

Hot Topics in Translational Endocrinology—Endocrine Research

Differential Regulation of Ovarian Anti-Müllerian Hormone (AMH) by Estradiol through α- and β-Estrogen Receptors

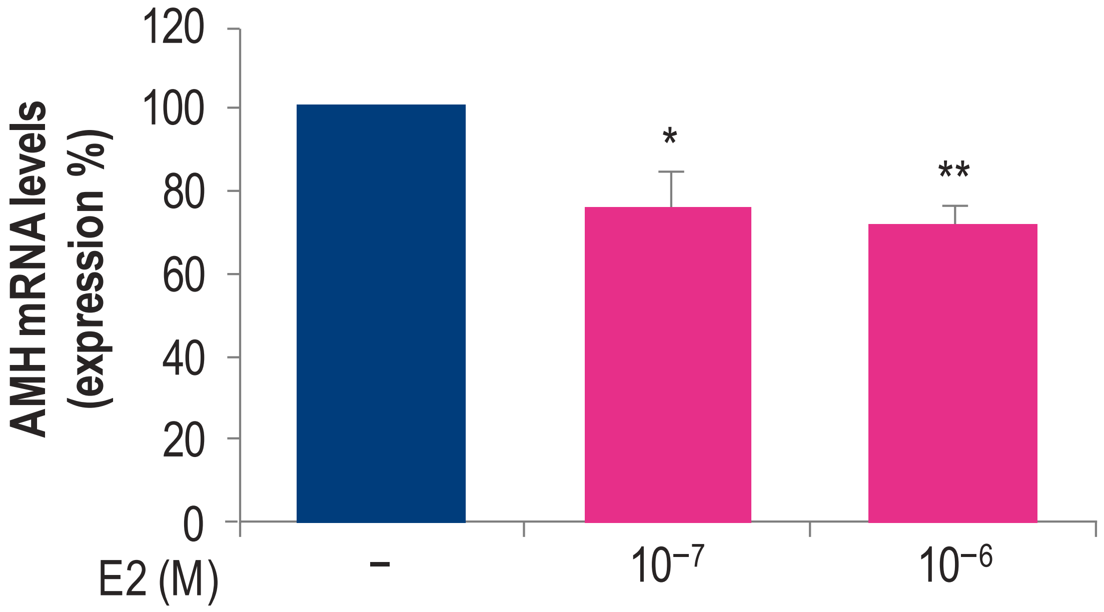
Michaël Grynberg,* Alice Pierre,* Rodolfo Rey, Arnaud Leclerc, Nassim Arouche, Laetitia Hesters, Sophie Catteau-Jonard, René Frydman, Jean-Yves Picard, Renato Fanchin, Reiner Veitia,# Nathalie di Clemente,# and Joëlle Taieb

- Depending on the ER, estradiol differentially regulates AMH expression
- Decreased AMH expression in the granulosa cell of growing follicles (primarily expressing ERβ) and during controlled ovarian hyperstimulation may be due to the effect of estradiol

ER, estrogen receptor.

Grynberg M, et al. J Clin Endocrinol Metab. 2012;97:E1649-57.

Estradiol downregulates AMH mRNA levels in primary cultures of human granulosa cells (lutein)



* $p < 0.05$.
** $p < 0.01$.

Grynberg M, et al. J Clin Endocrinol Metab. 2012;97:E1649-57.

Estrogen receptors: ERα and ERβ

- ERα expression:
  - Ubiquitous; highest mRNA levels in uterus, ovary, pituitary gland, male reproductive organs, white and brown adipose tissue, prostate, skin, skeletal muscle, aorta, kidney, gall bladder, and bone
- ERβ expression:
  - mRNA levels are highest in the ovary, lung, male reproductive organs, colon, brain, and kidney

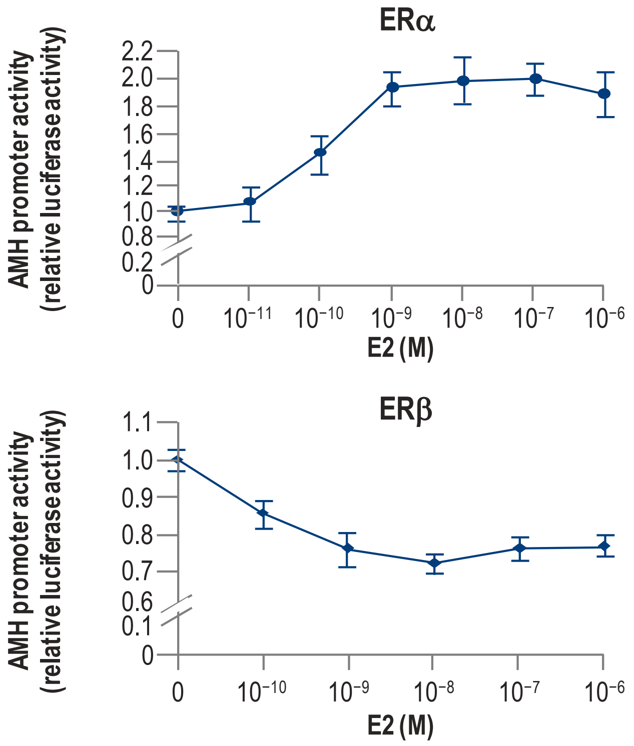
Function:

- ERα mediates most estrogen signalling in classic estrogen target tissues such as the uterus, mammary gland, and skeleton, whereas ERβ has a minor role in these tissues
- ERβ regulates signalling in the ovary, immune system, prostate, GI tract, and hypothalamus
- The two ERs have little functional overlap

GI, gastrointestinal.

AMH transcription is mediated by estrogen receptors

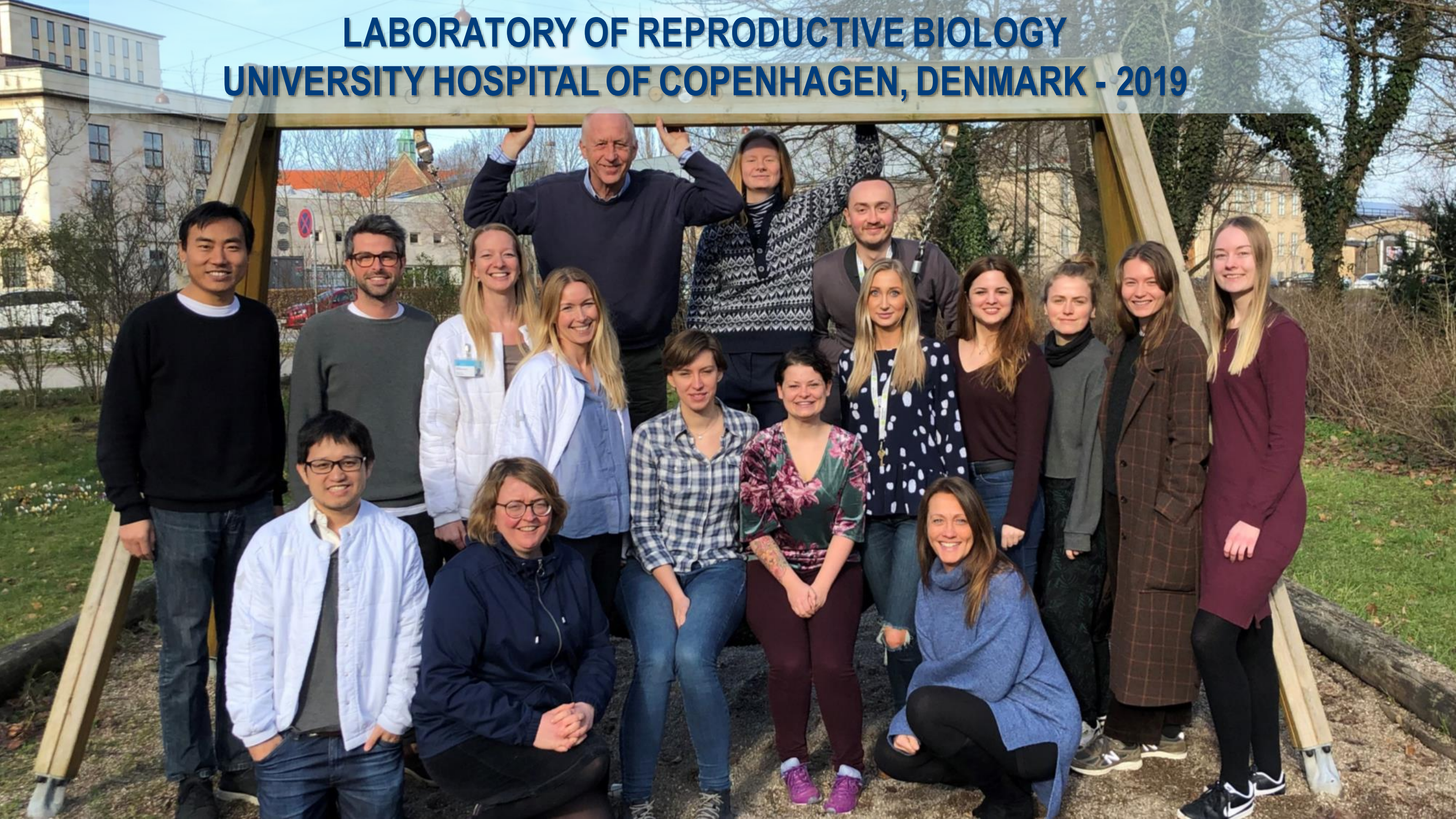
- Estradiol exerts opposite effects on AMH transcription through ERα and ERβ
- In particular, estradiol suppresses AMH expression in granulosa cells when ERβ is in excess compared with ERα, the most common situation encountered in vivo
- The effect of FSH on AMH expression is indirect and mediated by estradiol



Grynberg M, et al. J Clin Endocrinol Metab. 2012;97:E1649-57.

Conclusions

- AMH is a valuable tool for the precise diagnosis of PCOS based on biochemical parameters alone
- Area under the ROC curve is close to 100%
- LHR and LH prematurely expressed on granulosa cells from women with PCOS may cause downregulation of AR, and thereby FSHR, making follicles less sensitive to FSH
- Estradiol output is, therefore, reduced, causing a continued augmented AMH expression in follicles from women with PCOS after follicular selection
- AMH and estradiol exhibit a complicated interaction around follicular selection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