IMPROVING THE PREDICTION OF OVARIAN RESERVE AND RESPONSE IN ART USING BIOMARKERS

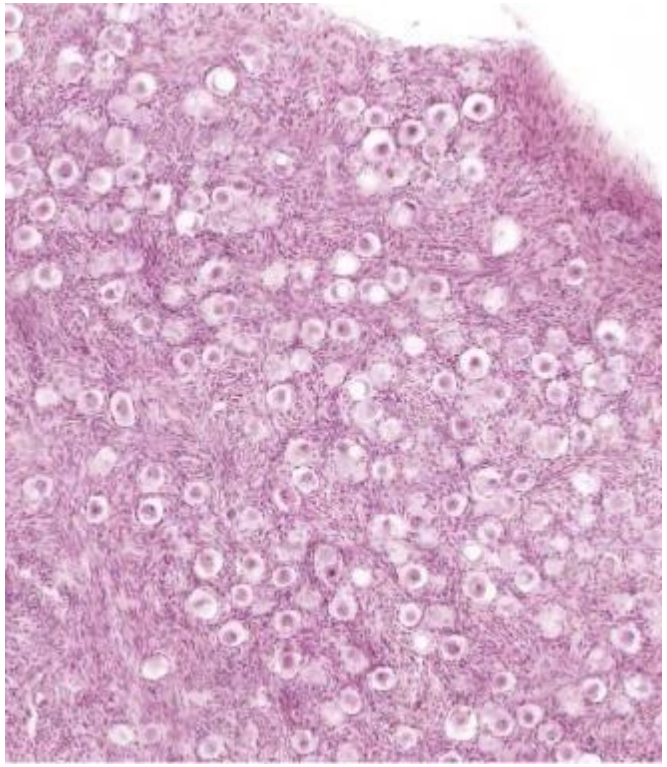
Christos Venetis
MD, FRANZCOG, MSc (Res), MSc (HCM), PhD
University of New South Wales
Sydney, Australia
c.venetis@unsw.edu.au

CENTRE FOR BIG DATA RESEARCH IN HEALTH

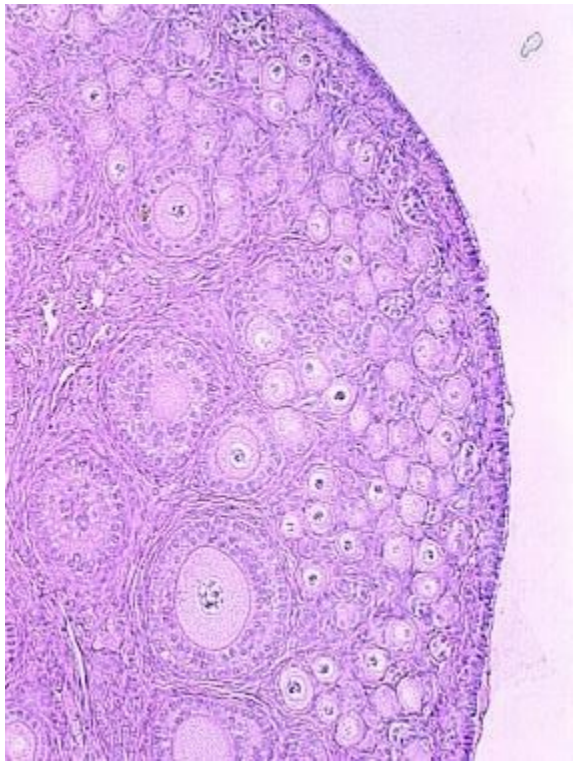
Disclosures

- Consultancy fees and Honoraria received for speaker engagements: Merck; Merck, Sharp & Dohme

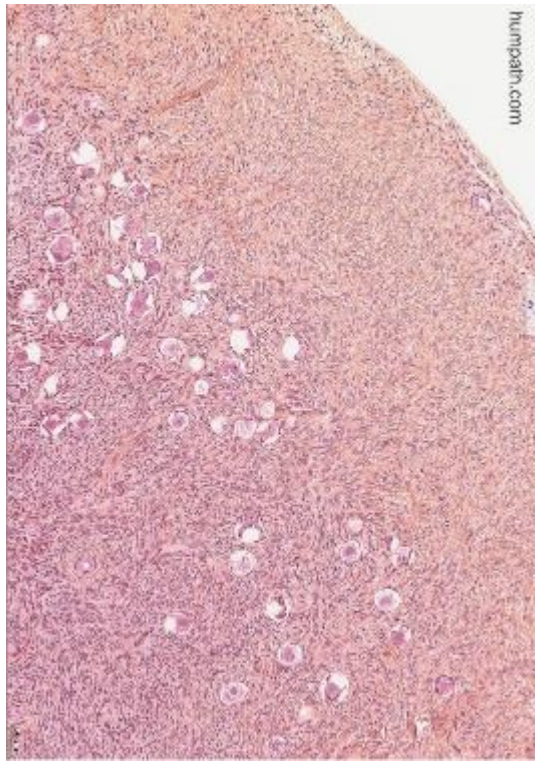
Ovarian reserve in humans (1)



Infant



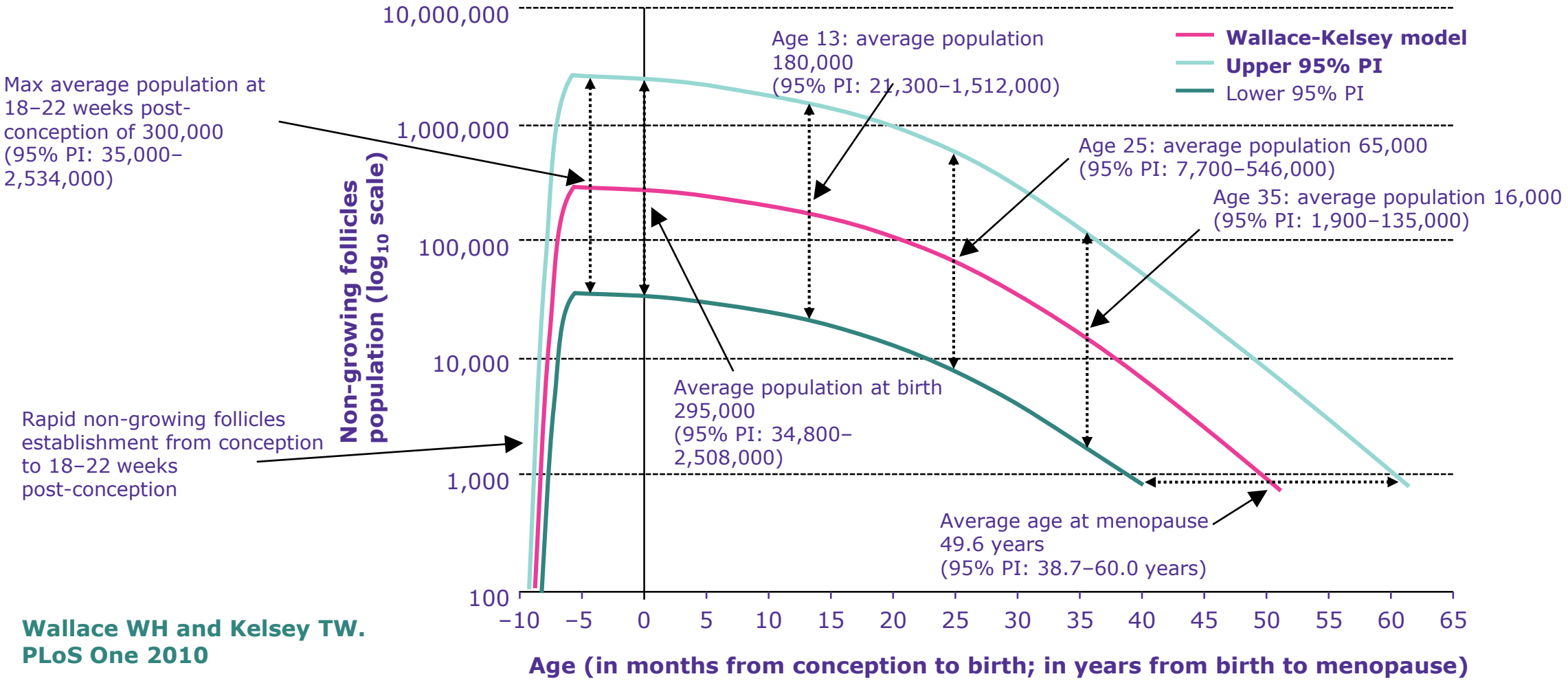
Reproductive age



Menopause

Dramatic loss of primordial follicles with advancing age

Ovarian reserve in humans (2)

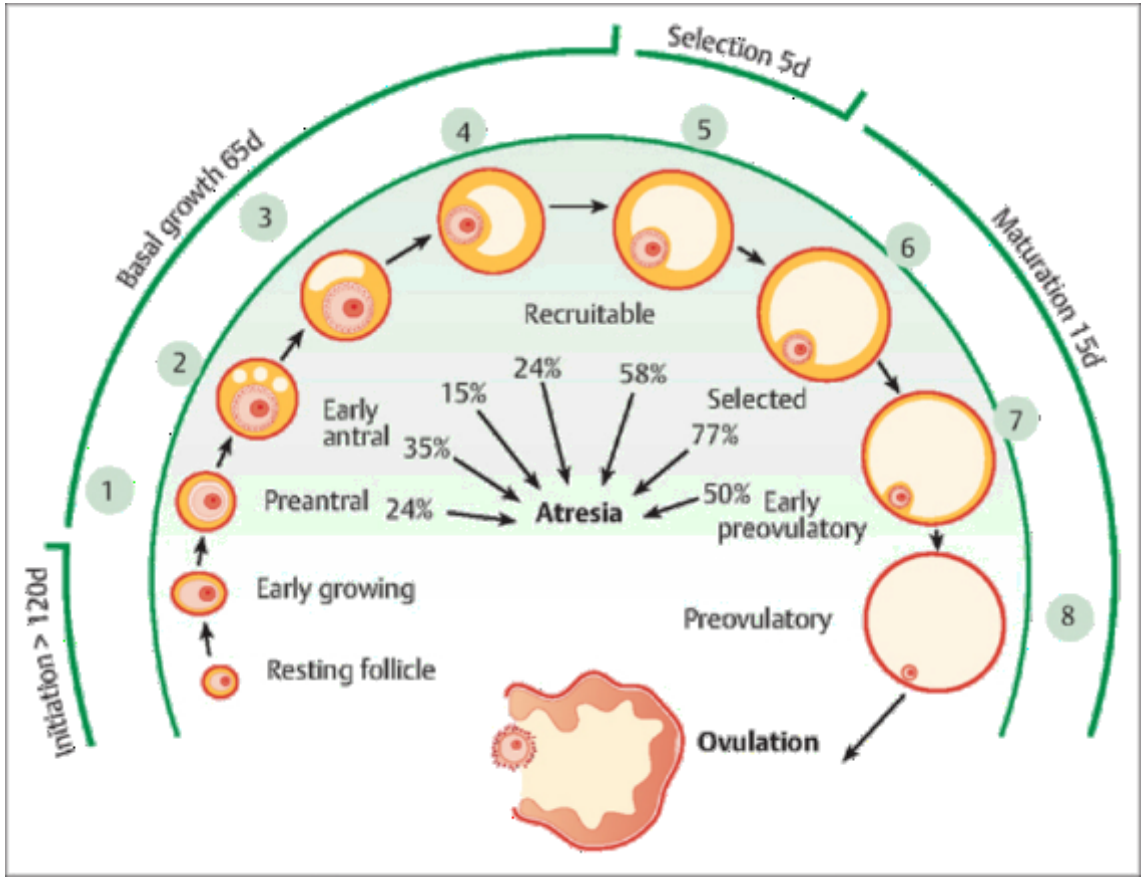


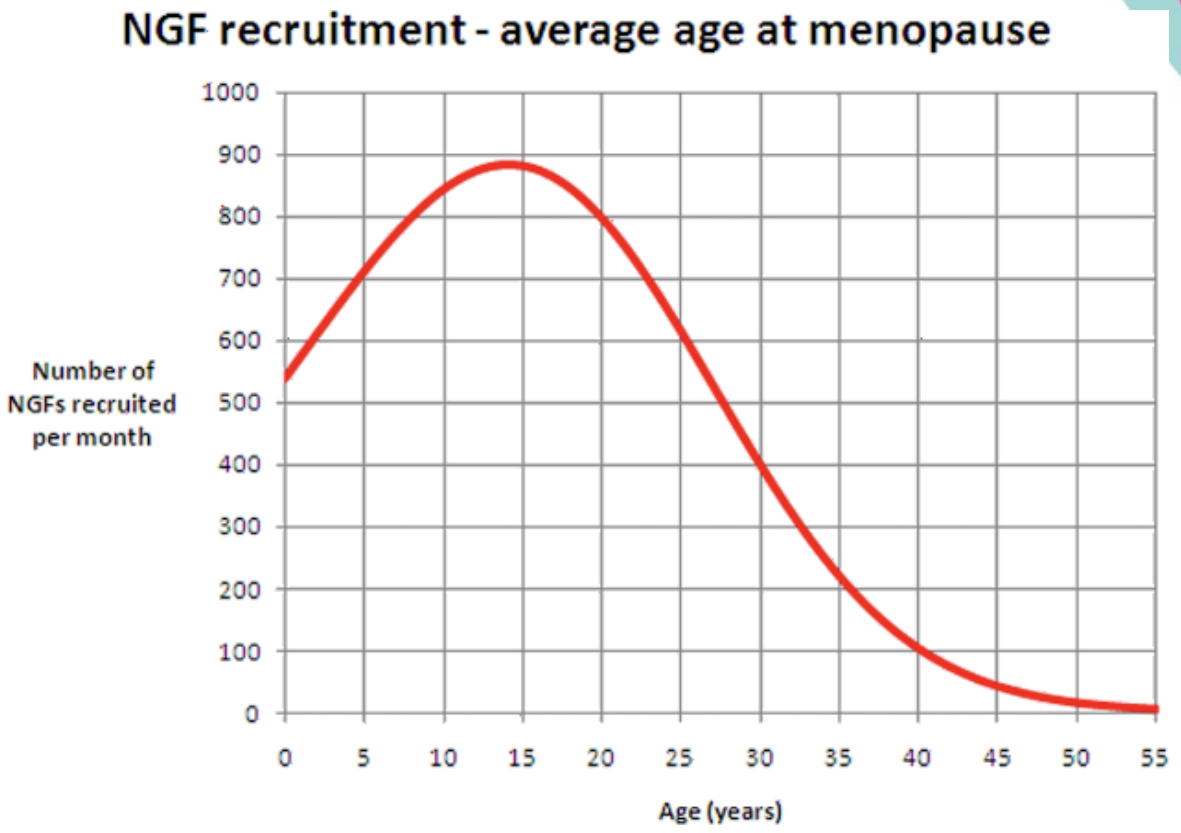
Wallace WH and Kelsey TW. PLoS One 2010

PI, prediction interval.

Figure reproduced from Wallace WH and Kelsey TW. PLoS One 2010;5:e8772.

Folliculogenesis/oogenesis in humans





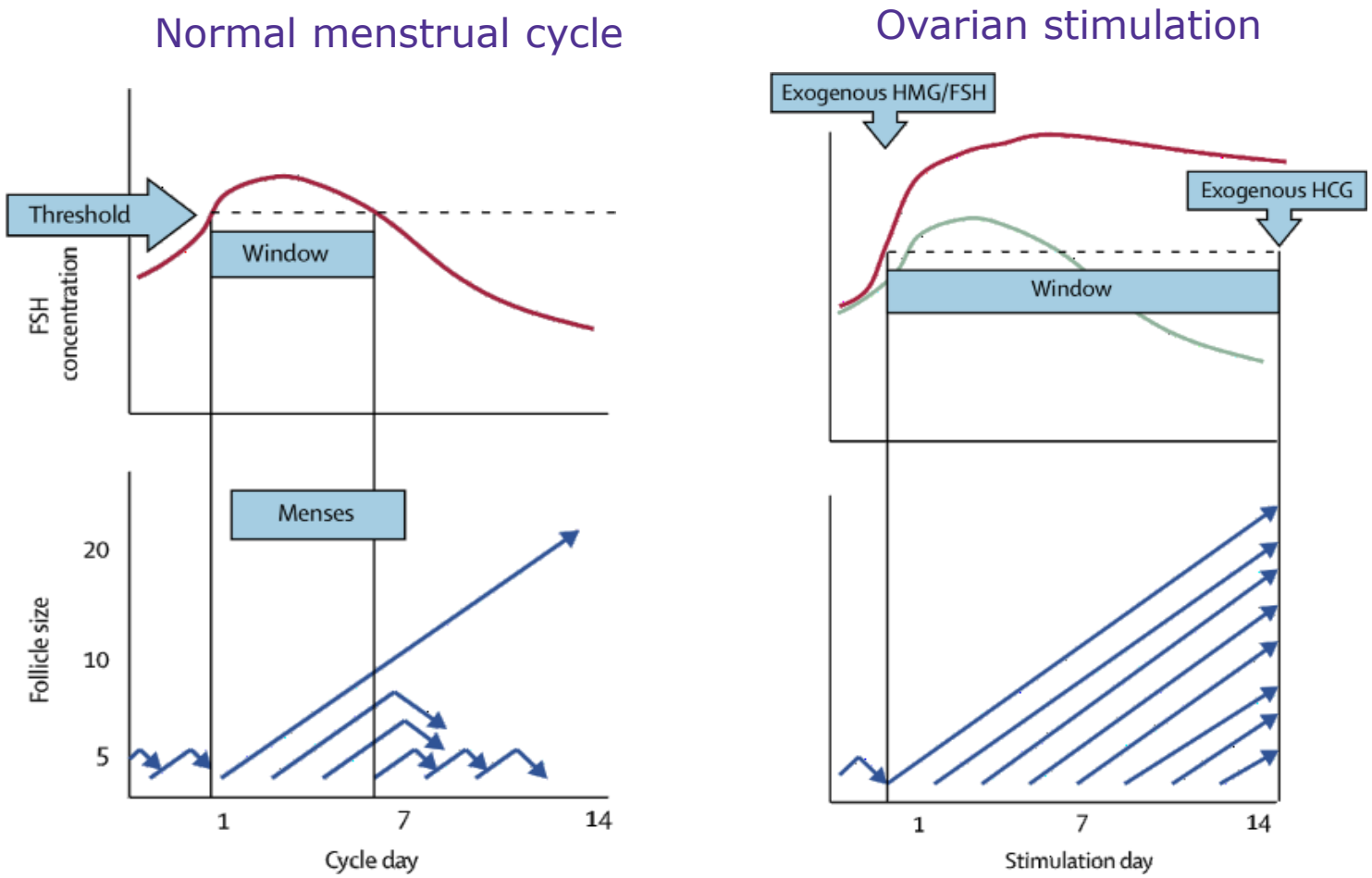
Wallace WH and Kelsey TW. PLoS One 2010

NGF, non-growing follicles.

Left image reproduced from https://obgynkey.com/the-menstrual-cycle-and-fertilization/
Right figure reproduced from Wallace WH and Kelsey TW. PLoS One 2010;5:e8772.

Ovarian stimulation

Follicular rescue



Fauser BC et al. Lancet 2005

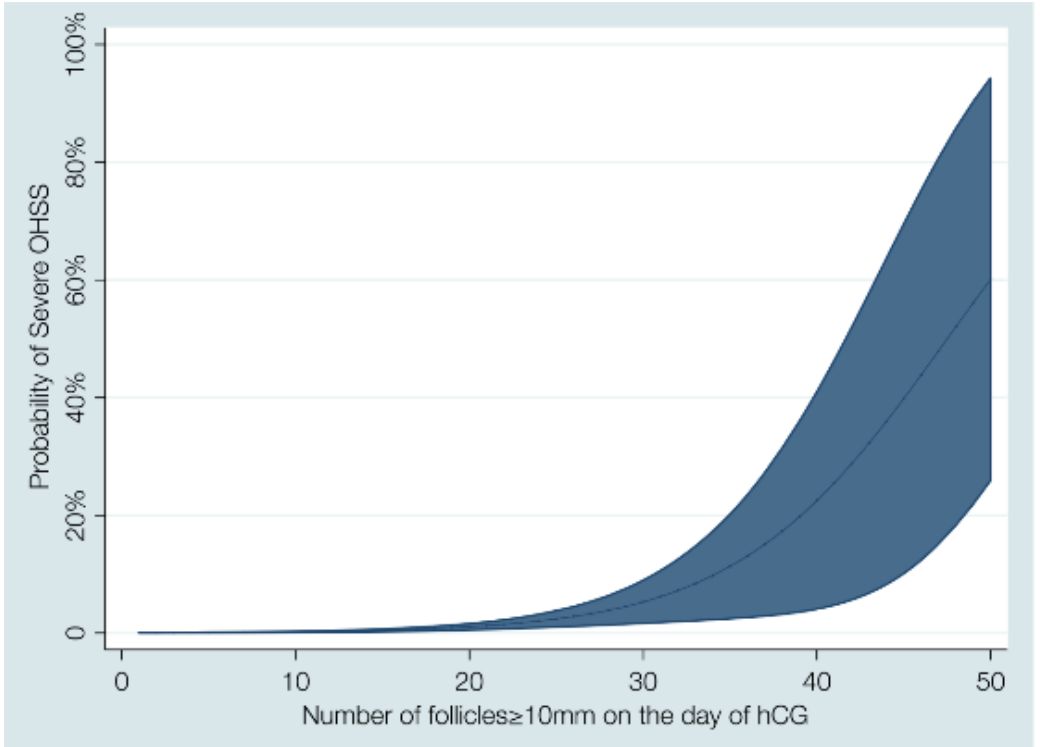
FSH, follicle-stimulating hormone; HCG, human chorionic gonadotropin; HMG, human menopausal gonadotropin.

Figure reproduced from Fauser BC, et al. Lancet 2005;365:1807–16.

Why is prediction of ovarian response important? (1)

Poor ovarian response is associated with disappointingly low pregnancy rates and is one of the most challenging patient groups to manage

Venetis C, et al. Ann NY Acad Sci 2010



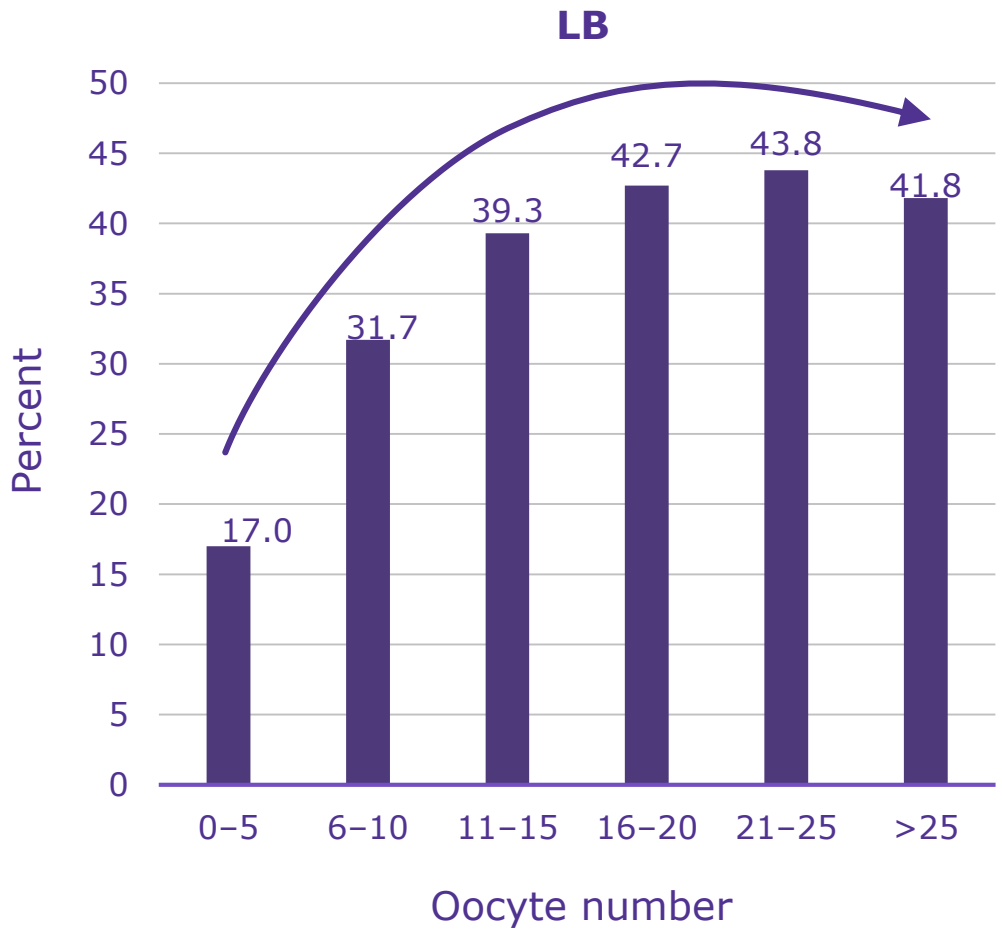
Increased number of follicles (>10 mm) on the day of triggering final oocyte maturation = increased risk of severe OHSS

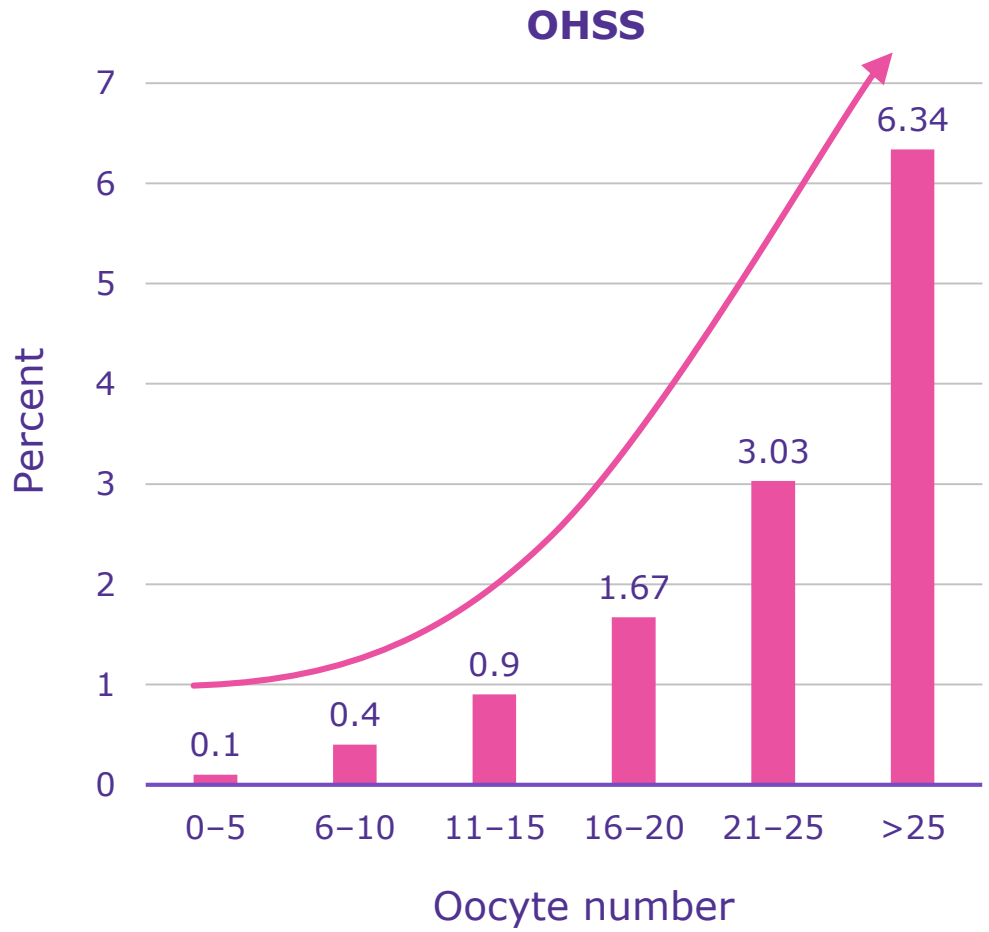
Tarlatzi TB, et al. JARG 2017

OHSS, ovarian hyperstimulation syndrome.

Venetis CA, et al. Ann NY Acad Sci 2010;1205:199–206;
Figure reproduced from Tarlatzi TB, et al. JARG 2017;34:1341–51.

Why is prediction of ovarian response important (2)





Note: percent LB and OHSS are per retrieved oocyte numbers per IVF cycle among SART members from 2008 to 2010.

LB, live birth.

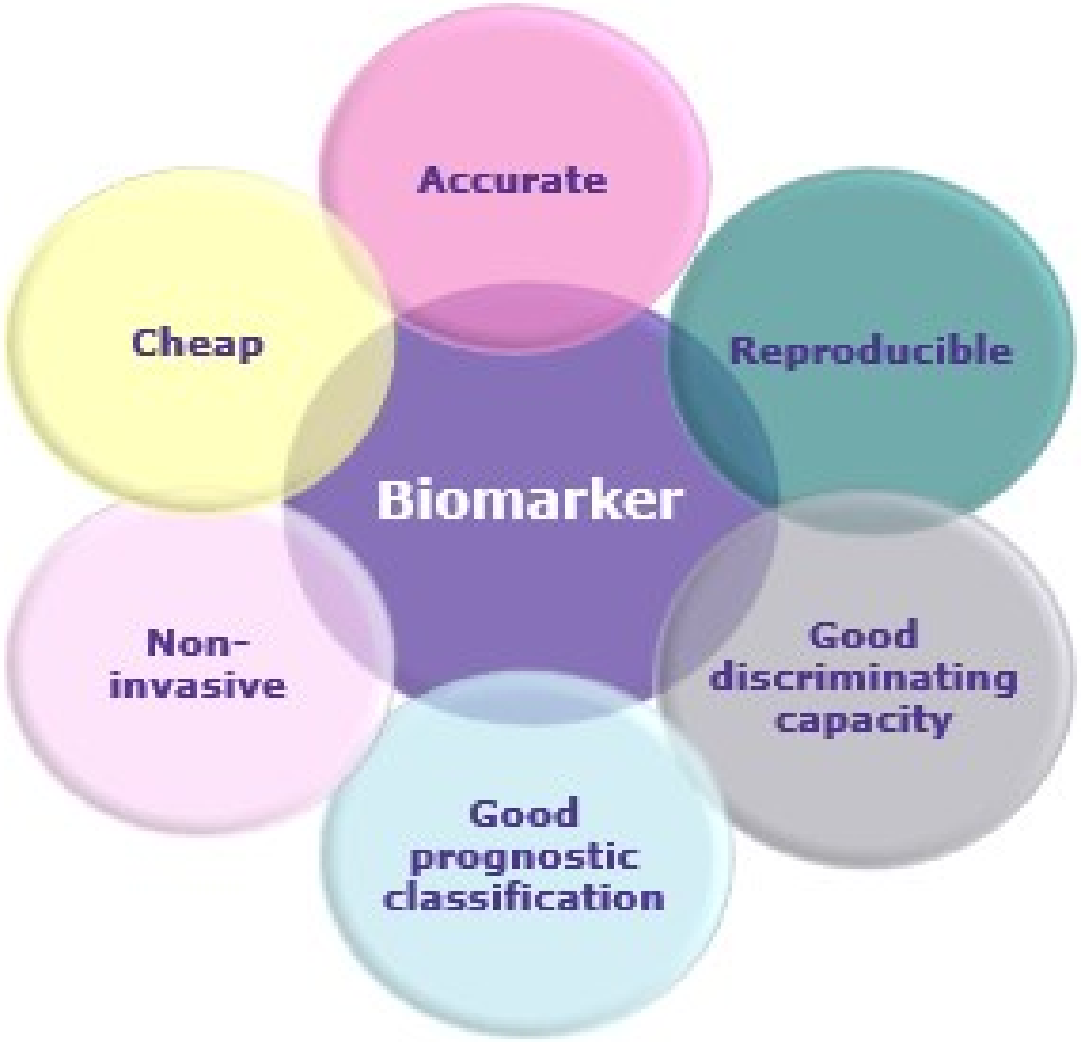
Steward RG et al. Fertil Steril. 2014;101:967–73.

Prediction of ovarian reserve and response…

…increases the efficiency and safety of ovarian stimulation

ICU, intensive care unit.

What makes a good predictor/biomarker?



Do we have reliable predictors/biomarkers of ovarian reserve and response?

- **Limited value**
- Basal FSH or LH
- Basal estradiol
- Inhibin B
- Ovarian volume
- Ovarian vascular flow

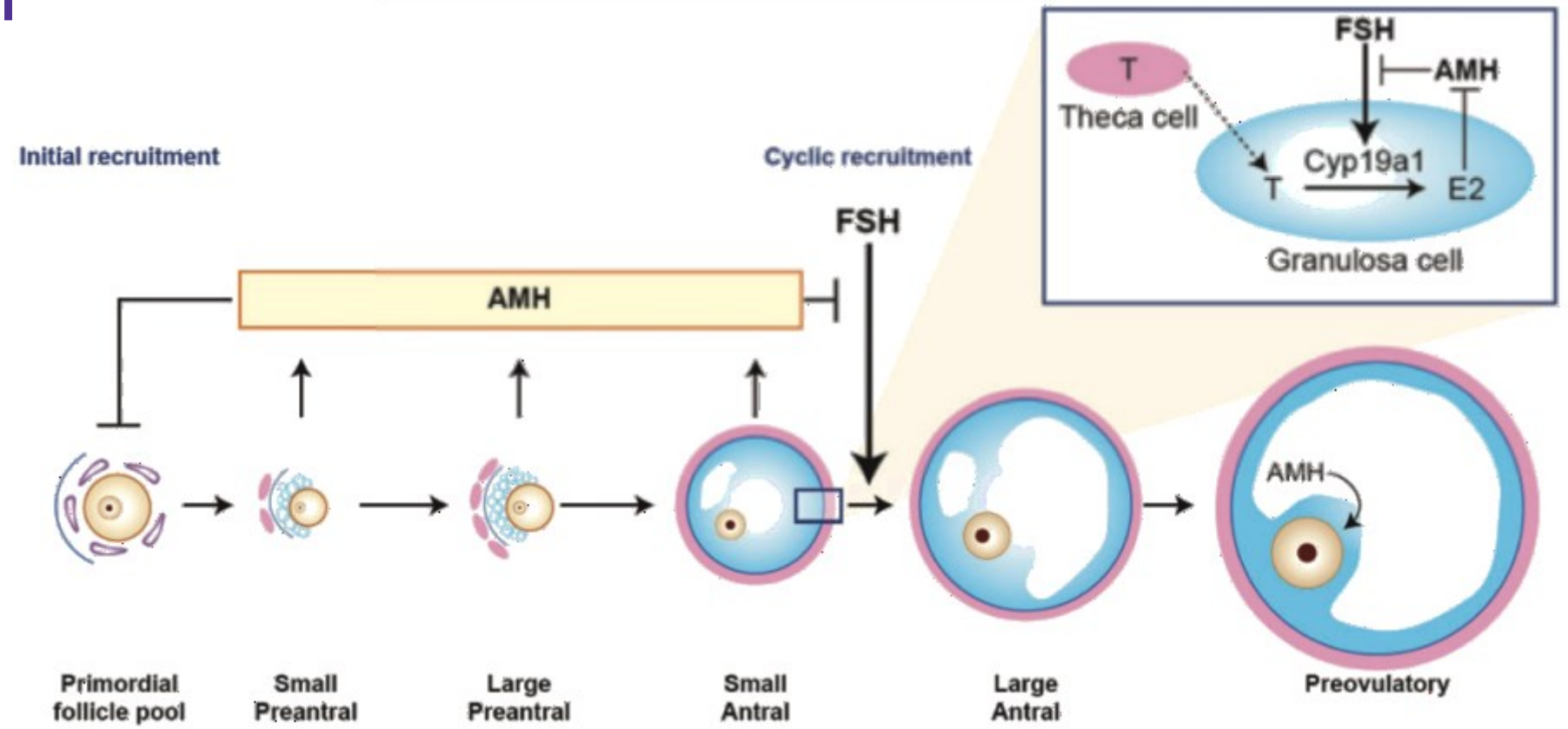
- **Most promising**
- Female age
- AFC
- AMH

AMH, anti-Müllerian hormone; AFC, antral follicle count; LH, luteinizing hormone.

Broekmans FJ, et al. Hum Reprod Update. 2006;12:685–718; Broer SL, et al. Hum Reprod Update. 2011;17:46–54; Broer SL, et al. Hum Reprod Update. 2013;19:26–36.

The era of AMH

What is AMH?



Dimeric glycoprotein
Member of the TGF-β superfamily

Cyp19a1, cytochrome P450 family 19 subfamily A member 1;
E2, estradiol.

Figure reproduced from Dewaily D, et al. Hum Reprod Update. 2014:370–85.

How is AMH measured? (1)

1990's: First report of measurement of serum AMH with ELISAs

Early 2000's: The first ultrasensitive AMH assay
(IOT assay/Beckman Coulter)

2006: An additional AMH ELISA assay using different epitopes was developed (DSL) – different result to IOT

2010: **AMH Gen II assay** developed by Beckman Coulter using the DSL antibodies – calibrated to IOT assay

DSL, Diagnostic Systems Labs; ELISA, enzyme-linked immunosorbent assay; IOT, Immunotech.

Dewaily D, et al. Hum Reprod Update. 2014:370–85.

How is AMH measured? (2)

Table I Anti-Müllerian hormone (AMH) assays characteristics.

Competitor assays	Beckman Coulter AMH Gen II (Kumar *et al.*, 2010; Beckman Coulter AMH Gen II ELISA package insert, 2013)	Beckman Coulter Immunotech AMH (Immunotech AMH (Beckman Coulter) package insert, 2012)	AnshLabs Ultrasensitive (AnshLabs UltraSensitive AMH/MIS ELISA package insert, 2014)	AnshLabs picoAMH (AnshLabs picoAMH ELISA package insert, 2014)	Roche Assay Elecsys® AMH (Gassner and Jung, 2014)	Beckman Coulter Access 2 IA AMH (Beckman Coulter, 2014)
Assay type	Manual	Manual	Manual	Manual	Automated	Automated
Imprecision	<8%	<14%	<6%	<6%	1.8–2.0%	2.87–4.34%
Sample type	Serum, plasma	Serum, plasma	Serum, plasma	Serum, plasma	Serum, Li-heparin plasma	Serum, Li-heparin plasma
Minimum sample volume	20 µl	25 µl	50 µl	100 µl	50 µl	20 µl
Incubation time	<3 h	3 h	2.5 h	4.5 h	18 min	39 min
Limit of detection (LoD)	0.08 ng/ml	0.14 ng/ml	0.023 ng/ml	0.0012 ng/ml	0.01 ng/ml	≤0.02 ng/ml
Limit of Quantification (LoQ)	0.16 ng/ml	0.35 ng/ml (Decanter *et al.*, 2014)	0.06 ng/ml	0.0039 ng/ml	0.03 ng/ml	≤0.08 ng/ml
Measurement range	0.16–22.5 ng/ml	0.42–21.0 ng/ml	0.06–11.6 ng/ml	0.003–0.75 ng/ml	0.01–23.0 ng/ml	0.02–24.0 ng/ml

Results are not directly comparable and have to be interpreted in an assay specific manner

Table reproduced from Iliodromiti S, et al. Hum Reprod Update. 2015;21:698–710.

Issues with AMH measurement?
Assay specific

- Manual assays:
- Substantial variability between labs/lack of standardization
- Recent field notices regarding complement interference
- Complement interference seems to be addressed with freezing storage

- Automated assays:
- Better standardization
- Recent tests seem to suggest that complement does not interfere with AMH measurement in automated protocols

Han X, et al. Hum Reprod. 2014;29:1042–8;
Iliodromiti S, et al. Hum Reprod Update. 2015;21:698–710.

Issues with AMH measurement? Inter/intra-individual

Inter-individual factors

- Age
- Ovarian surgery/ Endometriosis/ Prior gonadotoxic chemotherapy
- Ethnicity (Chinese, Black African, Hispanic and South Asian< Caucasian)
- Smoking
- Pregnancy, GnRH analogues, combined hormonal contraceptives
- Chronic disease/ malignancies

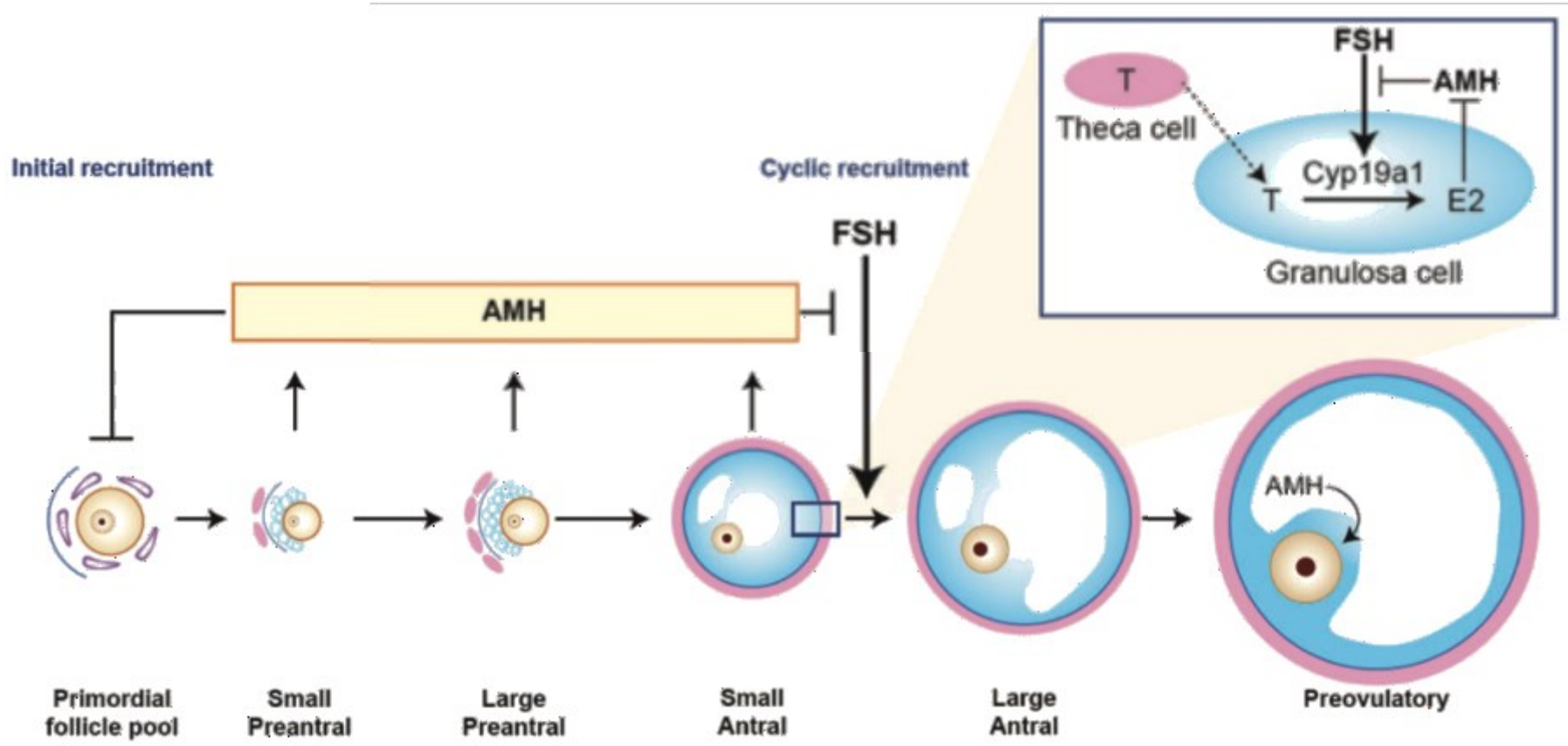
Intra-individual factors

- Minimal cyclic variation during the menstrual cycle (late follicular phase)
- Low variability between menstrual cycles

GnRH, gonadotropin-releasing hormone.

Dewaily D, et al. Hum Reprod Update. 2014:370–85;
Iliodromiti S, et al. Hum Reprod Update. 2015;21:698–710;
McCredie S, et al. Reprod Biomed Online. 2017;34:522–33.

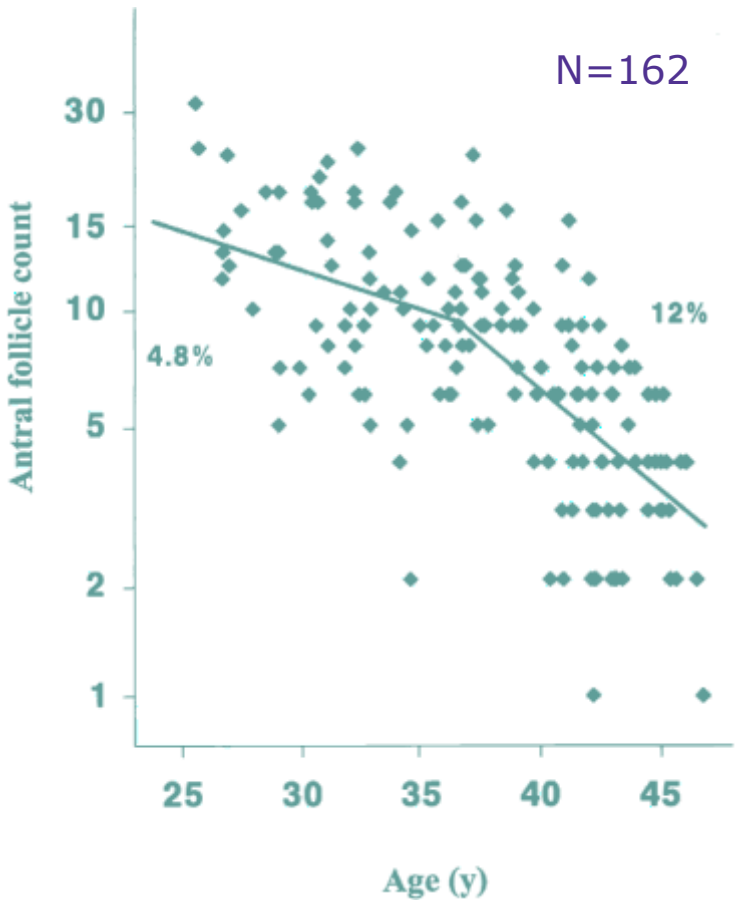
What is AFC?



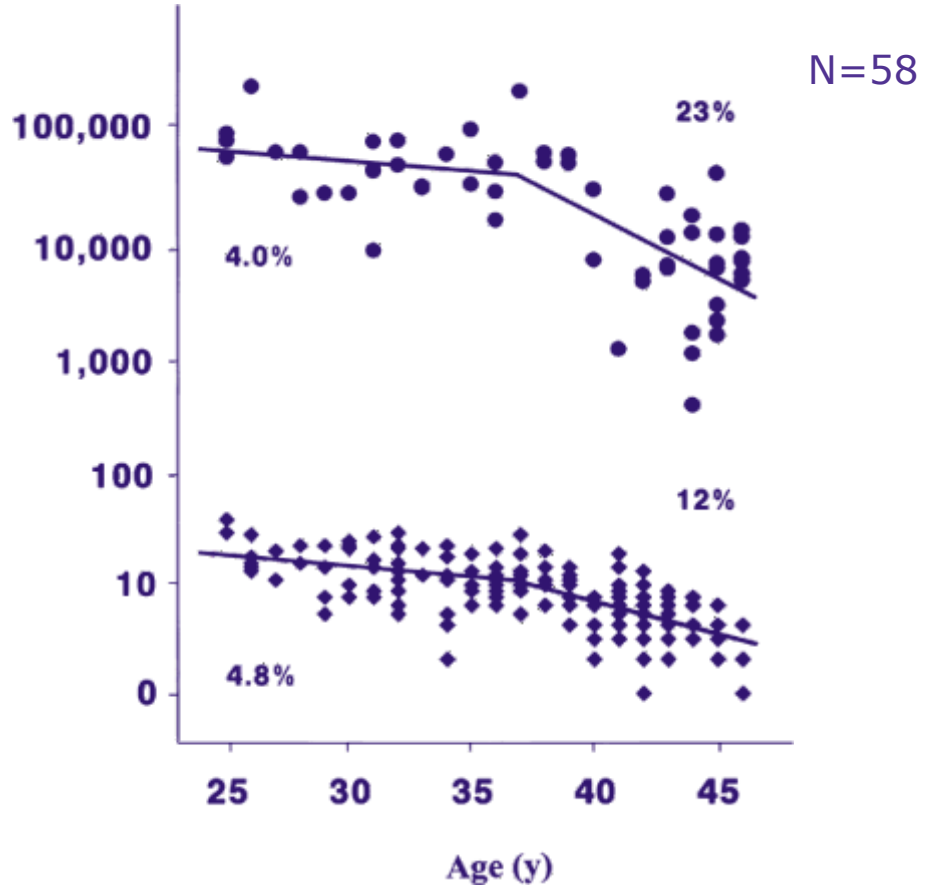
Follicles with an antrum, 2–10 mm in diameter

Figure reproduced from Dewaily D, et al. Hum Reprod Update. 2014:370–85.

Is AFC associated with ovarian reserve?



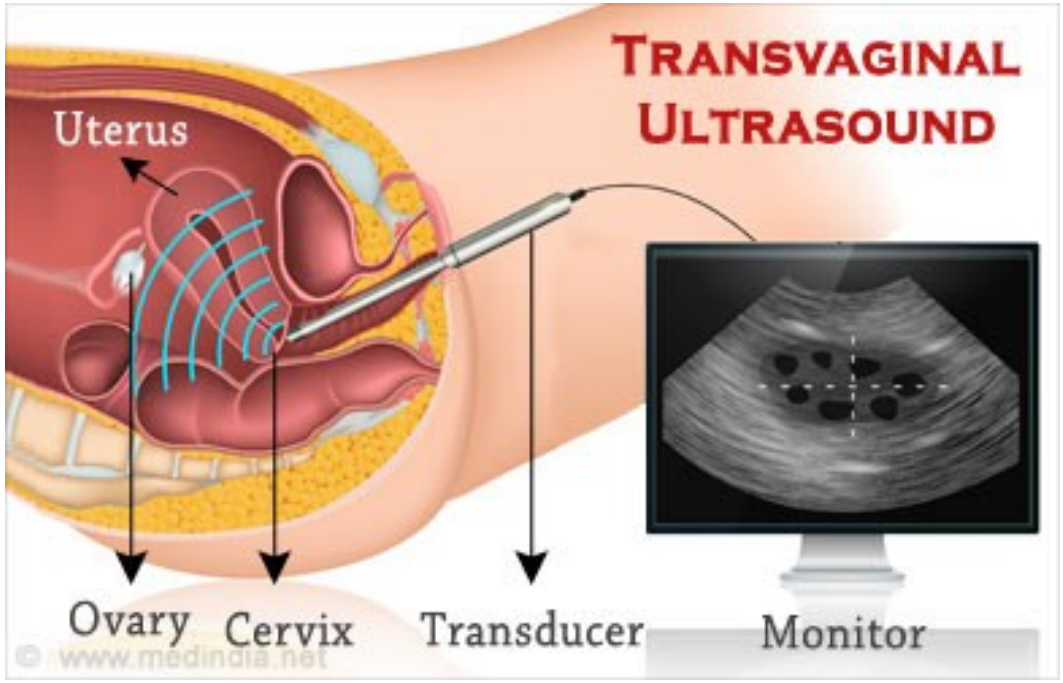
AFC declines with age

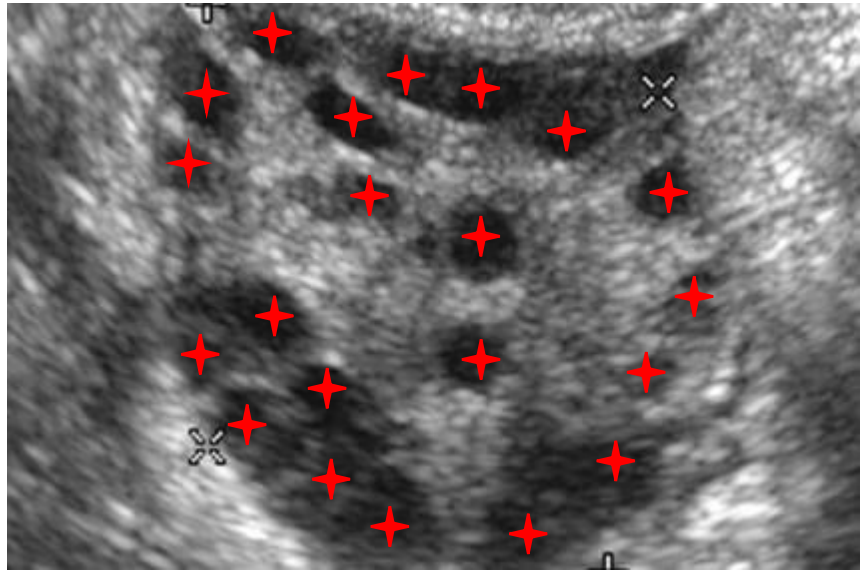


Similar pattern of loss of primordial follicles and AFCs with age

Figures reproduced from Scheffer GJ, et al. Fertil Steril. 1999;72:845–51.

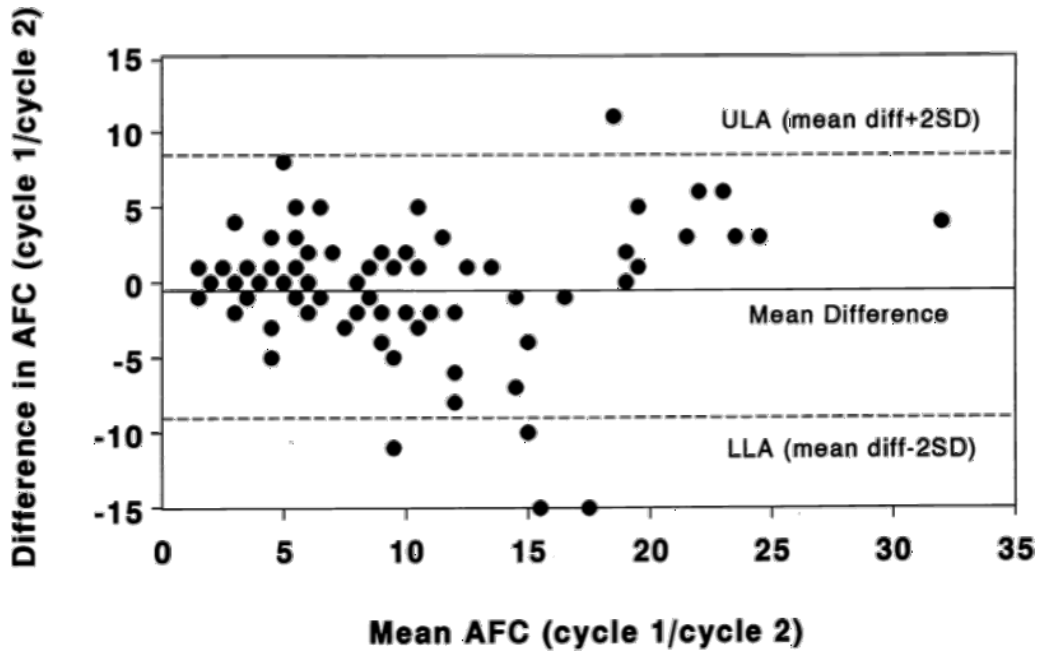
How is AFC measured?



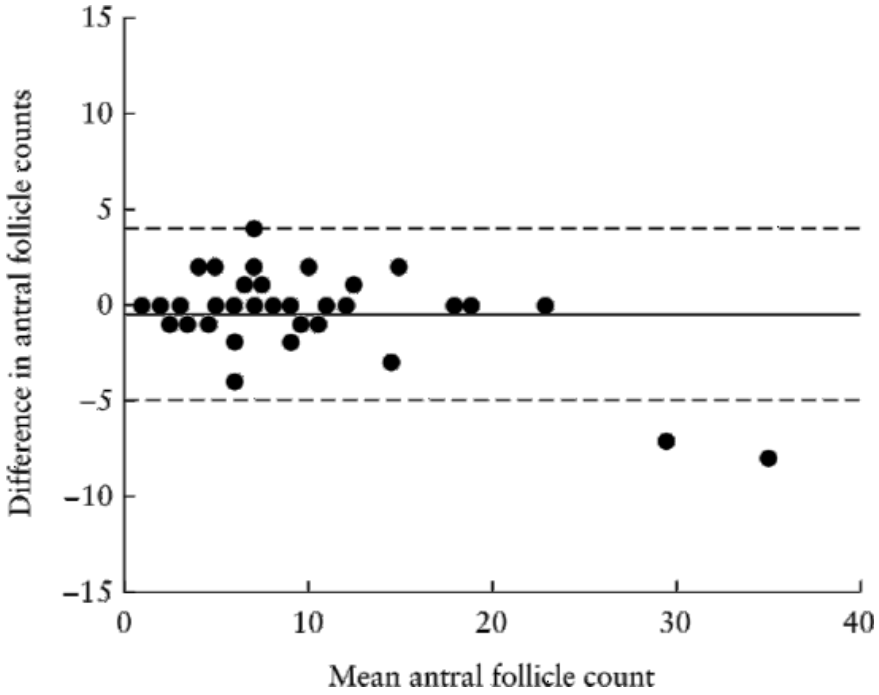


21 AFC

AFC measurement: Inter-cycle & inter-observer variability



Moderate inter-cycle agreement at the entire range of AFC



Moderate inter-observer variability increasing with higher AFCs

LLA, lower limit of agreement; ULA, upper limit of agreement.

Left graph reproduced from Scheffer GJ, et al. Fertil Steril. 1999;72:845–51; right graph reproduced from Scheffer GJ, et al. Ultrasound Obstet Gynecol. 2002;20(3):270–5.

AFC measurement: Pros and cons

Pros

- Allows the assessment of recruitable follicles for the specific cycle
- Allows the assessment of the potential of each ovary
- Provides information regarding the sizes of follicles
- Can provide information regarding other ovarian pathology (cysts etc.)

Cons

- Invasive/ TVUS
- Requires expensive equipment
- Performed at a specific time of the cycle
- Inter-operator variability/lack of standardization
- Inter-cycle variability
- May include atretic follicles

TVUS, transvaginal ultrasound.

Standardizing AFC measurement

- Patients with regular menstrual cycles with no coexisting ovarian pathologies
- Day 2–4 of cycle
- Include all follicles 2–10mm in diameter
- Have appropriately trained personnel
- Use TVUS with a probe of a minimum frequency of 7 MHz
- Systematically sweep each ovary
- Measure the largest follicle in two dimensions
- If that follicle is ≤ 10mm then count all round or oval transonic structures within the ovarian margins – combine the number of follicles in each ovary
- If the largest follicle is > 10mm measure the largest follicles until a follicle with a mean diameter of ≤ 10mm is identified – measure all follicles and subtract the ones > 10mm

Broekmans FJ, et al. Fertil Steril. 2010;94:1044–51.

Automated AFC measurement?

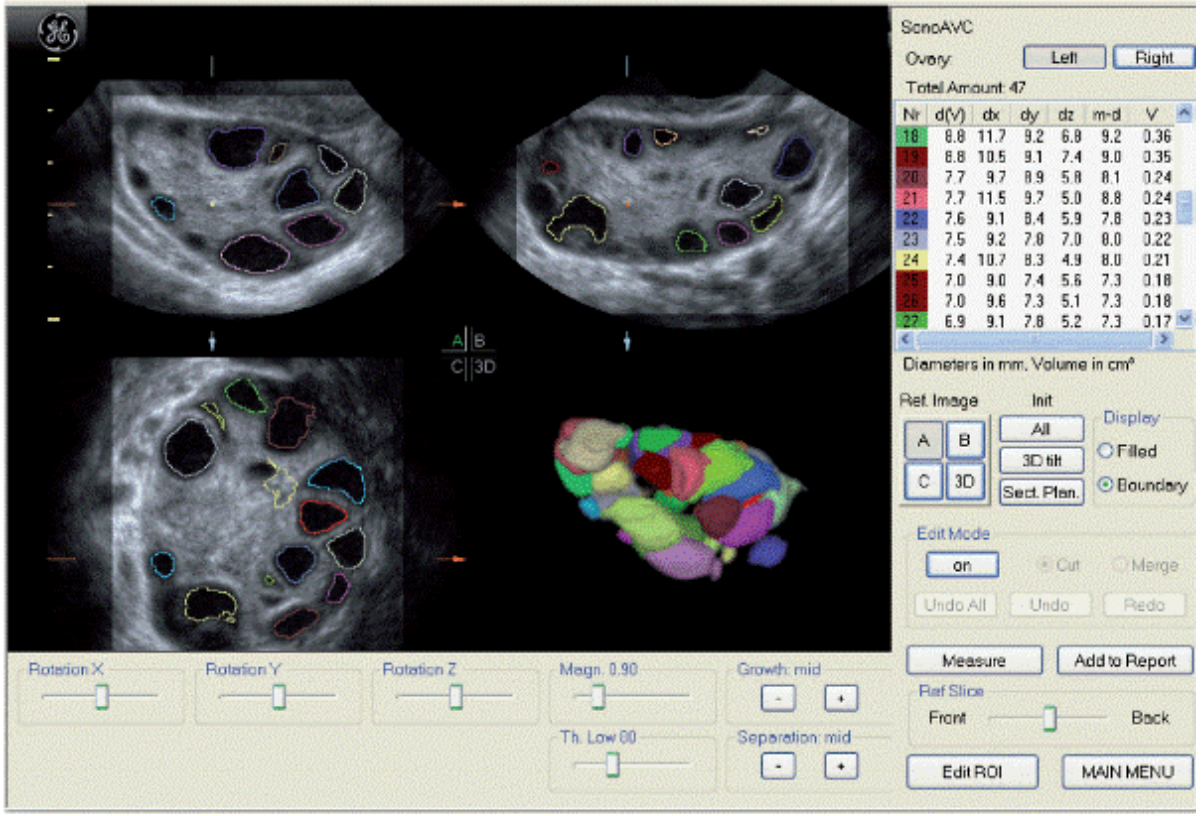


Figure 1. Application of SonoAVC™ on a polycystic ovary. Presence of more than 12 antral follicles measuring 2–9 mm are displayed within an ovary.

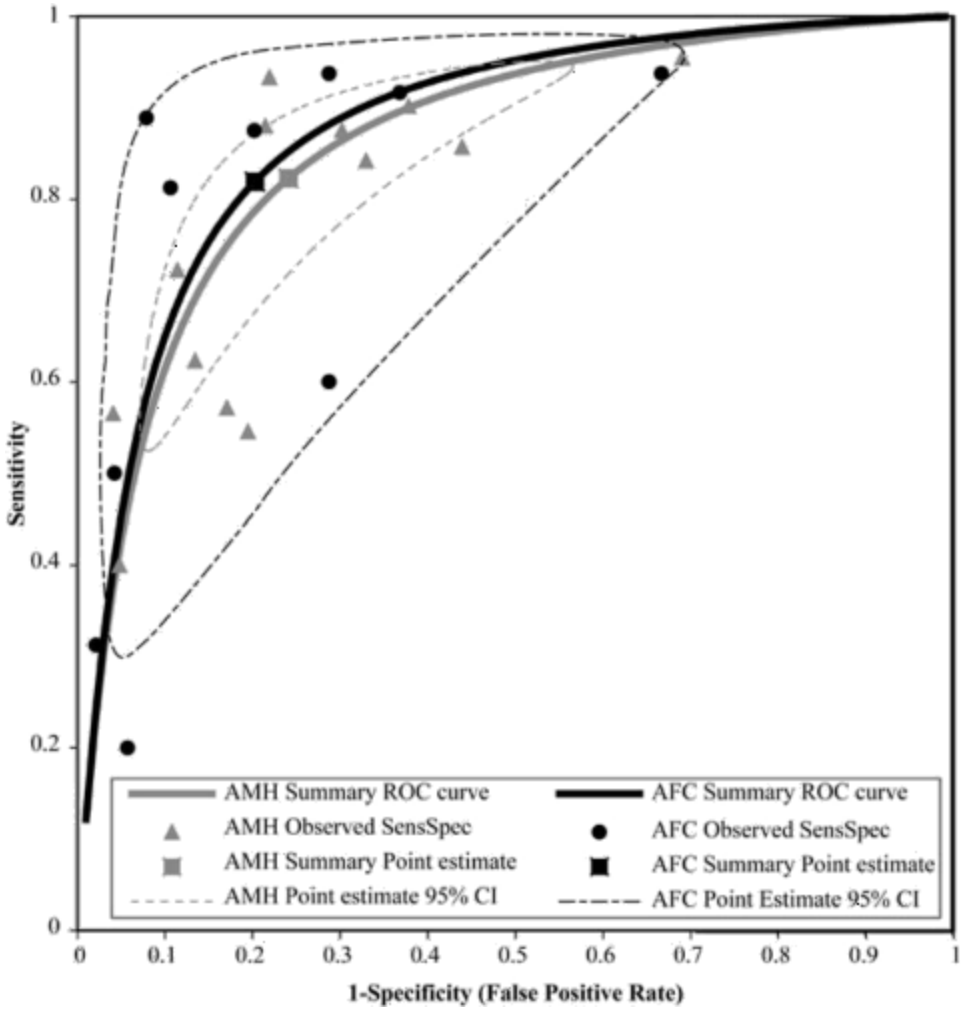
Accurate and reproducible automated system that determines the absolute AFC measurement and volume

Figure reproduced from Jayaprakasan K, et al. Imaging Med. 2010;2:135–50; Vandekerckhove F, et al. Front Surg. 2014;1:18.

AFC vs AMH: clinical data

Which one is best at predicting ovarian response after ovarian stimulation?

AFC vs AMH: hyper-response



Only AMH
6 studies

AMH + AFC
3 studies

AFC only
2 studies

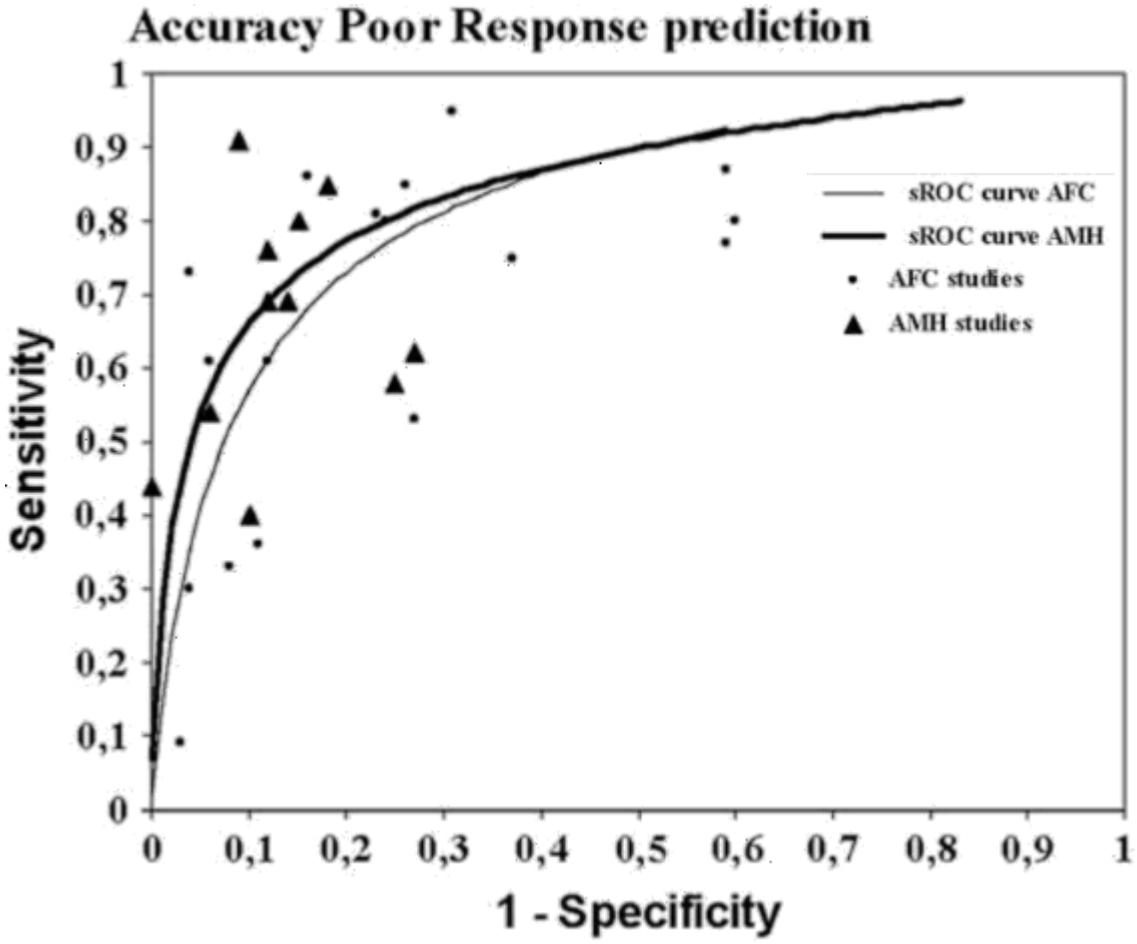
Predictor	Sensitivity	Specificity
AMH	82%	76%
AFC	82%	80%

"Both AMH and AFC are accurate predictors of excessive response to ovarian hyperstimulation.
Moreover, both tests appear to have clinical value"

ROC, receiver operating characteristic.

Figure reproduced from Broer SL, et al. Hum Reprod Update. 2011;17:46–54.

AFC vs AMH: poor response



Only AMH
13 studies

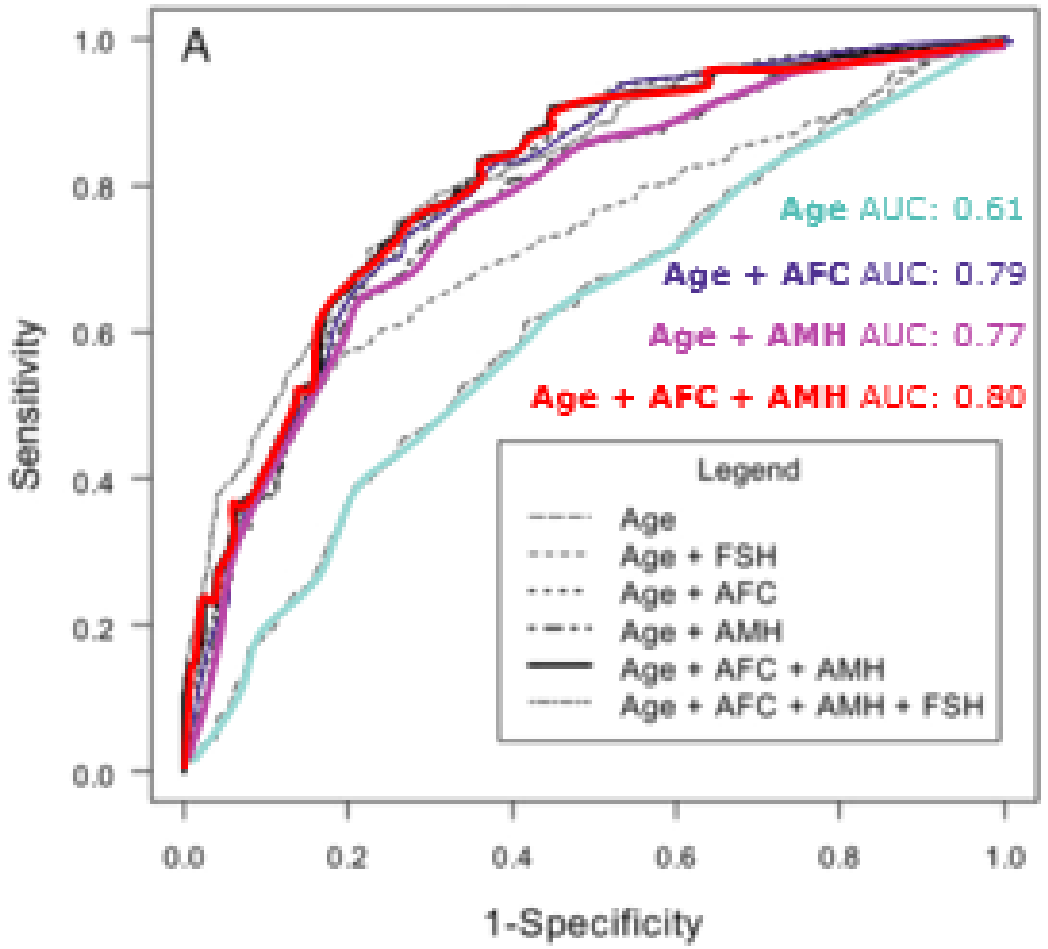
AFC only
17 studies

- *"Both AMH and AFC are accurate predictors of poor response to ovarian hyperstimulation"*
- *No significant difference seems to be present between the two tests*
- *Both tests appear to have clinical value*

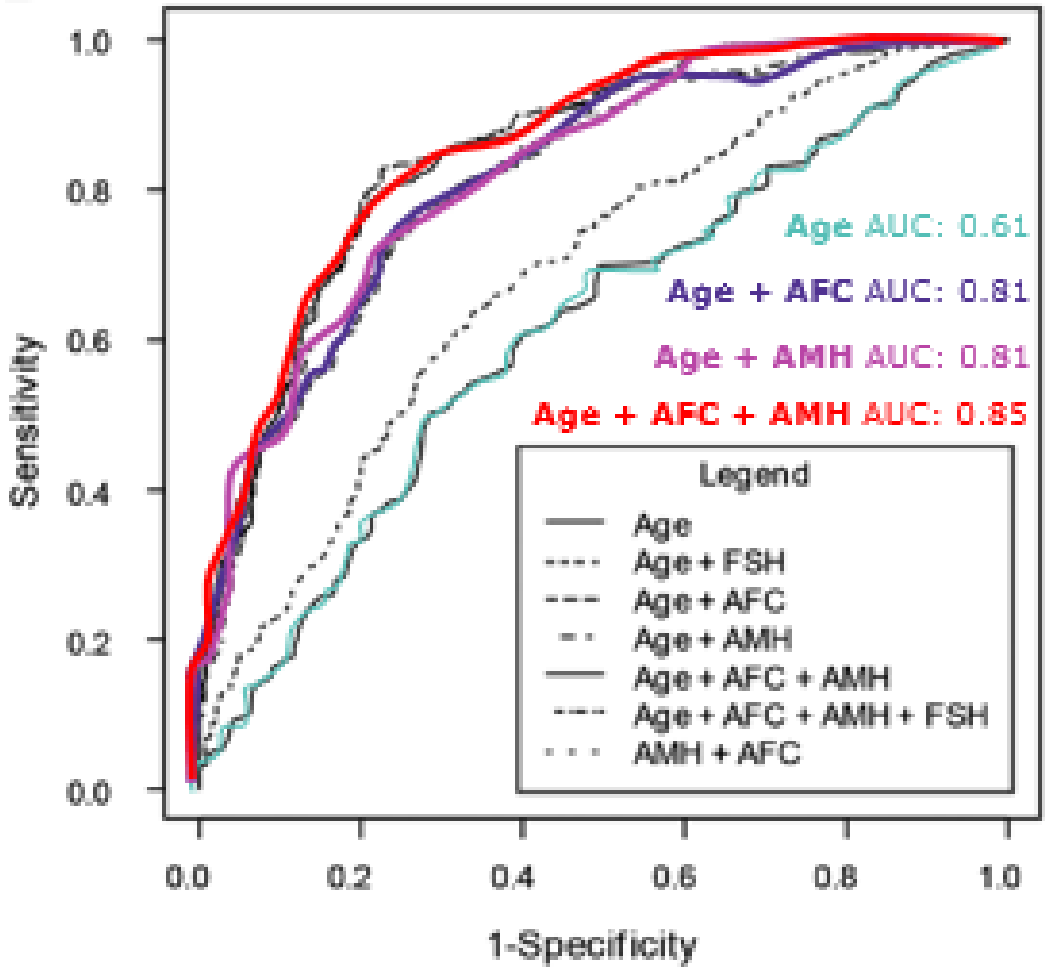
Figure reproduced from Broer SL, et al. Fertil Steril. 2009;91:705–14; Broer SL, et al. Hum Reprod Update. 2011;17:46–54.

What about combining ORTs? (1)

IPD Meta-analysis: 28 studies
Poor response



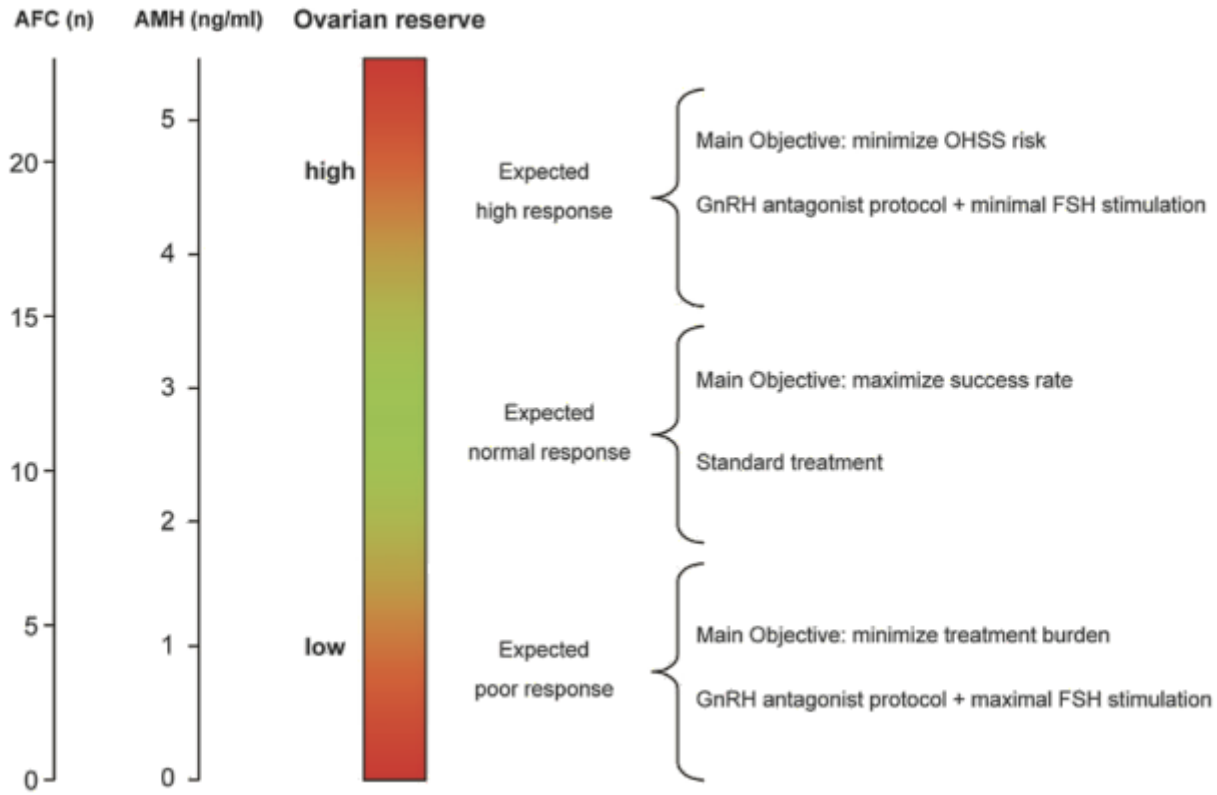
IPD Meta-analysis: 32 datasets
Hyper-response



AUC, area under the curve; IPD, individual patient data; ORT, ovarian reserve test.

Figures reproduced from Broer SL, et al. Hum Reprod Update. 2013;19(1):26–36 & Broer SL, et al. Fertil Steril. 2013;100:420-9.e7.

Purpose of ORTs

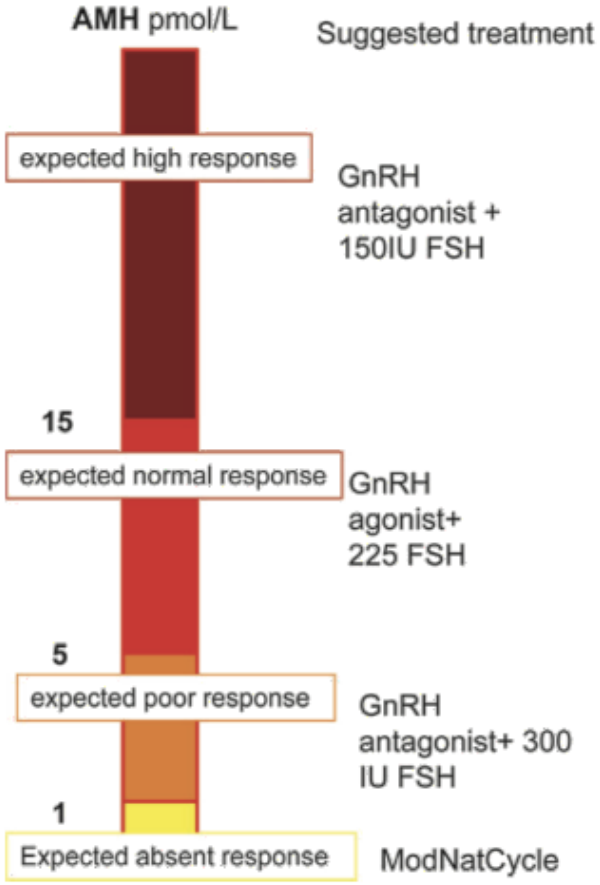


Nomograms

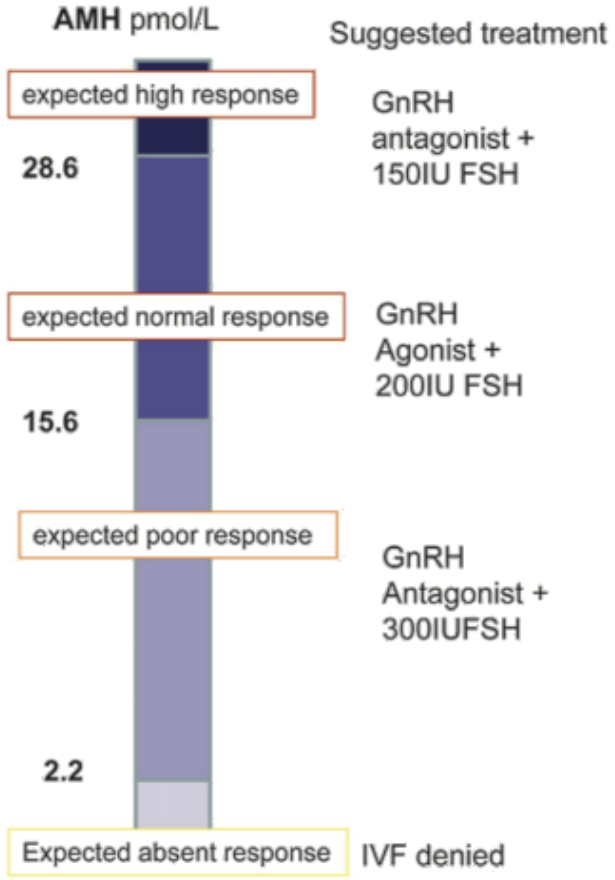
Note: ORT before the first IVF cycle would permit to categorize patients as expected poor-, normal- or hyper-responders. Since there is no evidence of superiority of one approach over another in the treatment of poor responders, the protocol associated with reduced discomfort and treatment burden should be preferred.
In hyper-responder patients, one of the most important objectives of medical counselling is to prevent OHSS. Hence the first line protocol would be based on administration of low doses of FSH in a GnRH-antagonist-based scheme

Figure reproduced from La Marca A, Sunkara SK. Hum Reprod Update. 2014;20:124–40.

Examples of ORT nomograms



Nelson SM et al. 2009



Yates AP et al. 2011

Yates AP, et al. Hum Reprod. 2011;26:2353–62;
Nelson SM, et al. Hum Reprod. 2009;24:867–75;
figure reproduced from La Marca A and Sunkara SK. Hum Reprod Update. 2014;20:124–40.

Prediction of response using ORT: RCTs? (1)

ORT algorithm vs. standard dose (150 IU)

Study	N	Population	Predictors	Aim	Stimulation protocol
Popovic-Todorovic et al., 2003	262	First IVF/ICSI, bFSH<12.5 IU/L, regular cycles, <39 yo	AFC, ovarian stromal blood flow, serum testosterone, smoking	5–14 oocytes	Long GnRH agonist
Olivennes et al., 2015	200	18–34 yo, Normoovulatory, BMI<30 kg/m^{2,} bFSH <12 IU/L	bFSH, age, BMI, AFC	COCs	Long GnRH agonist
Oudshoorn et al., 2017	1032*	First IVF/ICSI, AFC<11 or >15, regular cycles, no PCOS, <44 yo	AFC	CLBR	Long GnRH agonist or daily antagonist
Nyboe-Andersen et al., 2017	1329	First IVF/ICSI, bFSH: 1–15 IU/L, BMI: 17.5–32 kg/m^2, regular cycles, <40 yo	AMH (Roche)	OPR	GnRH antagonist fixed Day 6

*Data from two of the direct dose comparison studies conducted in tandem (Oudshoorn 2017 and Van Tilborg 2017) were included as one ORT-algorithm study (Oudshoorn 2017) in this analysis.
bFSH, basal follicle-stimulating hormone; BMI, body mass index; CLBR, cumulative live birth rate; ICSI, intracytoplasmic sperm injection; OPR, ongoing pregnancy rate; PCOS, polycytstic ovary syndrome; RCT, randomized controlled trial; yo, year's old.

Lensen SF, et al. Cochrane Database Syst Rev. 2018 Feb 1;2:CD012693.

Prediction of response using ORT: RCTs? (2)

ORT algorithm vs. standard dose (150 IU)

Outcome	RCTs/ N	Effect size (95% CI)	P-value	Interpretation
Live birth or ongoing pregnancy	4/2823	OR: 1.04 (0.88-1.23)	0.68	No association
Moderate or severe OHSS	4/2823	OR: 0.58 (0.34-1.00)	0.048	Negative association
Poor response	2/1294	OR:1.16 (0.90-1.50)	0.24	No evidence of an effect
Normal response	3/2623	OR: 1.22 (1.04-1.43)	0.013	Positive association
Hyper-response	2/1294	OR: 0.56 (0.42-0.76)	<0.001	Negative association
Cancellation due to poor response	4/2823	OR: 1.19 (0.89-1.60)	0.25	No evidence of an effect
Cancellation due to hyper response	4/2823	OR: 0.37 (0.24-0.57)	<0.001	Negative association
Total dose of FSH	3/1494	WMD: -157 (-215 to -98)	<0.001	Negative association

CI, confidence interval; OR, odds ratio; WMD, weighted mean difference.

Lensen SF, et al. Cochrane Database Syst Rev. 2018 Feb 1;2:CD012693.

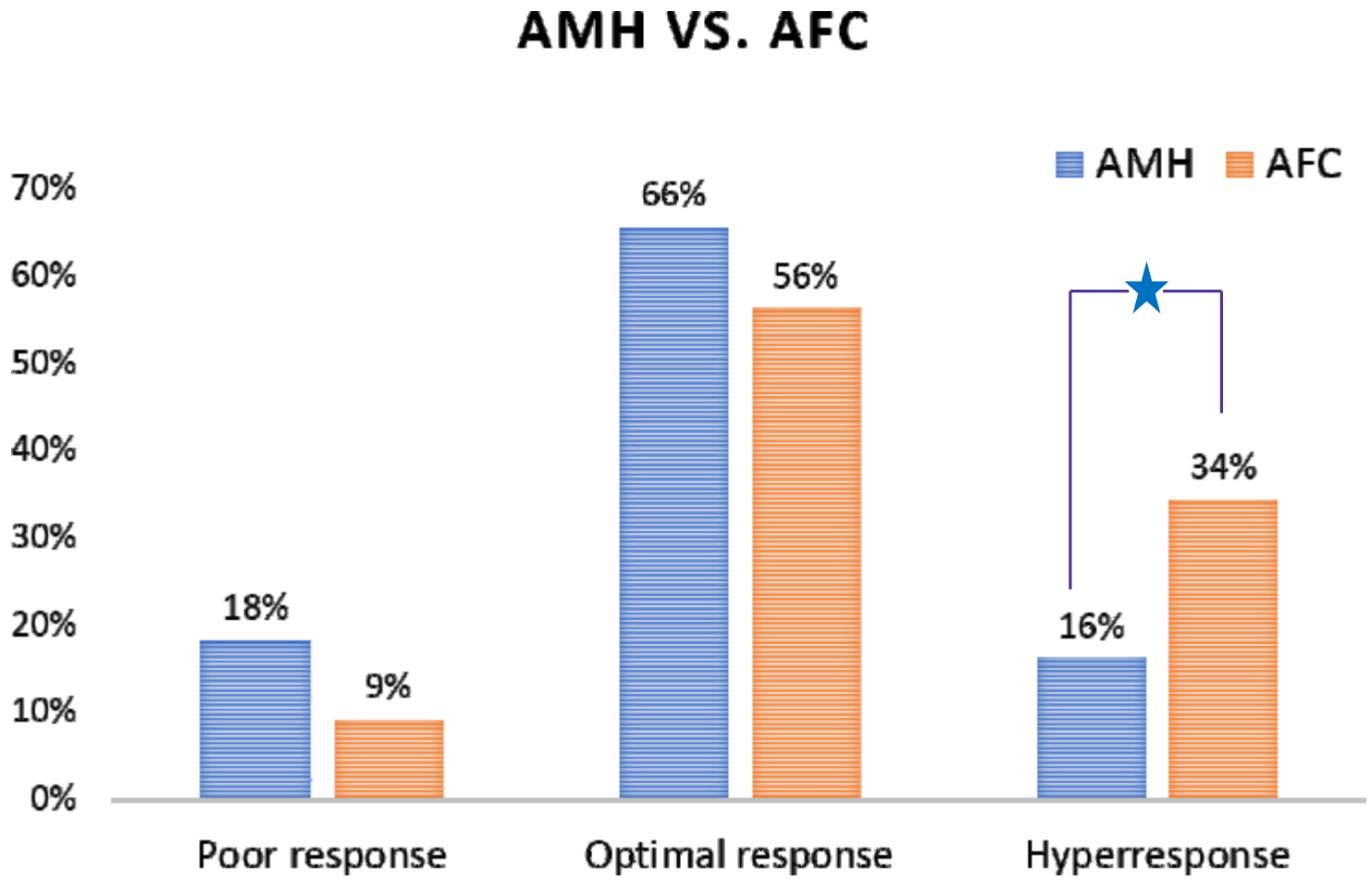
AMH vs. AFC based algorithm: RCT data

RCT: 368 patients

Starting dose of rFSH

AMH (ng/mL)
<0.7: 375 IU
0.7-2.1: 225 IU
>2.1: 150 IU

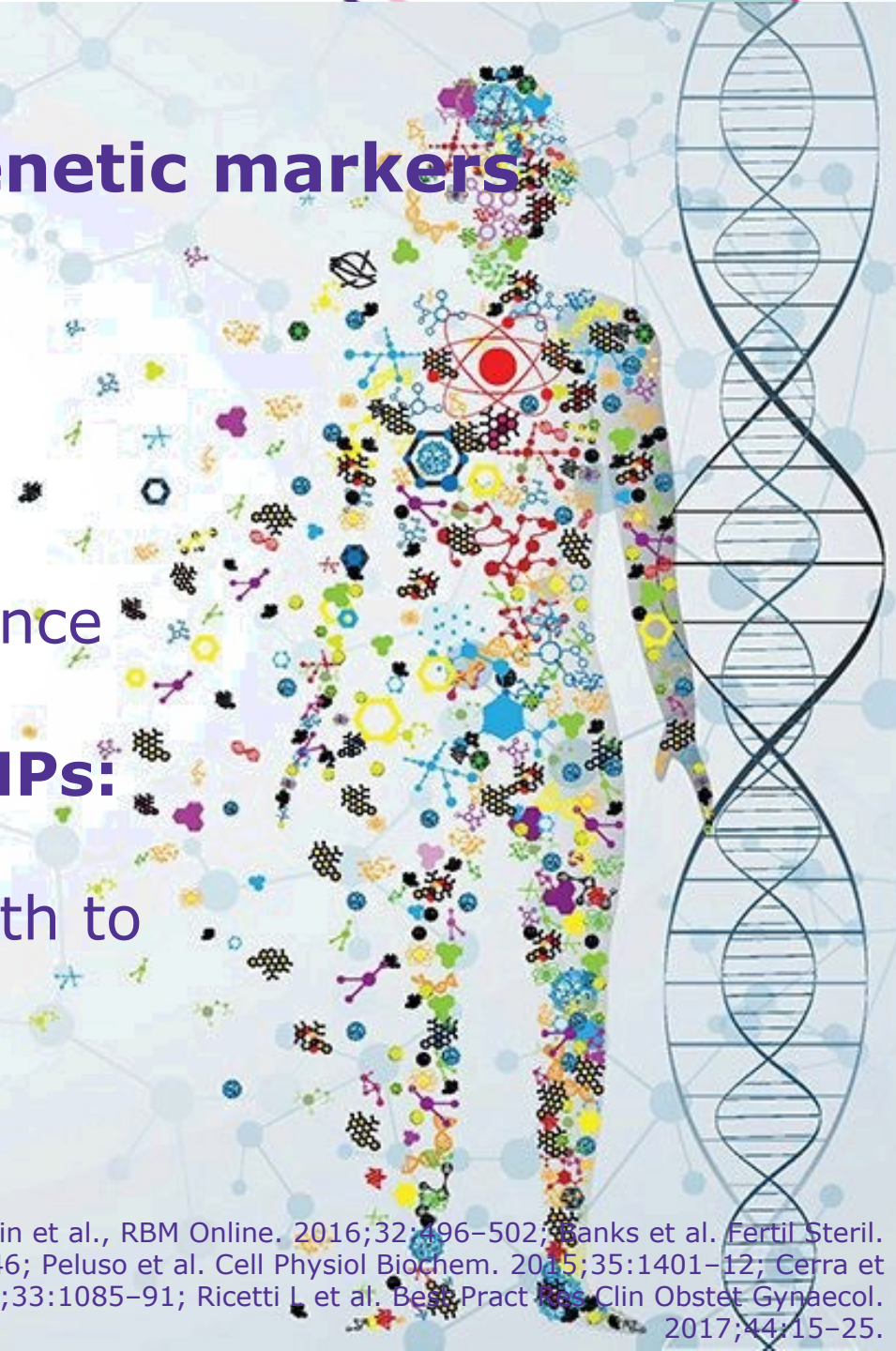
AFC
<6: 375 IU
6-15: 225 IU
>15: 150 IU



Lan VT, et al. Reprod Biomed Online. 2013;27:390–9.

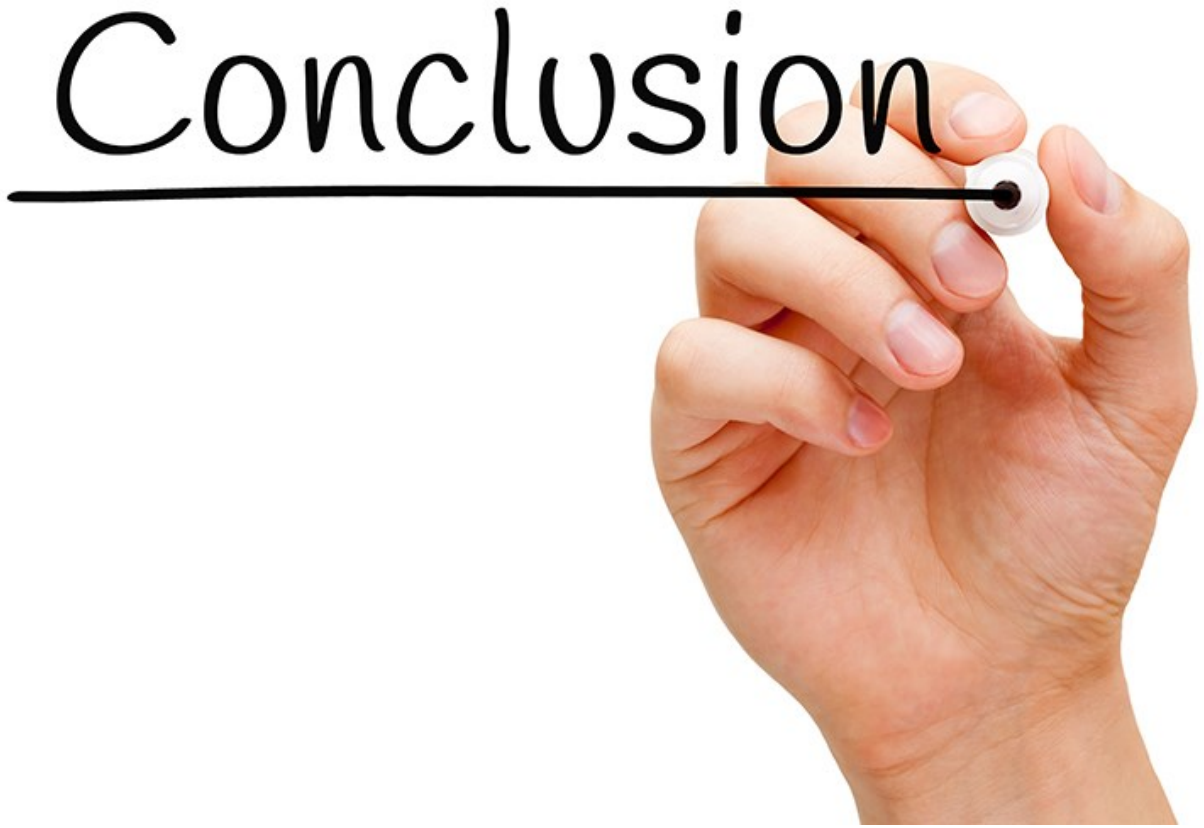
Prediction of response using ORT: genetic markers

- **FMR1 CGG repeat length:** Not associated with ovarian response or no added value in prediction of ovarian response
- **AMH and AMHR2 SNPs:** Conflicting evidence
- **Gonadotrophins and their receptors SNPs:** No strong association except for the p.Asn680Ser which modulates response both to endogenous and exogenous FSH



FMR1, fragile X mental retardation 1; SNP, single nucleotide polymorphism.

Morin et al., RBM Online. 2016;32:496–502; Banks et al. Fertil Steril. 2016;105(6):1537–46; Peluso et al. Cell Physiol Biochem. 2015;35:1401–12; Cerra et al. JARG. 2016;33:1085–91; Ricetti L et al. Best Pract Res Clin Obstet Gynaecol. 2017;44:15–25.



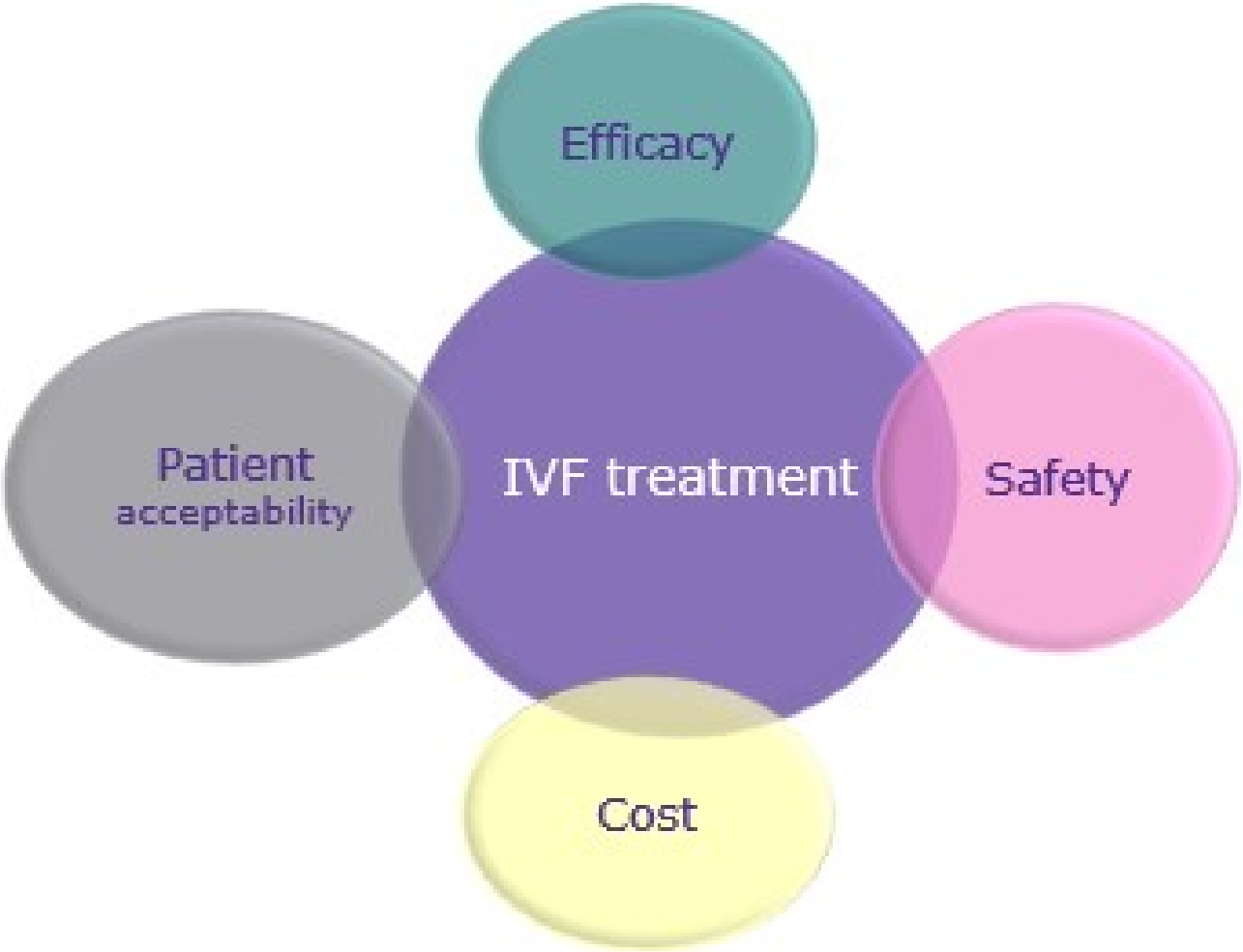
Conclusions (1)

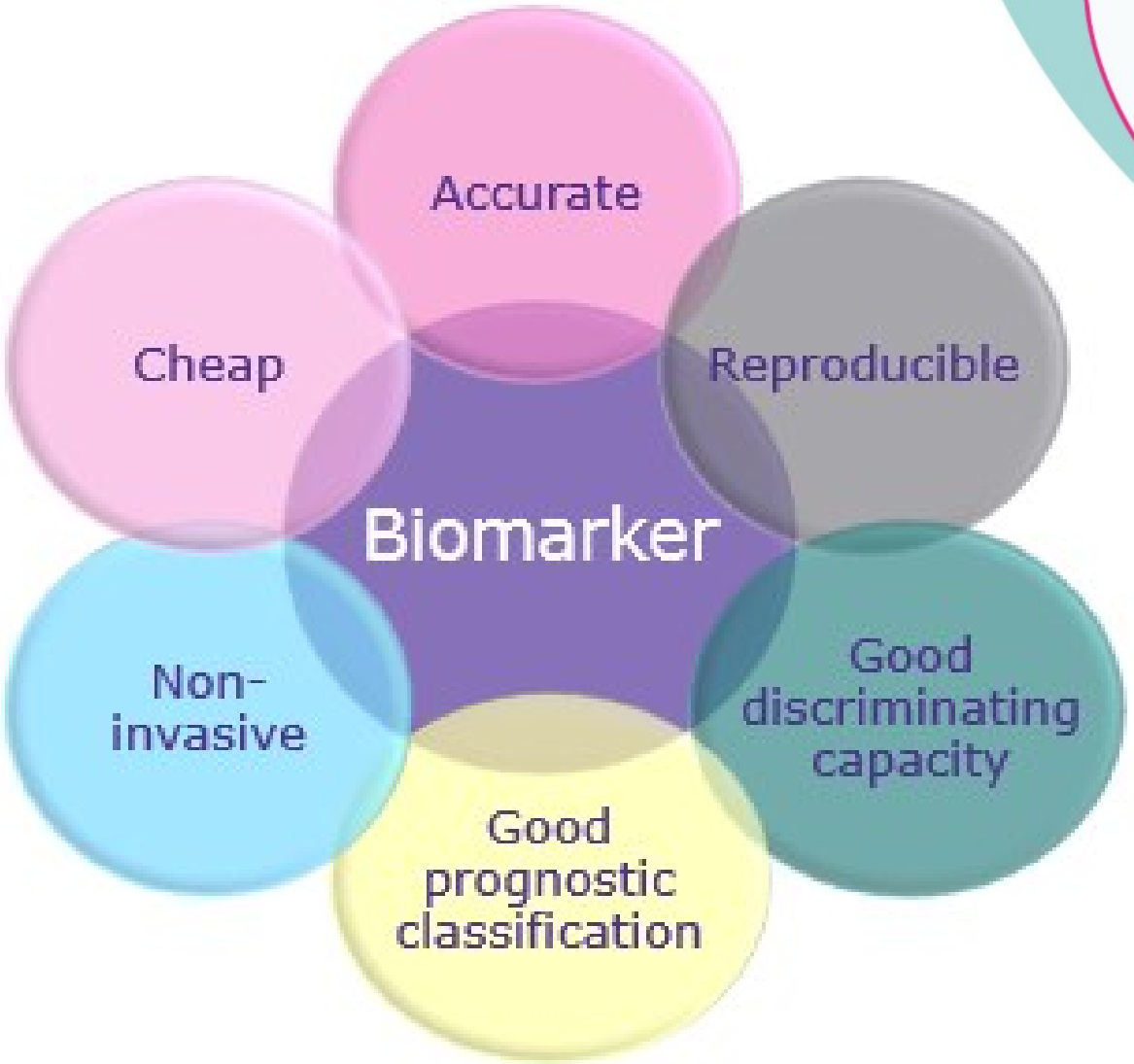
- Out of the many biomarkers that have been evaluated, **female age**, **AFC and serum AMH** seem to be the strongest predictors of ovarian response
- Both AMH and AFC have pros and cons, and issues with reproducibility; these might be resolved by standardizing AFC measurement and by using automated AMH assays
- AMH and AFC seem to have a similar capacity to predict poor or excessive response and can add to female age in predicting poor or excessive ovarian response

Conclusions (2)

- Using an ORT based algorithm to determine the starting dose is superior to 150 IU of rFSH in terms of OHSS prevention, prediction of normal or high response and cycle cancellation due to hyper-response
- One randomized study suggests that a dosing algorithm based on AMH is superior to one based on AFC when it comes to preventing hyper-response
- Genetic markers of ovarian response are still scantly reported in the literature and in most cases lack validation

Improving ART outcome using biomarkers





IMPROVING THE PREDICTION OF OVARIAN RESERVE AND RESPONSE IN ART USING BIOMARKERS

Christos Venetis
MD, FRANZCOG, MSc (Res), MSc (HCM), PhD
University of New South Wales
Sydney, Australia
c.venetis@unsw.edu.au

CENTRE FOR BIG DATA RESEARCH IN HEALTH