AMH & STIMULATION STRATEGY

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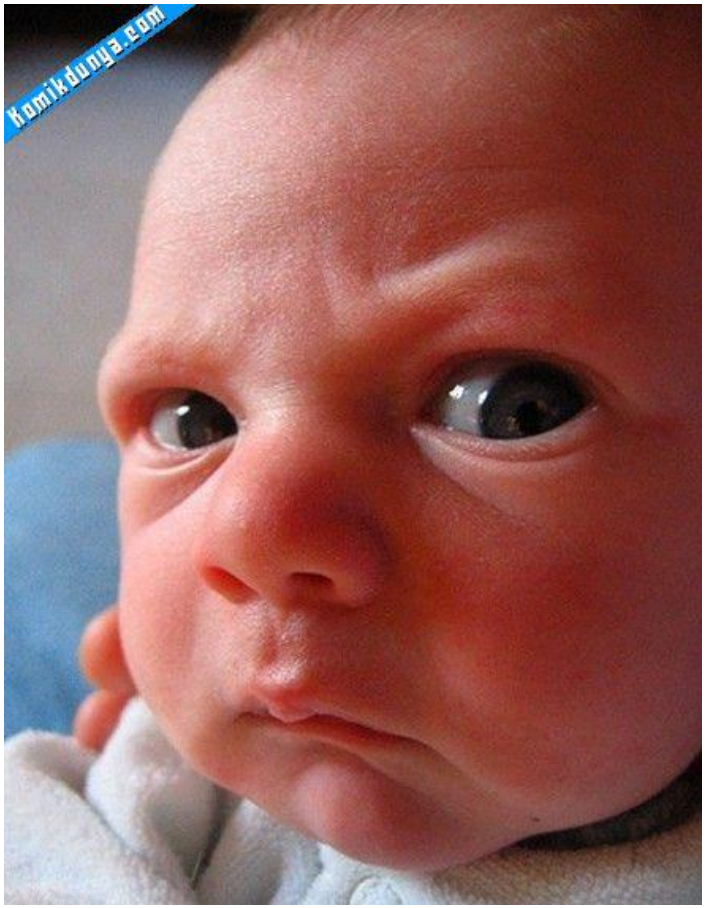
OUTLINE:

- IVF success
- i-COS
- Ovarian reserve
- Prediction of ovarian response
- AMH dictated COH protocols
- i-Gn dosage models



SUCCESS in IVF ?

ORIGINAL ARTICLE

Cumulative Live-Birth Rates after In Vitro Fertilization

and Alan S. Penzias, M.D.

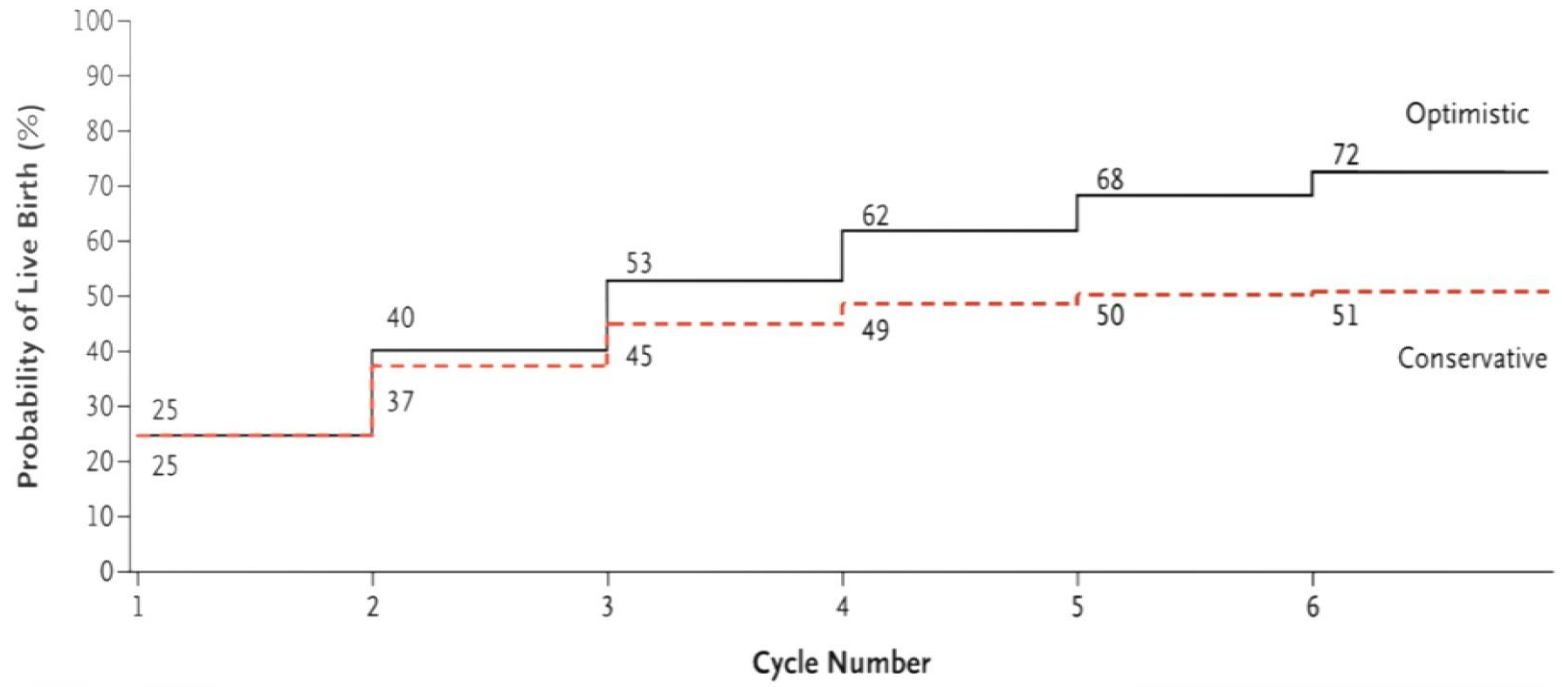


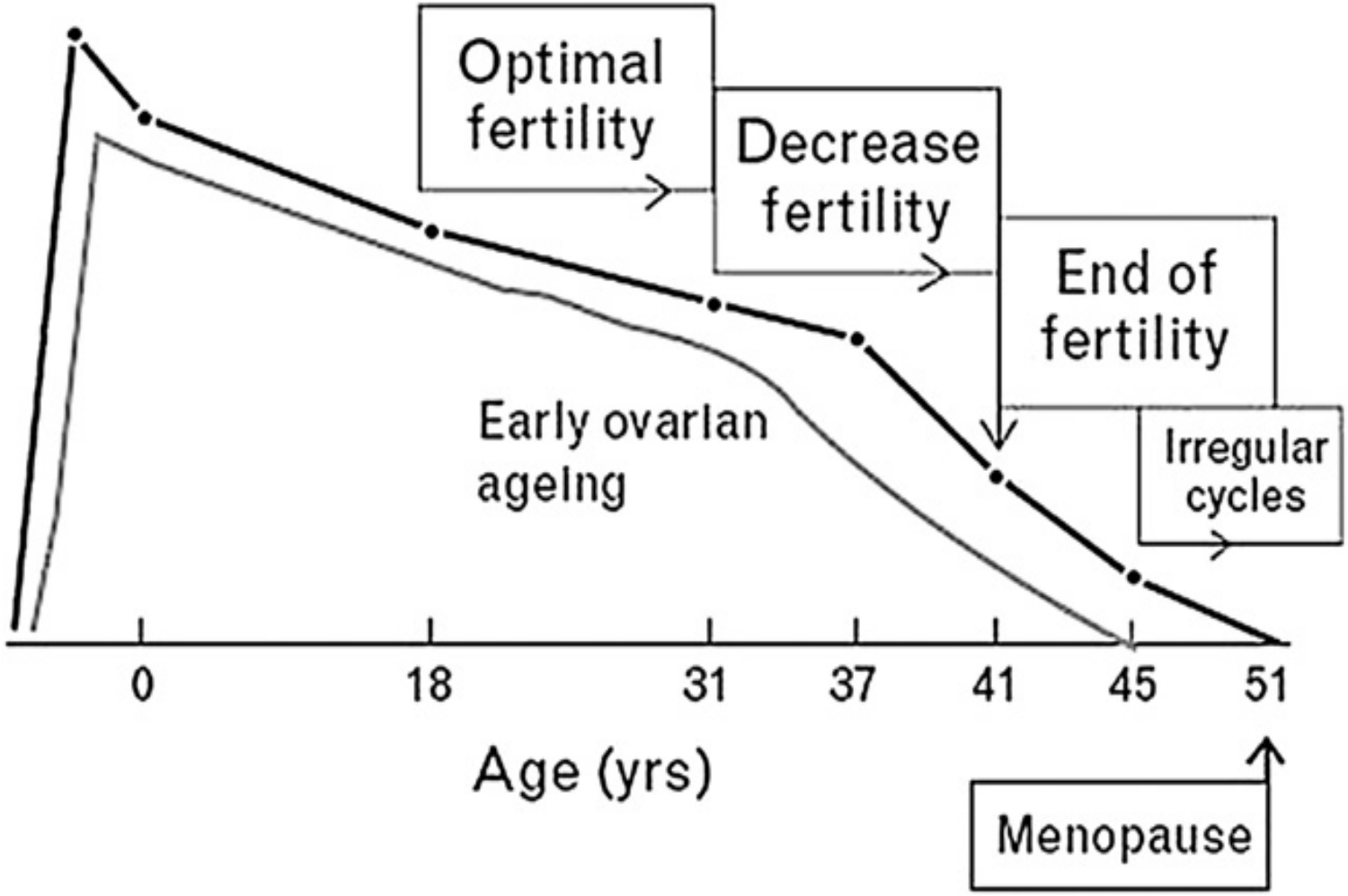
Figure 1. Kaplan–Meier Curves for Optimistic and Conservative Cumulative Live-Birth Rates among 6164 Women.

The optimistic cumulative live-birth rate is based on the assumption that patients who did not return for treatment had the same chance of a pregnancy resulting in a live birth as those who remained in treatment. The conservative cumulative live-birth rate assumes that patients who did not return for treatment did not have a pregnancy resulting in a live birth. These two curves show the best-case and worst-case estimates of the cumulative live-birth rate in the study population.

Woman age and fertility

- **It is generally considered that age is the primary driver of treatment success in IVF programmes**
  - By postponement of childbearing, a growing number of couples attempting pregnancy will experience reduced fecundability
- Strict embryo transfer policy

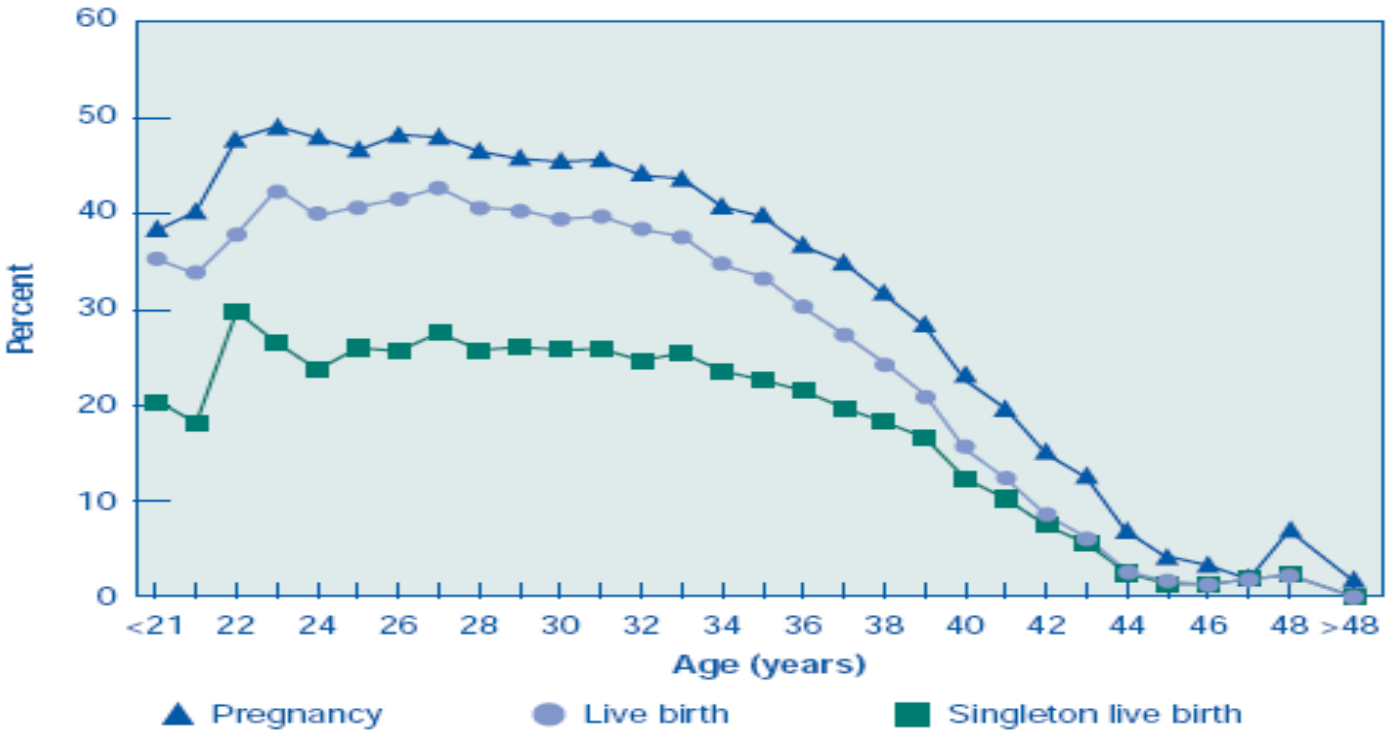
Woman age and fertility



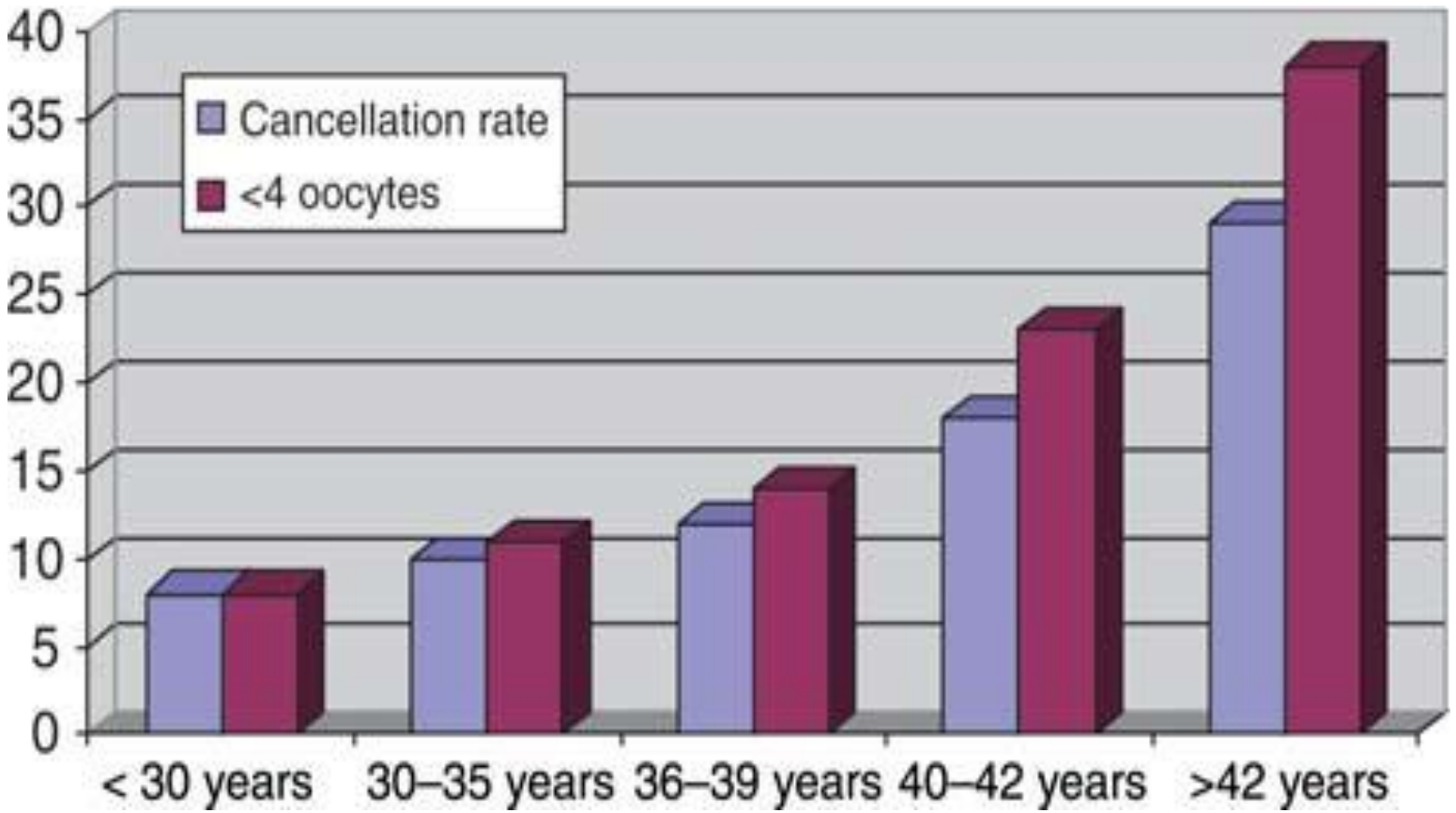
Woman age and fertility

success rates after ART

(2006 report - US Center for Disease Control and Prevention)



Woman age and fertility



Ferraretti, HR 2011

How many is better ?

- **Oocyte yield plays a critical role in predicting IVF success**

Human Reproduction, Vol.26, No.7 pp. 1768–1774, 2011
Advanced Access publication on May 10, 2011 doi:10.1093/humrep/der106

human reproduction ORIGINAL ARTICLE *Infertility*

Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles

Sesh Kamal Sunkara[1], Vivian Rittenberg[1], Nick Raine-Fenning[2], Siladitya Bhattacharya[3], Javier Zamora[4], and Arri Coomarasamy[5,*]

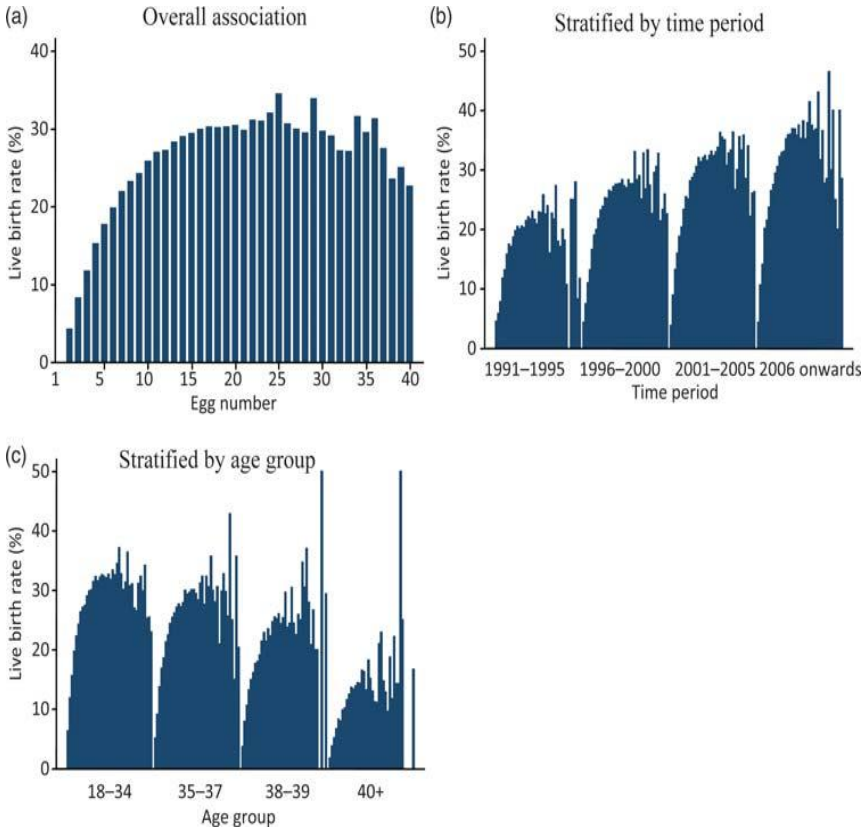


Figure 3 Association between egg number and live birth rate.

Association between the number of eggs and live birth

There was a strong association between the number of eggs and the LBR (Fig. 3a) which rose with increasing number of eggs up to ~15, plateaued between 15 and 20 eggs and steadily declined beyond 20 eggs. The same pattern was observed in all four of the time periods. For a given number of eggs, LBRs increased over time (Fig. 3b) but decreased with increasing age (Fig. 3c).

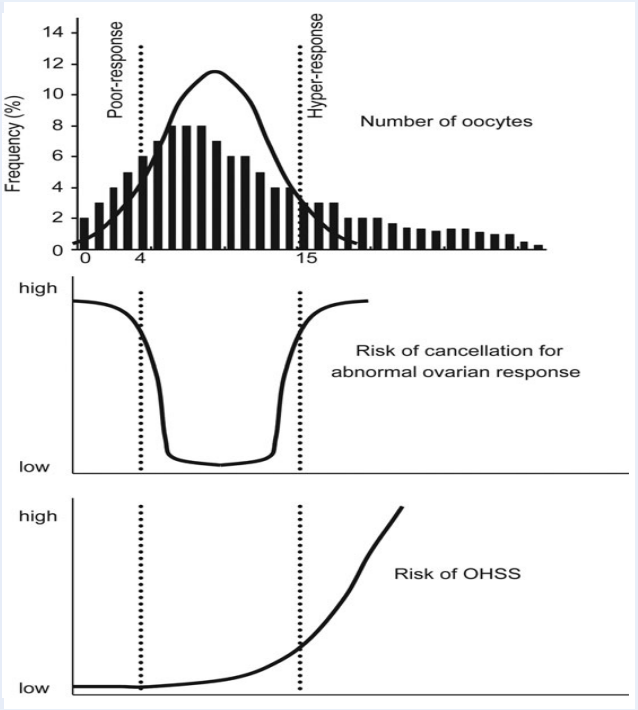


Figure 1 The objective of the individualization of the treatment strategy would be to possibly increase the percentage of patients with a number of retrieved oocytes considered appropriate, hence reducing the number of women at high risk of cycle cancellation and ovarian hyperstimulation syndrome (OHSS). Top of the figure: Bars indicate the actual frequency of retrieved oocytes as derived by Sunkara *et al.* (2011). The line indicates the ideal frequency of retrieved oocytes, characterized by a very high percentage of women with an appropriate oocyte yield.

Human Reproduction Vol.23, No.9 pp. 2050–2055, 2008 doi:10.1093/humrep/den219
Advance Access publication on June 10, 2008

Why do couples drop-out from IVF treatment? A prospective cohort study

M.F.G. Verberg[1,4], M.J.C. Eijkemans[1,2], E.M.E.W. Heijnen[1], F.J. Broekmans[1], C. de Klerk[3], B.C.J.M. Fauser[1] and N.S. Macklon[1]

- The causes of drop-out are summarize:
- the principal reason for dropping-out was the physical or psychological burden of treatment (28%).
- In 14% of drop-out patients, the primary reason for stopping treatment was a poor prognosis identified by a

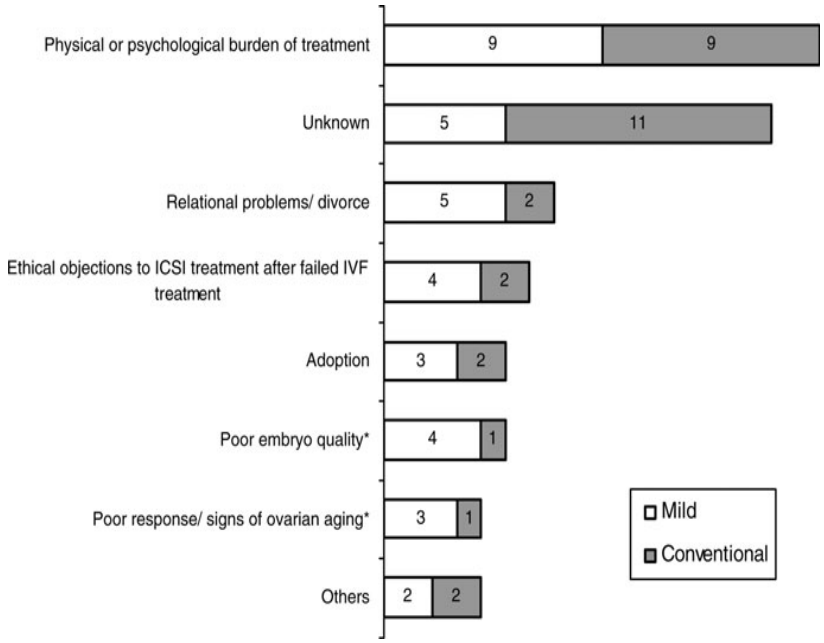
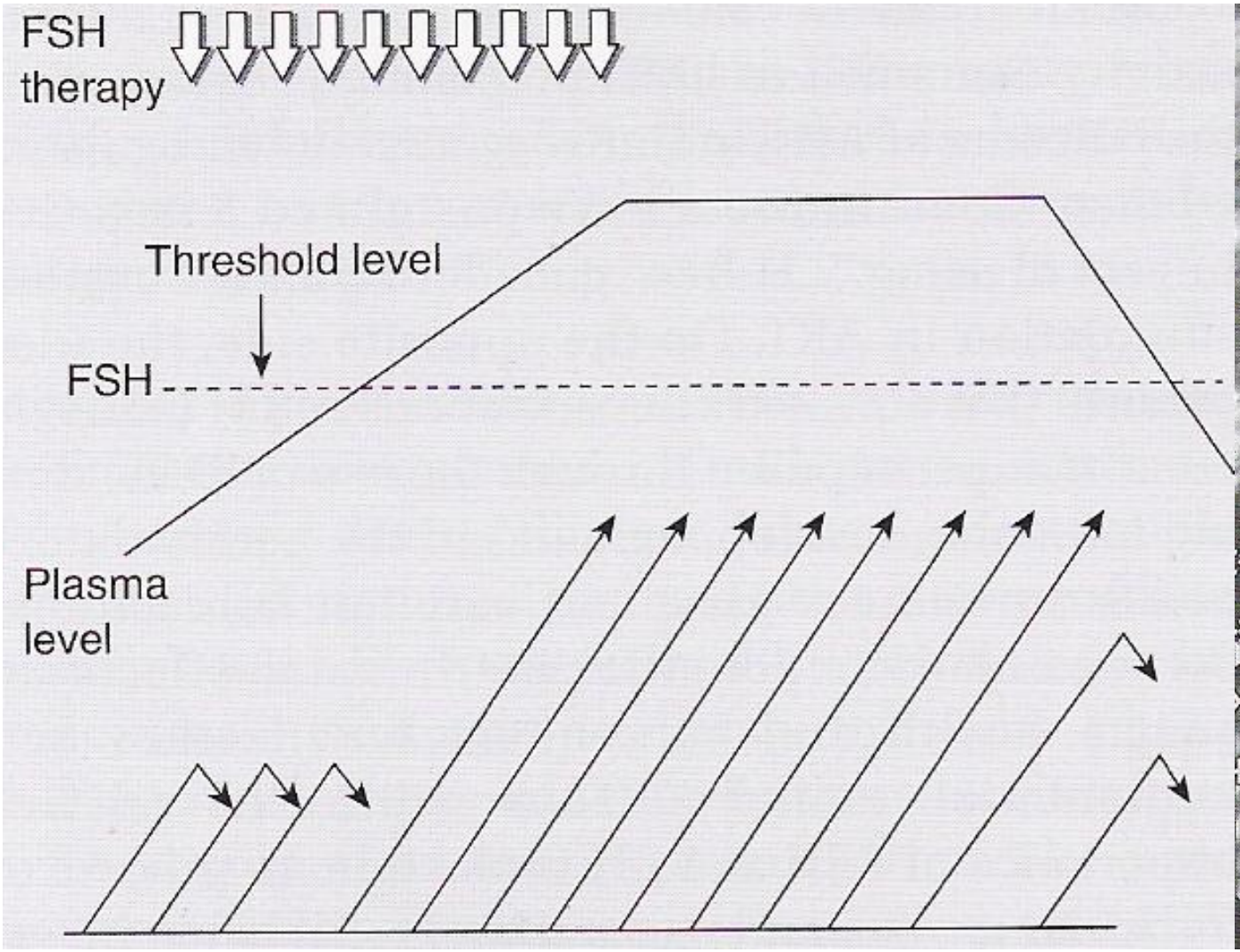
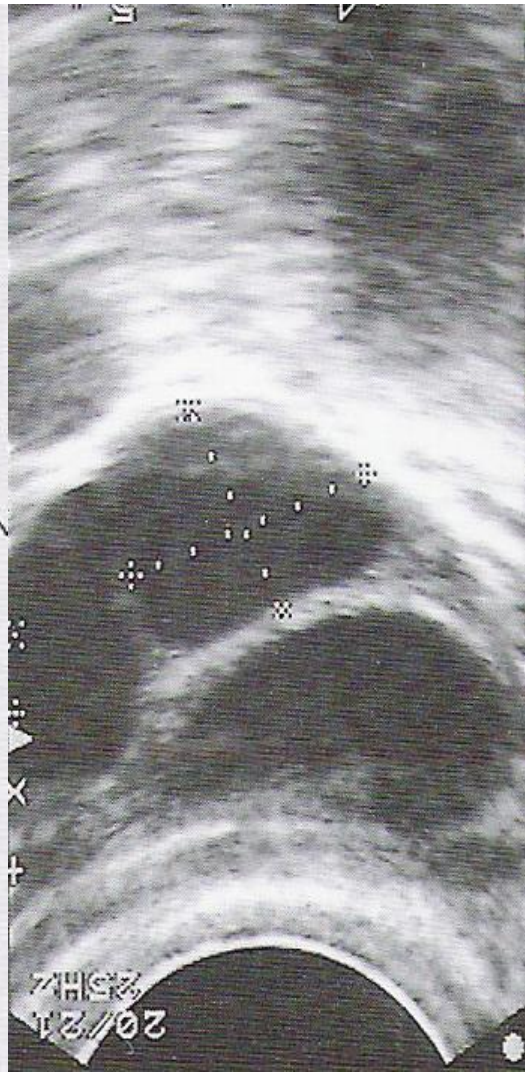


Figure 3: Causes for drop-out from IVF treatment in each treatment protocol. *actively censored.

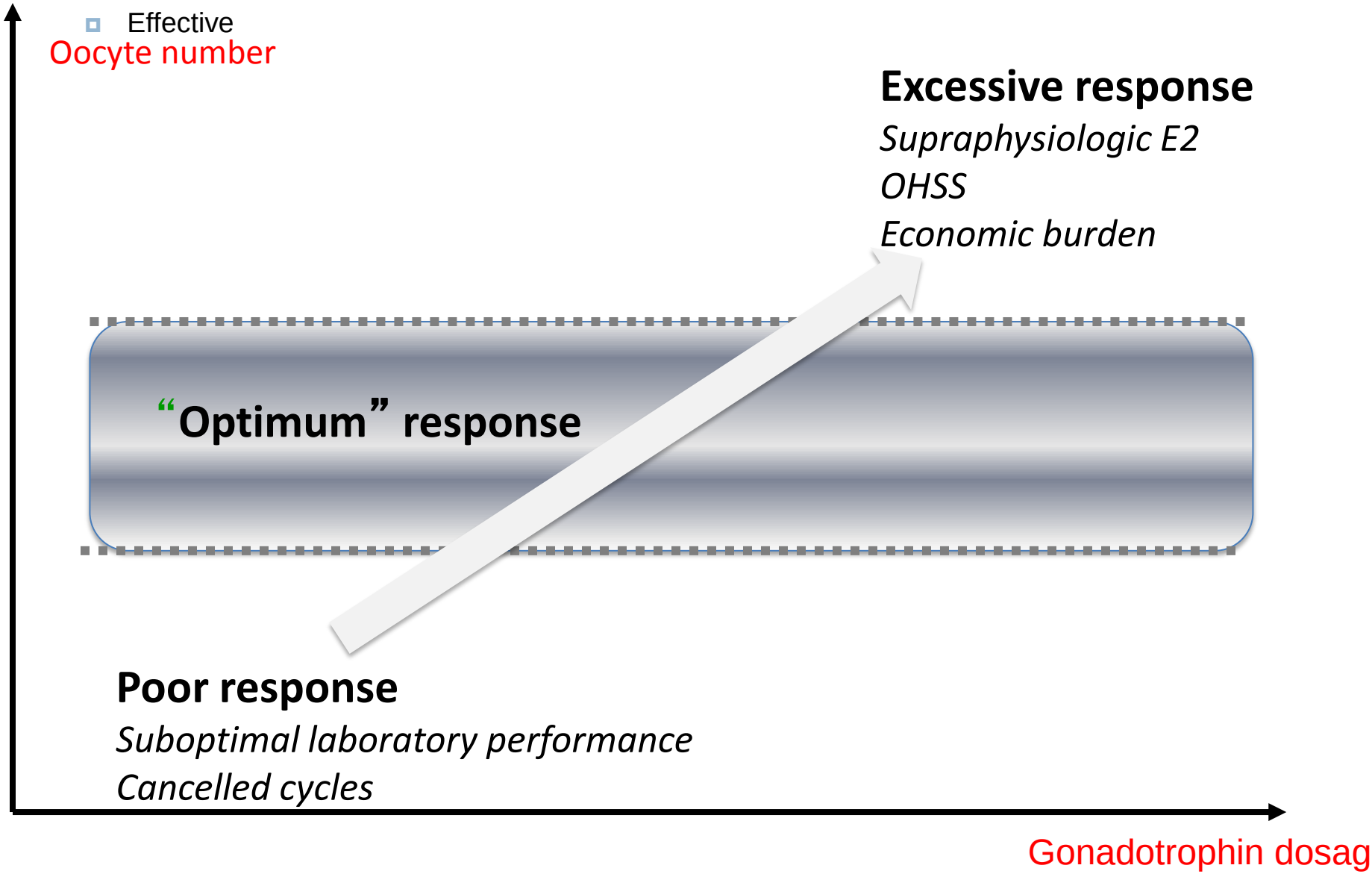
40% of couples abandon IVF after a single cycle

COH – IVF
:Multifollicular ovulation induction





- It is evident that patients have different ovarian responses to the same ovarian stimulation
- The ability to predict this variation in ovarian response is very useful in making ovarian stimulation
  - Safe
  - Effective



Ovarian response prediction ?

- **For patients predicted to have a poor ovarian response**:
  - clinicians may decide to counsel patients not to proceed with treatment or
  - alter their treatment protocol or
  - even to suggest egg donation at an early stage in their management
- **For patients anticipated to have an excessive ovarian response**:
  - clinicians can provide guidance on the potential risks associated with treatment
  - in addition to increased monitoring during treatment, and
  - can recommend alterations in treatment schedules accordingly

Treatment individualization: iCOS

Accurate prediction of ovarian response

- ✓ enable clinicians to give women more accurate information about the expected outcome of IVF treatment
- ✓ enable individualization of the therapeutic strategy

The main aim of treatment individualization in IVF is

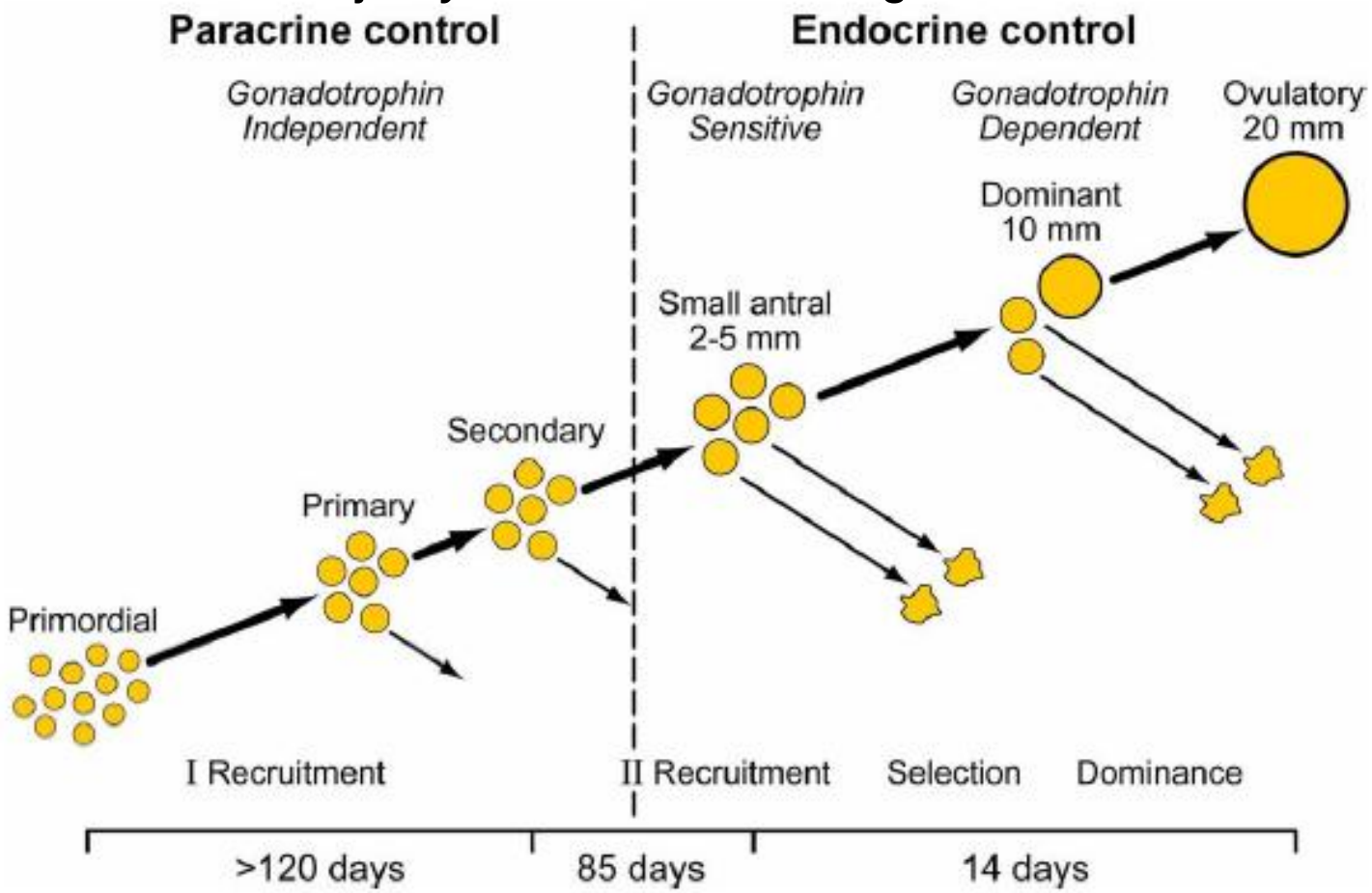
- ✓ to maximize the success
- ✓ to minimize the risk of OHSS
- ✓ to minimize cycle cancellation

Ovarian response prediction ?

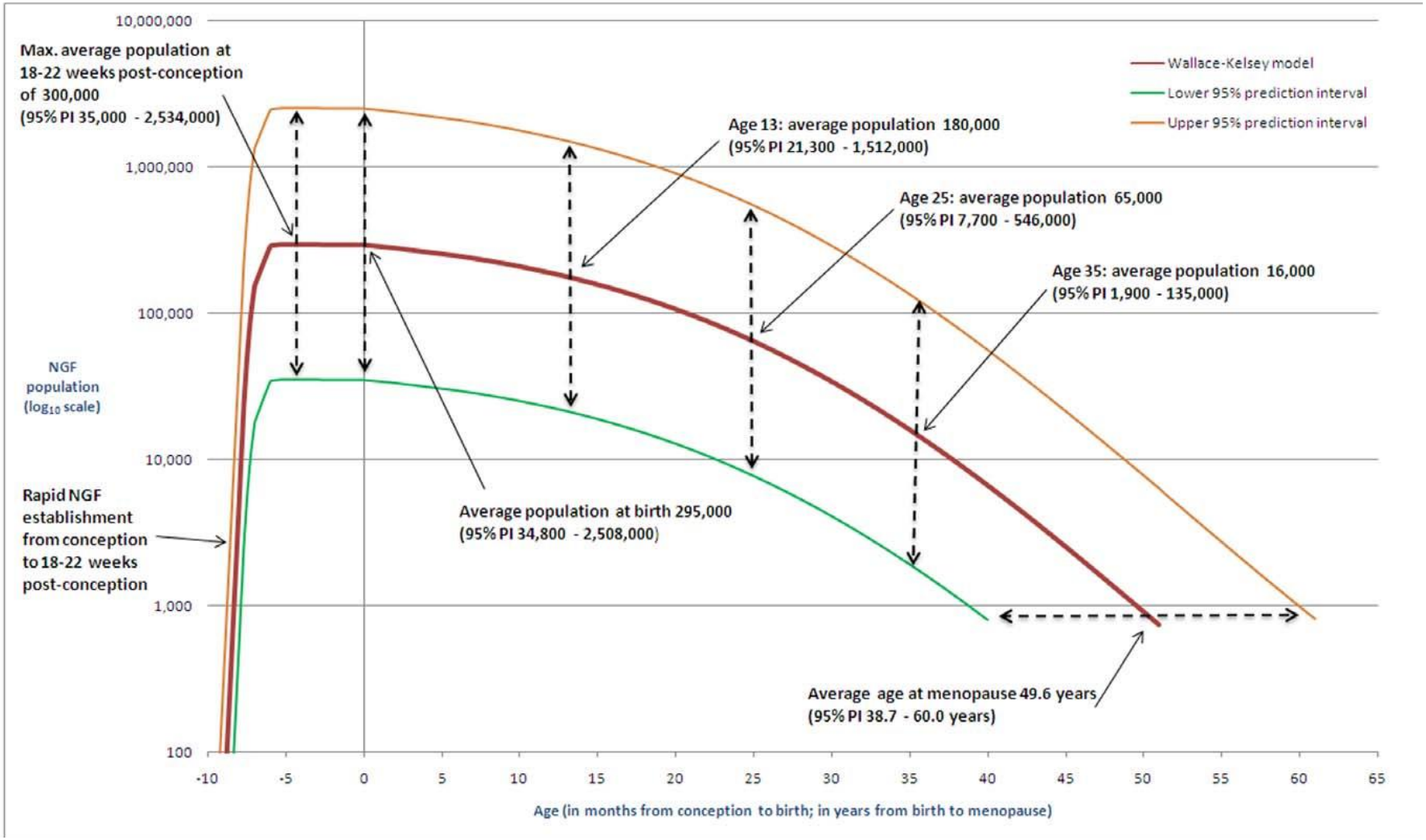
- **Choice is likely to be empirical**
- Age
- BMI
- Previous cycle response

- Ovarian reserve tests
  - USG: AFC
  - Biochemical: FSH, AMH

- Our current understanding of female reproductive function is that the ovary contains a limited number of primordial follicles and that their depletion marks the menopause.
- The remaining primordial follicle pool is referred to as the ovarian reserve.
- Throughout life, until their numbers are exhausted, primordial follicles leave the primordial follicle pool to enter the growing pool, with the vast majority intended to undergo atresia.



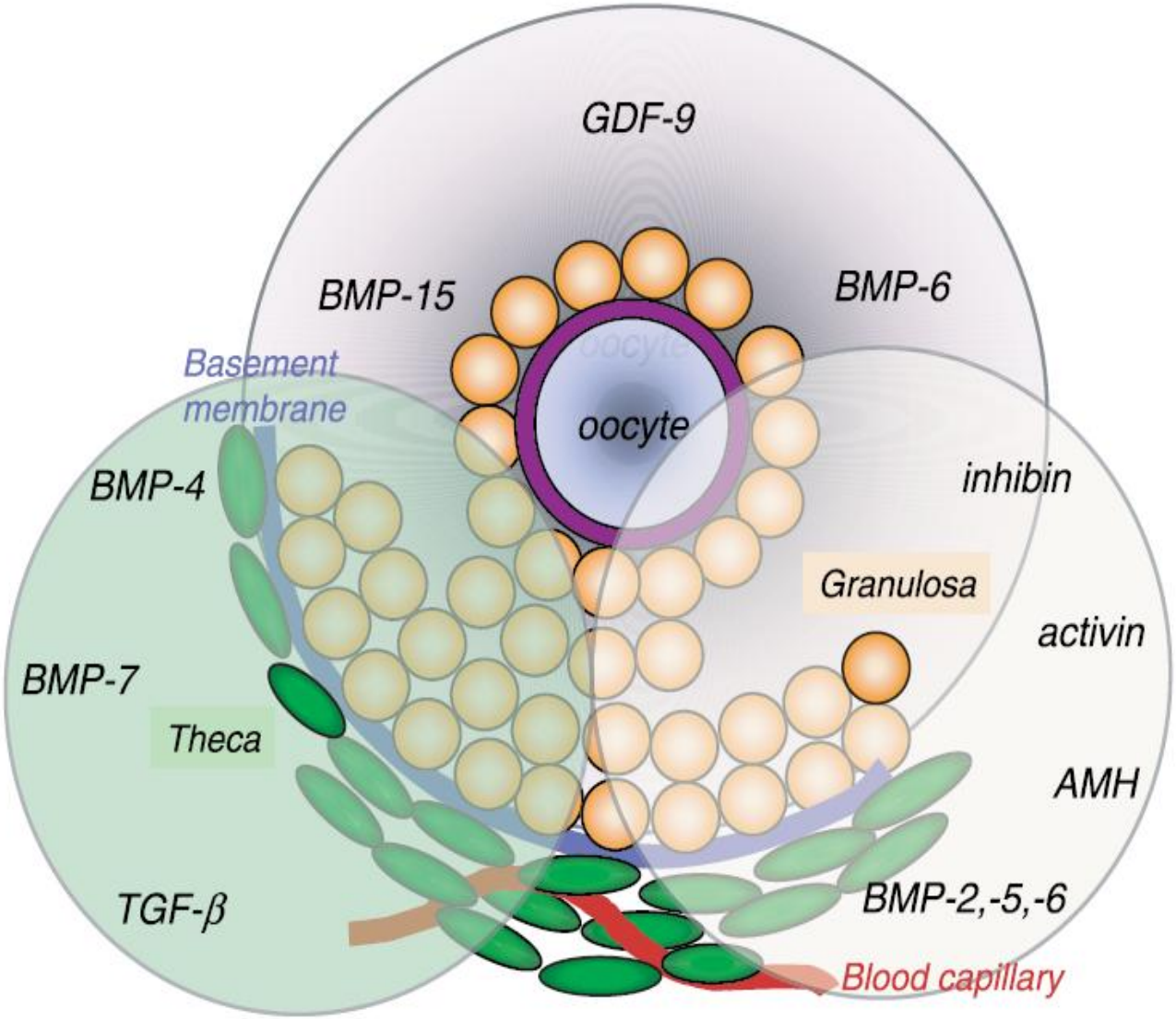
Ovarian reserve: Non-growing follicles



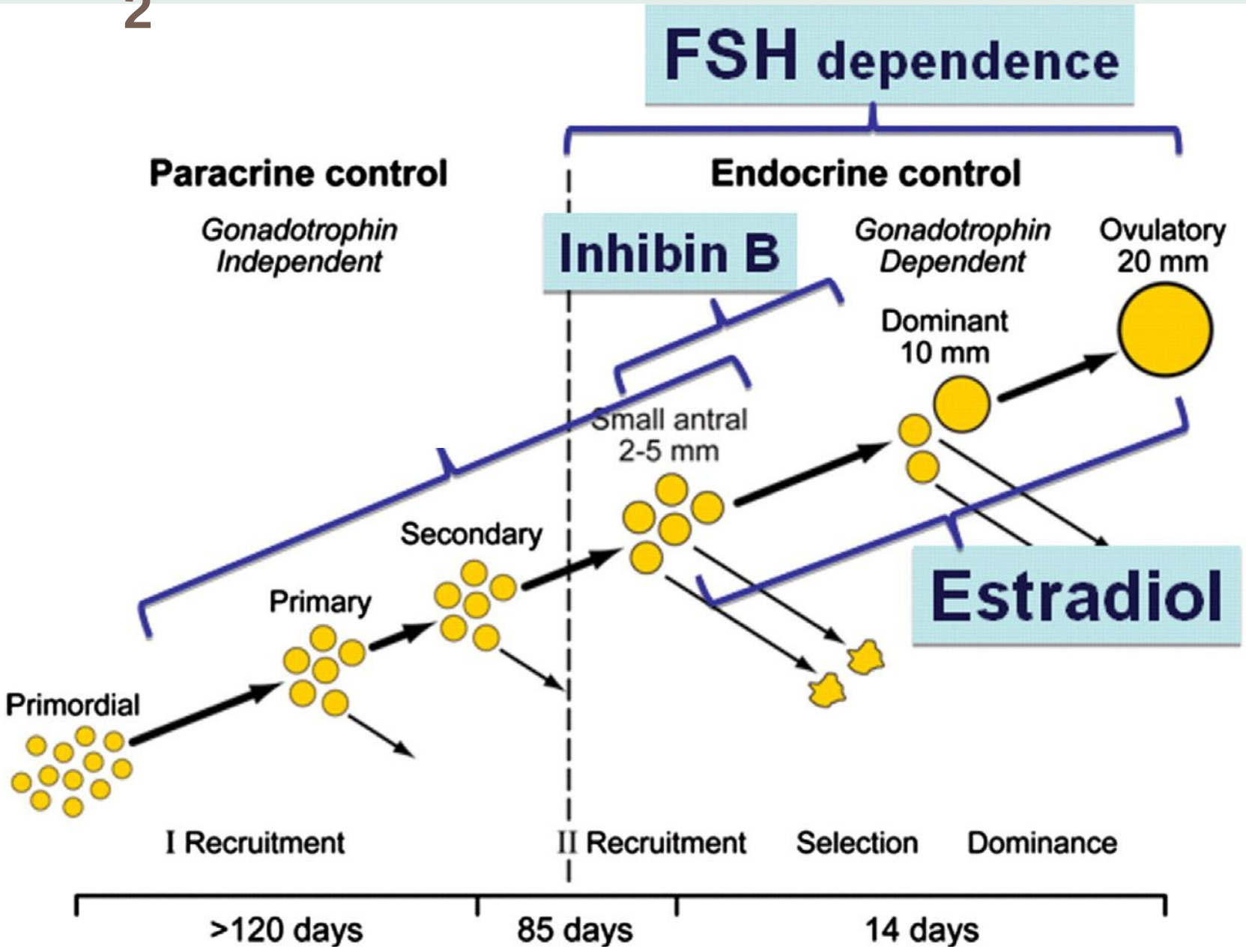
Basal FSH

- has to be done in the early follicular phase
- requires concomitant E2 determination
- it requires a functioning hypothalamic–pituitary–gonadal system
- an elevated FSH is a sufficiently specific marker of low response to ovarian stimulation
- it does not detect high ovarian reserve, a known risk factor for ovarian hyperstimulation

Search for a better marker



Timing of granulosa cell secretion of AMH, Inhibin B, and E_2 during folliculogenesis



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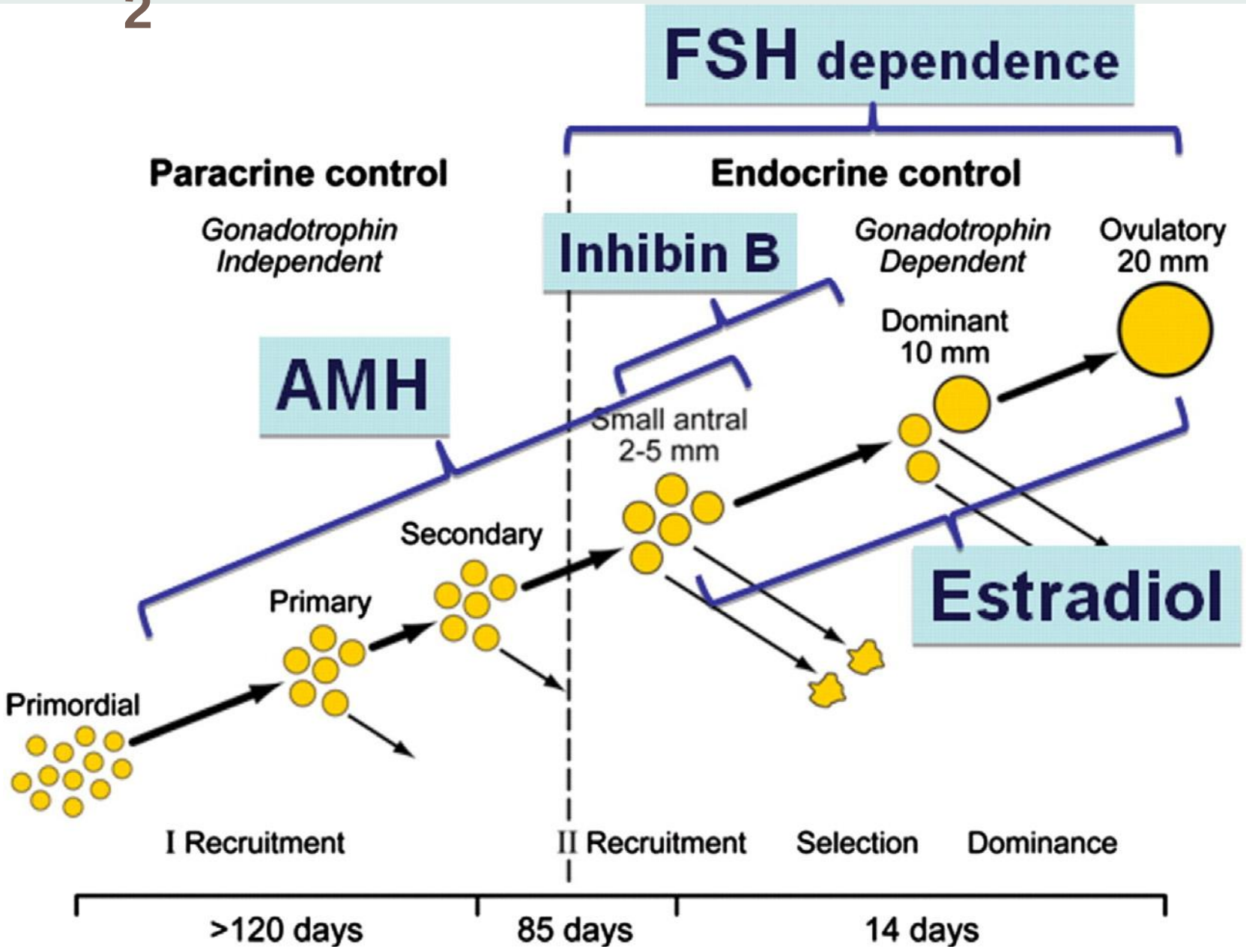


Anti-Müllerian hormone

- AMH is a glycoprotein within the transforming growth factor-[beta] family.
- It was first described in 1947 by Jost as a gonadal factor produced by Sertoli cells in the male embryo causing regression of the Müllerian ducts.
- Expression of AMH in the ovary was first reported by Hutson 30 years ago

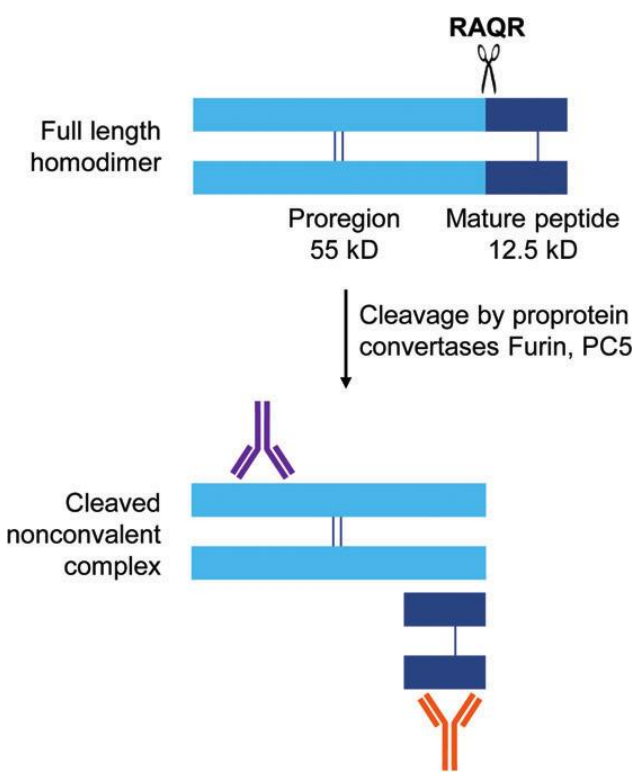
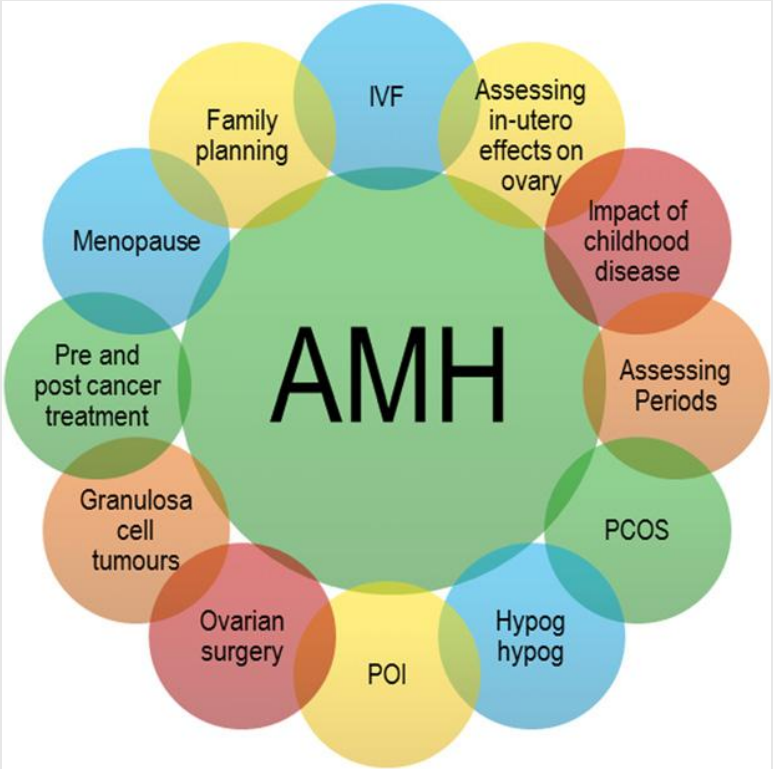


Figure 2 Schematic depicting the processing of AMH. AMH is produced as a precursor protein consisting of disulphide-linked monomers. Upon cleavage by prohormone convertases, the protein is cleaved into pro- and mature homodimers, which remain non-covalently associated. AMH ELISAs have been developed to detect AMH in the circulation. The regions that are recognized by the monoclonal antibodies used in the ultrasensitive IOT assay and the Gen II assay (previously DSL) are indicated. For the Gen II assay, the capture antibody recognized the mature region and the detector antibody recognizes the proregion.

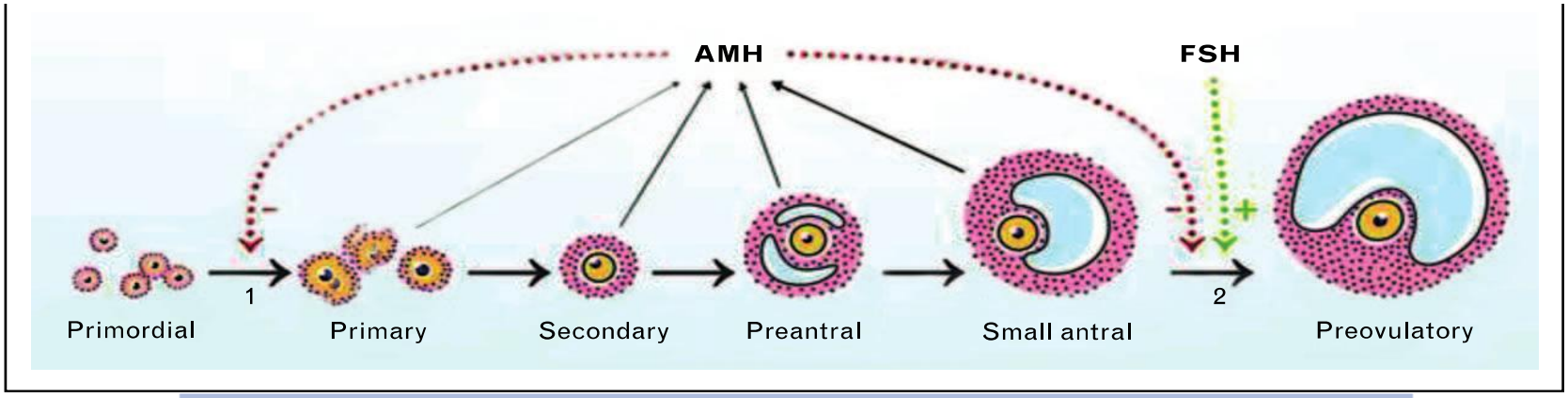
Anti-Müllerian hormone

- Over the last 10 years, after the development of commercially available assays, there has been a rapidly growing interest in the clinical utility of AMH measurements in female reproductive function.

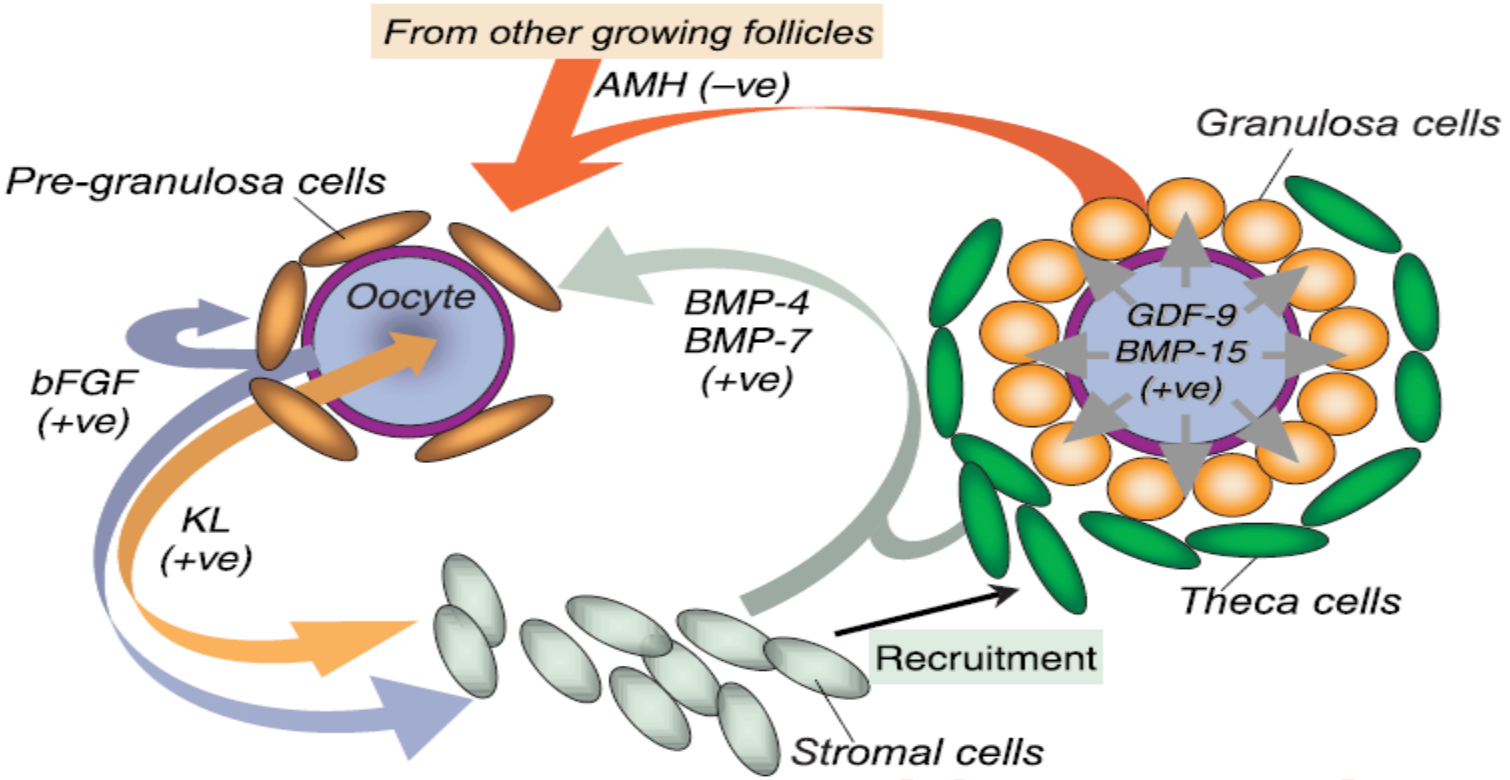


Potential clinical applications of antimüllerian hormone (AMH) by health-care providers. IVF = in vitro fertilization; PCOS = polycystic ovary syndrome; hypog hypog = hypogonadotrophic hypogonadism; POI = premature ovarian insufficiency.

Nelson. Biomarkers of ovarian response. Fertil Steril 2013.



Primordial follicle ➡ Primary follicle

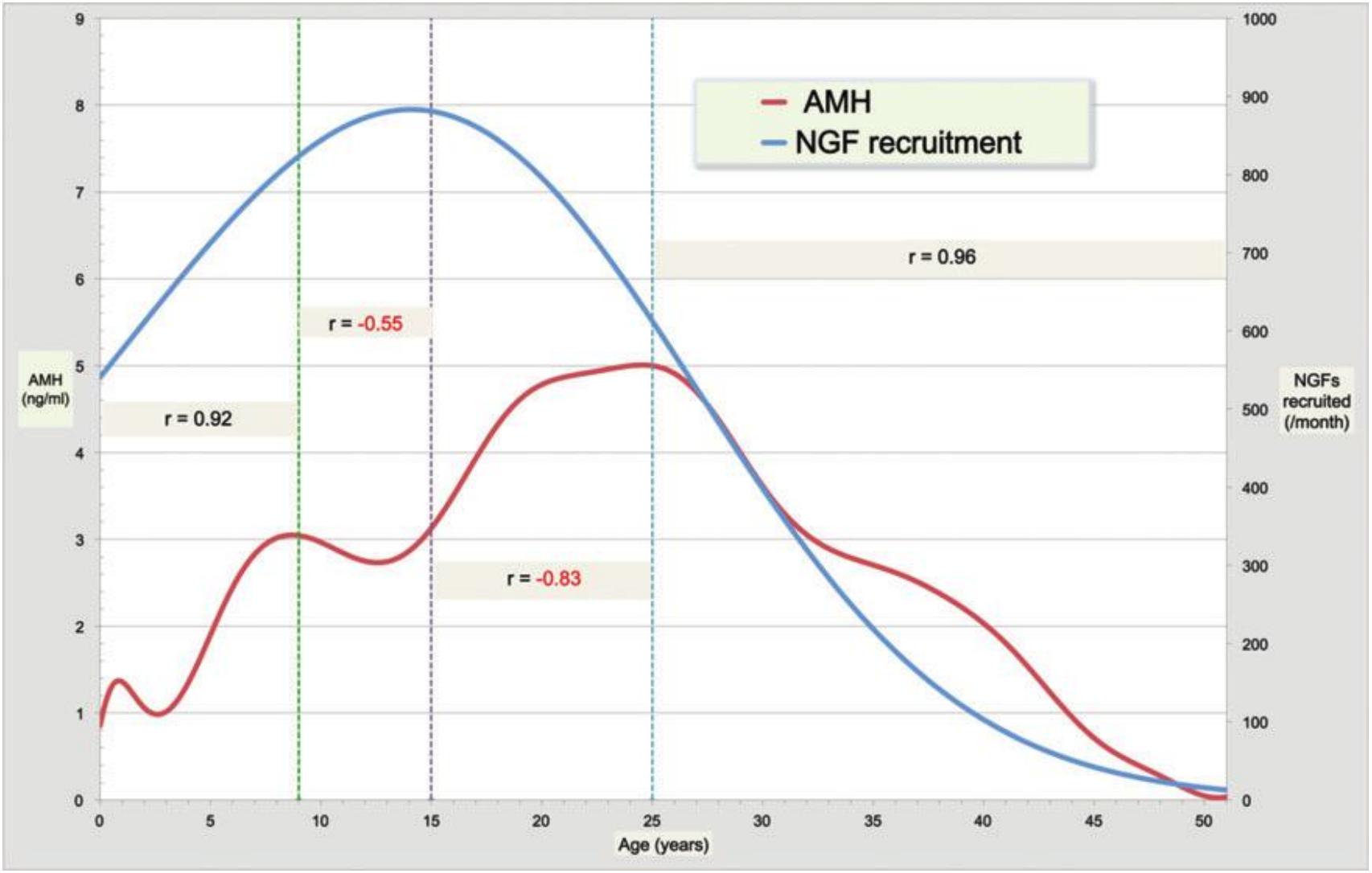


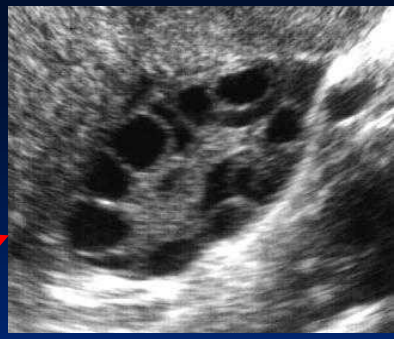
AMH attenuates this promotion

Comparison of ovarian reserve markers FSH and AMH.

Feature	FSH	AMH
Site of secretion	Anterior pituitary	Granulosa of pre- and small antral follicles
Temporal change indicating ovarian aging	Latest	Earliest
Timing requirement	Cycle day 2–4 only	Any cycle day
Need for concomitant assay	E_2	None
Cycle to cycle variability	High	Low
Sensitivity for low response	Moderate	Moderate
Sensitivity for high response (risk of OHSS)	None	High
Specificity for low response	High	High
Specificity for high response	None	High
Age-specific values	Limited	Extensive information
Methodology	Automated (1 h)	ELISA (6 h)

AMH and follicular recruitment profile across the human reproductive lifespan





Circulating AMH

?

6-10 mm

2-5 mm

0,1-2 mm

Pre-antral follicles

Primary follicles

Primordial pool

Antral Follicle Count

Human Reproduction Update, Vol.19, No.1 pp. 26–36, 2013
Advanced Access publication on November 27, 2012 doi:10.1093/humupd/dms041

human reproduction update

Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach

Simone L. Broer[1,2,*‡], Jeroen van Disseldorp[1,2‡], Kimiko A. Broeze[1,2], Madeleine Dolleman[1,2], Brent C. Opmeer[1,2], Patrick Bossuyt[1,2], Marinus J.C. Eijkemans[1,2], Ben-Willem J. Mol[1,2], and Frank J.M. Broekmans[1,2] on behalf of the IMPORT study group[**]

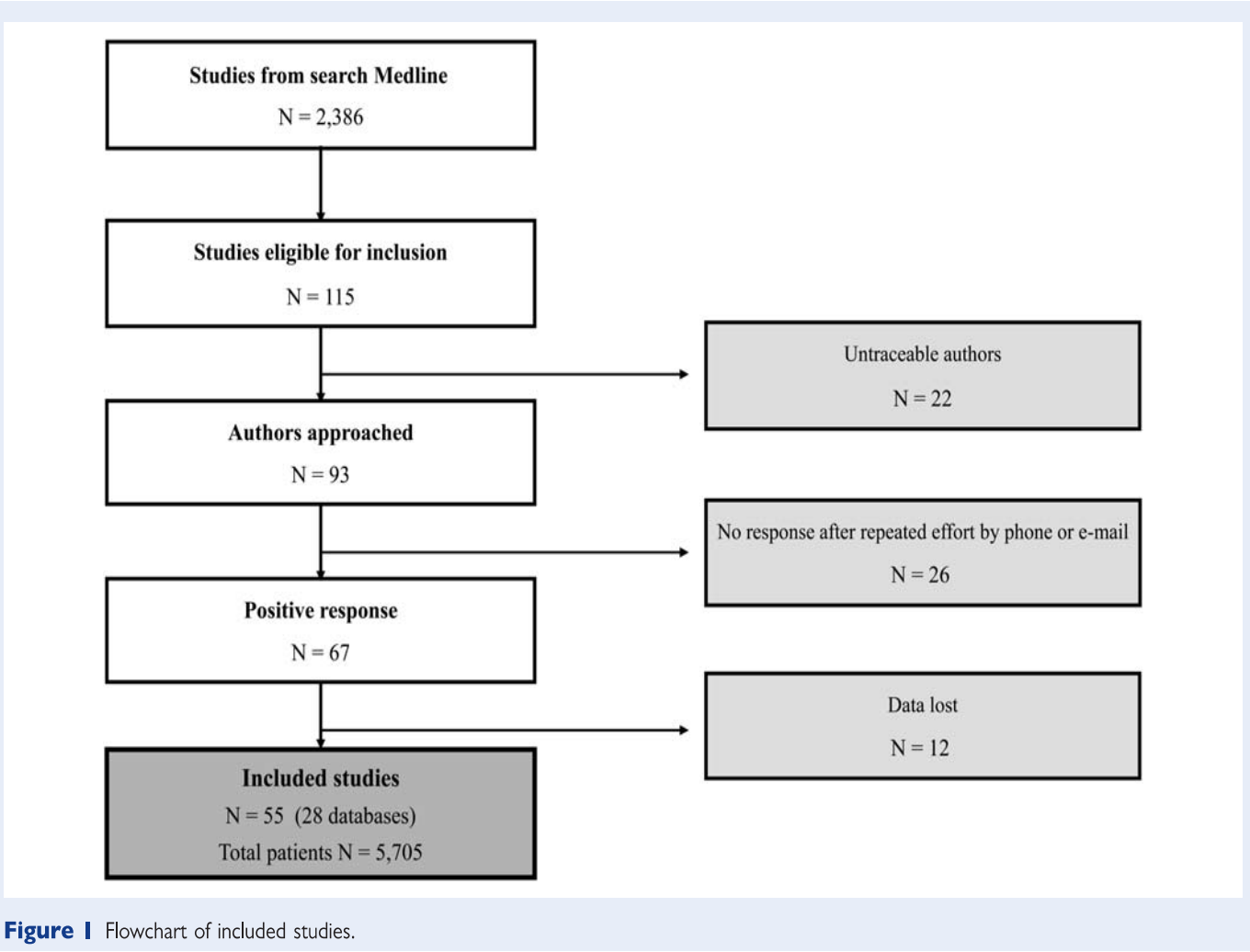


Figure 1 Flowchart of included studies.

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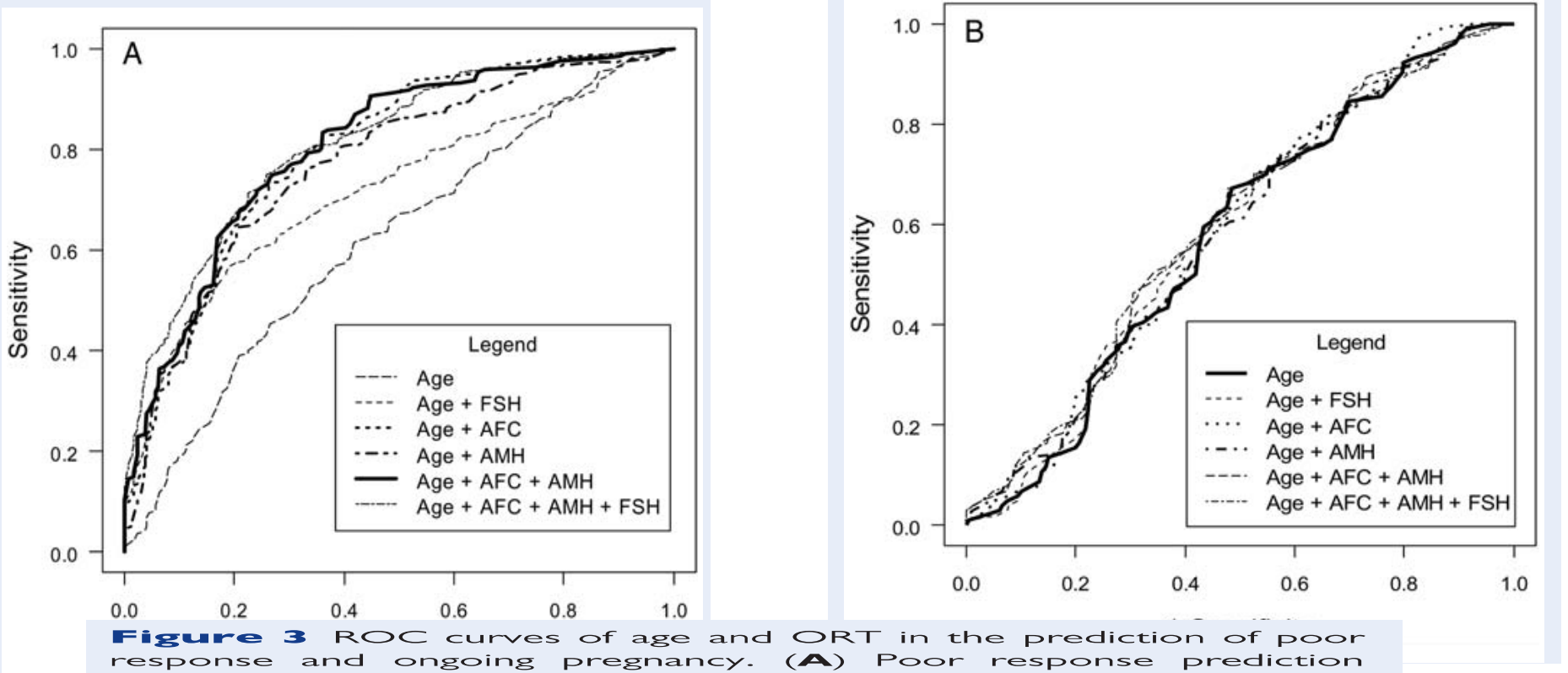


Figure 3 ROC curves of age and ORT in the prediction of poor response and ongoing pregnancy. (**A**) Poor response prediction based on age and ORT. The ROC curves of age or age combined with a single or more ORT are depicted. The ROC curves for 'Age + AMH', 'Age + AMH + AFC' and 'Age + AMH + AFC + FSH' run toward the upper left corner, indicating a good capacity to discriminate between normal and poor responders at certain cut-off levels. (**B**) Ongoing pregnancy prediction based on age and ORT. The ROC curves age or age combined with one or more ORT run almost parallel to or even cross the $X = Y$ line, indicating that the tests are useless for pregnancy prediction. AFC, antral follicle count; AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone; ORT, ovarian reserve test; ROC, receiver-operating characteristic.

Prediction of an excessive response in in vitro fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: an individual patient data meta-analysis

Simone L. Broer, M.D., Ph.D.,[a] Madeleine Dólleman, M.D.,[a] Jeroen van Disseldorp, M.D., Ph.D.,[a] Kimiko A. Broeze, M.D.,[b] Brent C. Opmeer, Ph.D.,[c] Patrick M. M. Bossuyt, Ph.D.,[c] Martinus J. C. Eijkemans, Ph.D.,[d] Ben Willem Mol, M.D., Ph.D.,[b] and Frank J. M. Broekmans, M.D., Ph.D.,[a] on behalf of the IPD-EXPORT Study Group

Objective: To evaluate whether ovarian reserve tests (ORTs) add prognostic value to patient characteristics, such as female age, in the prediction of excessive response to ovarian hyperstimulation in patients undergoing IVF, and whether their performance differs across clinical subgroups.

Design: Authors of studies reporting on basal FSH, antimüllerian hormone (AMH), or antral follicle count (AFC) in relation to ovarian response to ovarian hyperstimulation were invited to share original data. Random intercept logistic regression models were used to estimate added value of ORTs on patient characteristics, while accounting for between-study heterogeneity. Receiver operating characteristic regression analyses were performed to study the effect of patient characteristics on ORT accuracy.

Setting: In vitro fertilization clinics.

Patient(s): A total of 4,786 women for the main analysis, with a subgroup of 1,023 women with information on all three ORTs.

Intervention(s): None.

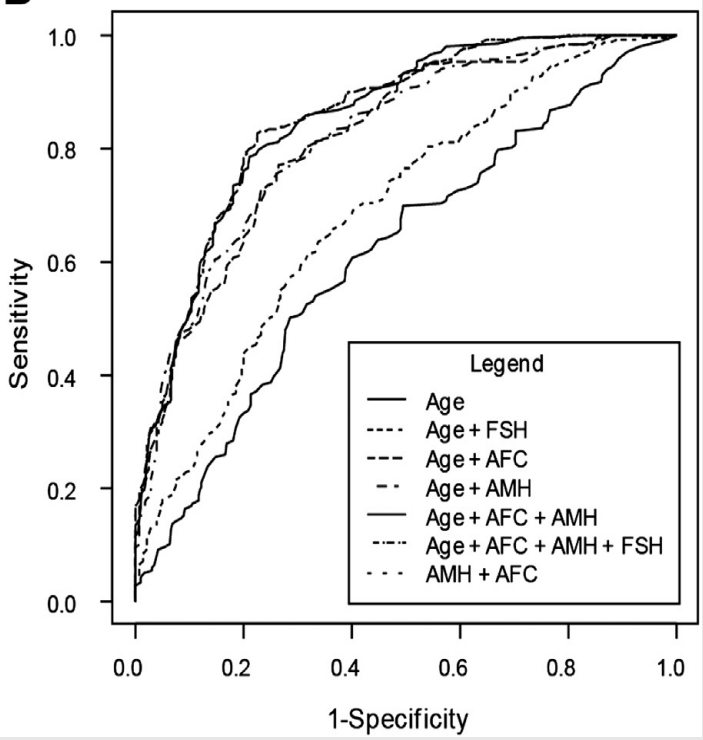
Main Outcome Measure(s): Excessive response prediction.

Result(s): We included 57 studies reporting on 32 databases. Female age had an area under the receiver operating characteristic curve of 0.61 for excessive response prediction. Antral follicle count and AMH significantly added prognostic value to this. A model with female age, AFC, and AMH had an area under the receiver operating characteristic curve of 0.85. The combination of AMH and AFC, without age, had similar accuracy. Subgroup analysis indicated that FSH performed significantly worse in predicting excessive response in higher age groups, AFC did significantly better, and AMH performed the same.

A

	Three-test study group (N= 1,023)				Total study group (N=4,786)			
	AUC	95% CI	P value	N	AUC	95% CI	P value	N
Univariable analysis								
Age	0.61	0.54 - 0.68	NA	1023	0.61	0.58 - 0.64	NA	4650
FSH	0.66	0.60 - 0.73	0.071	1023	0.64	0.61 - 0.67	0.026	4254
AFC	0.79	0.74 - 0.85	< 0.001	1023	0.73	0.69 - 0.77	< 0.001	2524
AMH	0.81	0.76 - 0.87	< 0.001	1023	0.82	0.77 - 0.86	< 0.001	1890
Multivariable analysis								
Age & FSH	0.68	0.62 - 0.75	< 0.001	1023	0.67	0.64 - 0.71	< 0.001	4254
Age & AFC	0.81	0.76 - 0.87	< 0.001	1023	0.75	0.71 - 0.79	< 0.001	2524
Age & AMH	0.81	0.76 - 0.87	< 0.001	1023	0.81	0.77 - 0.85	< 0.001	1890
Age & AMH & AFC	0.85	0.80 - 0.90	< 0.001	1023	0.85	0.80 - 0.90	< 0.001	1024
Age & AMH & AFC & FSH	0.85	0.80 - 0.90	< 0.001	1023	0.85	0.80 - 0.90	< 0.001	1023
AMH & AFC	0.85	0.80 - 0.90	< 0.001	1023	0.85	0.80 - 0.90	< 0.001	1024

B



Areas under the curve and ROC curves of prediction models of age and ovarian reserve tests for the prediction of an excessive response. (A) Areas under the curves of prediction models of age and ovarian reserve tests for the prediction of an excessive response. The AUCs of the univariable and multivariable models of age or ORTs in the prediction of an excessive response are shown. In the univariable analysis it is shown that both AMH and AFC have high accuracy, whereas FSH only has moderate accuracy. In the multivariable models the added value to the AUC of an ORT on female age is shown; the *P* value indicates whether this added value is significant in comparison with the model based on age alone. Adding any of the ORTs shows a significant rise in the AUC. Moreover, the added value of adding several ORTs to female age is shown. The model including age, AFC, and AMH reached the maximum predictive power. Addition of FSH to this model did not improve the predictive accuracy (*P*=.725). However, a model with AMH and AFC alone has a comparable AUC. (B) Receiver operating characteristic curves of age and ORTs in the prediction of an excessive response. The ROC curves of age and age combined with a single or more ORTs are depicted. The ROC curves for Age + AMH, Age + AFC, Age + AMH + AFC, and Age + AMH + AFC + FSH run toward the upper left corner of the ROC space, indicating a good capacity to discriminate between normal and excessive responders at certain cutoff levels. Receiver operating characteristic curves in the three-test study group (n = 1,023).

Broer. Excessive response prediction in IVF. Fertil Steril 2013.

AMH: 0.7–1.3 ng/ml may be considered acceptable for the prediction of poor response in IVF

AFC cut-off <5–7 may be considered acceptable for the prediction of poor response in IVF

Table I Cut-off values of anti-Mullerian hormone (AMH) for the prediciton of poor- and hyper response in IVF cycles.

Study	Design	*n*	Assay used	Cut-off value		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Conversion to AMH gen II assay[a]	
				ng/ml	pmol/l					ng/ml	pmol/l
Poor response											
van Rooij *et al.* (2002)	Prospective	119	IBC	0.3[b]	2.1	60	89			0.3[b]	2.1
Muttukrishna *et al.* (2004)	Prospective	69	IBC	0.1[b]	0.7	87.5	72.2			0.1[b]	0.7
Muttukrishna *et al.* (2005)	Retrospective	108	IBC	0.2[b]	1.4	87	64			0.2[b]	1.4
Tremellen *et al.* (2005)	Prospective	75	IBC	1.1	8.1[b]	80	85			1.1	8.1[b]
Peñarrubia *et al.* (2005)	Prospective	80	IBC	0.68	4.9[b]	53	96			0.68	4.9[b]
Ebner *et al.* (2006)	Prospective	141	IBC	1.66[b]	11.9	69	86			1.66[b]	11.9
Ficicioglu *et al.* (2006)	Prospective	50	DSL	2.5[b]	17.9	90.9	90.9			3.47	24.8
La Marca *et al.* (2007a)	Prospective	48	IBC	0.75[b]	5.4	80	93			0.75[b]	5.4
Fréour *et al.* (2007)	Prospective	69	IBC	1.3[b]	9.3	44	100			1.3[b]	9.3
Smeenk *et al.* (2007)	Prospective	80	IBC	1.4[b]	10	62	73			1.4[b]	10
McIlveen *et al.* (2007)	Prospective	84	IBC	1.25[b]	8.9	58	75			1.25[b]	8.9
Kwee *et al.* (2008)	Prospective	110	DSL	1.4[b]	10	76	86			1.94	13.9
Nakhuda *et al.* (2007)	Prospective	77	DSL	0.35[b]	2.5	90.1	81.8			0.48	3.5
Lekamge *et al.* (2007)	Retrospective	126	IBC	1.96	14[b]	73	73			1.9	14[b]
Nelson *et al.* (2007)	Prospective	340	DSL	0.7	5[b]	75	91			0.97	6.95
Gnoth *et al.* (2008)	Prospective	132	DSL	1.26[b]	9	97	41			1.75	12.51
Jayaprakasan *et al.* (2008b)	Prospective	135	DSL	0.99[b]	7.1	100	73			1.37	9.8
Riggs *et al.* (2008)	Retrospective	123	DSL	0.83[b]	5.9	83	79			1.15	8.2
Elgindy *et al.* (2008)	Prospective	33	IBC	2.7[b]	19.3	83.3	82.4			2.7[b]	19.3
Nardo *et al.* (2009)	Prospective	165	DSL	1[b]	7.1	87	67			1.39	9.8
Barad *et al.* (2009)	Retrospective	76	DSL	0.5[b]	3.6	87	84			0.69	5
Riggs *et al.* (2011)	Retrospective	78	DSL	1.5[b]	10.7	86	78	16	99	2.1	14.8
Al-Azemi *et al.* (2011)	Prospective	356	IBC	1.36[b]	9.7	75.5	74.8			1.36[b]	9.7
Lee *et al.* (2011a, b)	Prospective	172	IBC	1.08[b]	7.7	95	76			1.08[b]	7.7
Buyuk *et al.* (2011)	Retrospective	73	DSL	0.6[b]	4.3	70	70			0.83	6
Kunt *et al.* (2011)	Prospective	180	DSL	2.97[b]	21.2	100	89.6			4.1	29.4
Lee *et al.* (2011a)	Retrospective	1538	DSL	0.68[b]	4.8	64.7	85.1	92	47.8	0.94	6.67
Fridén *et al.* (2011)	Retrospective	127	DSL	0.7	5[b]	75	75			0.97	6.95
Yoo *et al.* (2011)	Retrospective	91	IBC	0.95[b]	6.8	73.3	82.1			0.95[b]	6.8
Tolikas *et al.* (2011)	Prospective	90	DSL	2.74[b]	19.6	69	70.5			3.8	27.2
Bonilla-Musoles *et al.* (2012)	Retrospective	143	IBC	1.3	9.28[b]	69	64			1.3	9.28[b]
Anckaert *et al.* (2012)	Retrospective	731	IBC	2.29	16.4[b]	81	83			2.29	16.4[b]
Satwik *et al.* (2012)	Prospective	198	DSL	2[b]	14.3	20	98			2.78	19.9

Table II Cut-off values of antral follicle count (AFC) for the prediciton of poor- and hyper response in IVF cycles.

Study	Design	*n*	AFC cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Poor response							
Chang *et al.* (1998)	Prospective	149	3	73	96		
Sharara and McClamrock (1999)	Prospective	127	4	53	73		
Frattarelli *et al.* (2000)	Retrospective	278	10	87	41		
Hsieh *et al.* (2001)	Prospective	372	3	61	94		
Nahum *et al.* (2001)	Prospective	224	6	95	69		
Frattarelli *et al.* (2003)	Prospective	267	4	30	96		
Järvelä *et al.* (2003)	Prospective	45	4	86	84		
Yong *et al.* (2003)	Prospective	46	4	9	97		
Bancsi *et al.* (2004)	Prospective	120	4	61	88		
Durmusoglu *et al.* (2004)	Retrospective	91	6.5	85	74		
Ng *et al.* (2005)	Prospective	131	4	33	92		
Muttukrishna *et al.* (2005)	Retrospective	108	5	89	39		
Ficicioglu *et al.* (2006)	Prospective	44	7	77	41		
Soldevila *et al.* (2007)	Prospective	327	8	62	74.8	59.1	77
Jayaprakasan *et al.* (2007)	Prospective	100	7	100	92.6		
Kwee *et al.* (2008)	Prospective	110	6	41	95	75	
Melo *et al.* (2009)	Prospective	1074	12	71.1	69.2	83.3	52.6
Jayaprakasan *et al.* (2010a, b)	Prospective	135	11	93	88		
Tolikas *et al.* (2011)	Prospective	90	4.5	72.4	80.3		
Bonilla-Musoles *et al.* (2012)	Retrospective	143	7	72	75		
Mutlu *et al.* (2013)	Retrospective	192	5.5	91	91		
Polyzos *et al.* (2013)	Retrospective	210	8	72.2	84.6		
Hyper response							
Ng *et al.* (2000)	Prospective	128	9	60	71		
van Rooij *et al.* (2002)	Prospective	114	14	92	63		
Eldar-Geva *et al.* (2005)	Prospective	56	14	94	33		
Kwee *et al.* (2007)	Prospective	110	14	81	89		
Aflatoonian *et al.* (2009)	Prospective	159	16	89	92		
Ocal *et al.* (2011)	Retrospective	82	8[a]	78	65	52	86
Polyzos *et al.* (2013)	Retrospective	210	16	80	84.5		

[a]Prediction of ovarian hyperstimulation syndrome; AFC, antral follicle count.

AMH: 3.52 and 3.9 ng/ml acceptable cut-off values for the prediction of hyper response in IVF

AFC value of >16, with a sensitivity of 89% and a specificity of 92%, for the prediction of high response

Lee *et al.* (2012)	Prospective	162	IBC	1.08[b]	7.7	85.8	78.6			1.08[b]	7.7
Honnma *et al.* (2012)	Retrospective	456	IBC	1.4	10[b]	72.2	75.7			1.4	10[b]
Mutlu *et al.* (2013)	Prospective	192	DSL	0.94[b]	6.7	71	85			1.3	9.3
Arce *et al.* (2013)	Retrospective	759	AMH gen II	1.68	12[b]	92	83			1.68	12[b]
Polyzos *et al.* (2013)	Retrospective	210	AMH gen II	1.37[b]	9.78	74.1	77.5			1.37[b]	9.78
Hyper response											
van Rooij *et al.* (2002)	Prospective	114	IBC	3.5[b]	25	40	95			3.5[b]	25
Eldar-Geva *et al.* (2005)	Prospective	53	IBC	3.5[b]	25	72	89			3.5[b]	25
La Marca *et al.* (2007a)	Prospective	48	IBC	2.6[b]	18.6	86	56			2.6[b]	18.5
Kwee *et al.* (2008)	Prospective	110	DSL	5[b]	35.7	53	91			6.95	49.6
Nelson *et al.* (2007)	Prospective	340	DSL	3.5	25[b]	60	94.9			4.8	34.7
Riggs *et al.* (2008)	Retrospective	123	DSL	1.59[b]	11.3	84	67			2.21	15.7
Lee *et al.* (2008)	Prospective	262	DSL	3.36[b]	23.9	62	87			4.67	33.2
Nardo *et al.* (2009)	Prospective	165	DSL	3.5[b]	25	88	70			4.8	34.7
Aflatoonian *et al.* (2009)	Prospective	159	IBC	4.83[b]	34.5	93	78			4.83[b]	34.4
Riggs *et al.* (2011)	Retrospective	78	DSL	3	21.4	70	71			4.17	29.7
Ocal *et al.* (2011)	Retrospective	695	DSL	3.3[b]	23.6	90	71	61	94	4.6	32.6
Honnma *et al.* (2012)	Retrospective	456	IBC	2.46	17.6[b]	69	75			2.46	17.6[b]
Anckaert *et al.* (2012)	Retrospective	731	IBC	4.17	29.8[b]	82.5	70.4			4.17	29.8[b]
Lee *et al.* (2012)	Prospective	162	IBC	3.57	25.5	94.4	83.3			3.57	25.5
Arce *et al.* (2013)	Retrospective	759	AMH gen II	3.9	28[a]	78	67			3.9	28[a]
Polyzos *et al.* (2013)	Retrospective	210	AMH gen II	3.52[b]	25.1	89.5	83.8			3.52[b]	25.1

PPV, positive predictive value; NPV, negative predictive value.
[a]Values from the original study have been converted to the recent AMH gen II assay by using conversion factor reported in Wallace *et al.* (2011) and Kumar *et al.* (2010).
[b]Indicates the unit of measurement used in the study.

Table II Cut-off values of antral follicle count (AFC) for the prediciton of poor- and hyper response in IVF cycles.

Study	Design	*n*	AFC cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
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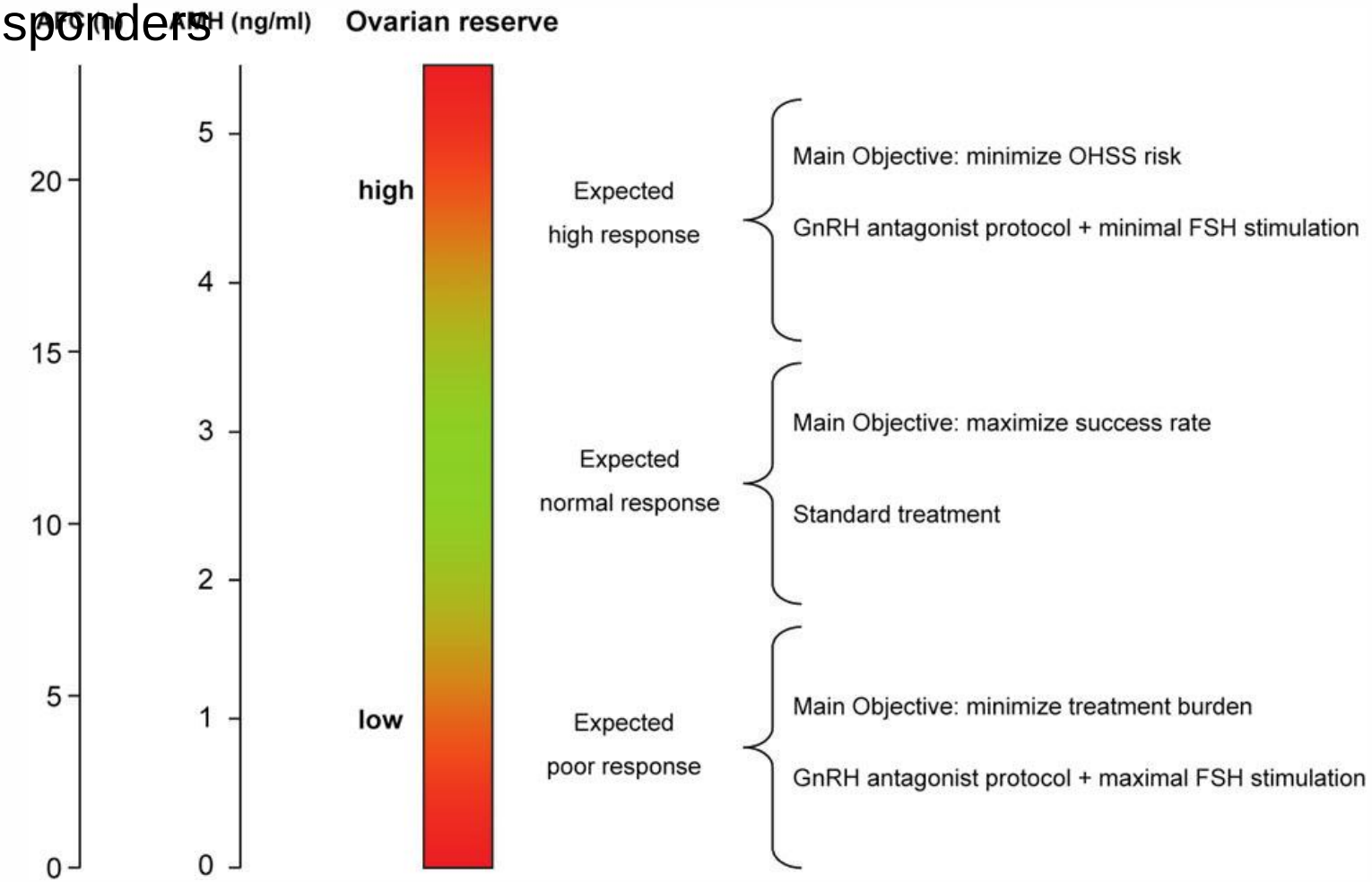
Aflatoonian *et al.* (2009)	Prospective	159	16	89	92		
Ocal *et al.* (2011)	Retrospective	82	8[a]	78	65	52	86
Polyzos *et al.* (2013)	Retrospective	210	16	80	84.5		

[a]Prediction of ovarian hyperstimulation syndrome; AFC, antral follicle count.

Ovarian reserve testing 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

Comparison of characteristics of the most widely used markers of ovarian reserve

Characteristics for a Good Marker	Age	AMH	FSH	AFC
Prediction of poor response	+	+++	++	+++
Prediction of hyper response	+	+++	+	+++
Low inter-cycle variability	+++	++	−	++
Low intra-cycle variability	+++	++	−	++
Applicable to all patients	+++	++	+	+
Economic	+++	−	−	−

- Serum AMH and AFC both seem to be the most reliable predictors of ovarian ageing
  - **they are equivalent in terms of their accuracy in predicting ovarian response**
  - **but none of the currently employed tests of ovarian reserve can reliably predict pregnancy success**

Prediction of ovarian response

- Therefore, other factors might influence the choice of test:
- Advantages of AMH include
  - intracycle stability and
  - the fact that concentrations can be determined from blood obtained during routine IVF testing
- In contrast, AFC needs to be determined early in the follicular phase of the cycle by a skilled ultrasound operator and the measurement requires standardization

AFC

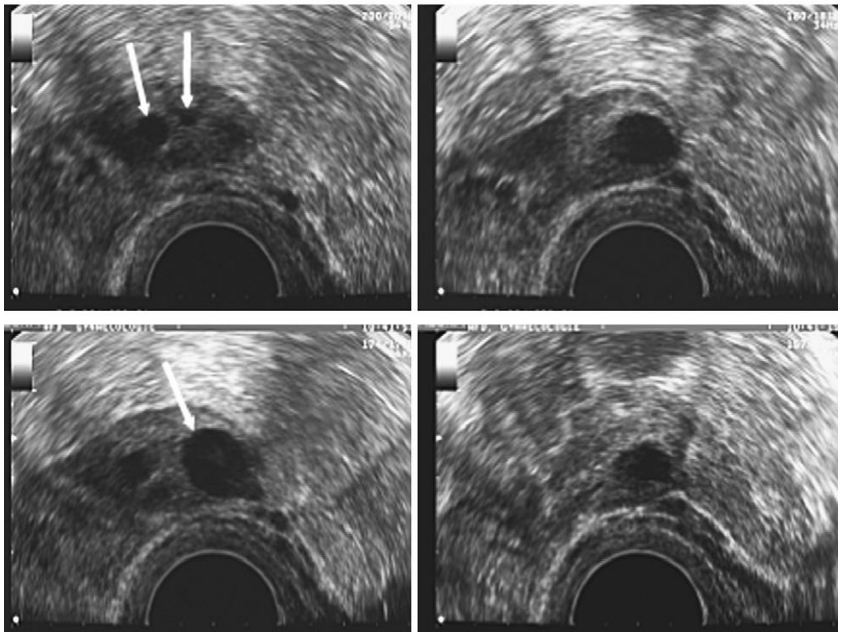
Table 2 The basic clinical and technical requirements for assessment of the AFC in clinical practice (reproduced with permission from Broekmans et al.

Considerations for the assessment of the AFC in clinical practice

Clinical considerations

Select patients with regular menstrual cycles with no co-existing pathological condition that could technically affect the counting of follicles, such as ovarian endometriosis or previous ovarian surgery

Count follicles between days 2 and 4 of a spontaneous menstrual or oral contraceptive cycle to avoid the effect of intra-cycle variation
Include all antral follicles of 2-10 mm in diameter



Technical considerations

A limited number of personnel, appropriately trained in transvaginal sonography should perform AFCs in each unit
Real-time, two-dimensional imaging is adequate

Use a transvaginal transducer
Use a probe with a minimum frequency of 7 MHz, which is maintained in an adequate condition and able to resolve a structure of 2 mm in diameter

Use a systematic process for counting antral follicles:

1. Identify the ovary
2. Explore the dimensions in two planes (perform a scout sweep)
Decide on the direction of the sweep to measure and count follicles
3. Measure the largest follicle in two dimensions

A. If the largest follicle is ≤10 mm in diameter:

i. Start to count from outer ovarian margin of the sweep to the opposite margin
ii. Consider every round or oval transonic structure within the ovarian margins to be a follicle
iii. Repeat the procedure with the contralateral ovary
iv. Combine the number of follicles in each ovary to obtain the AFC

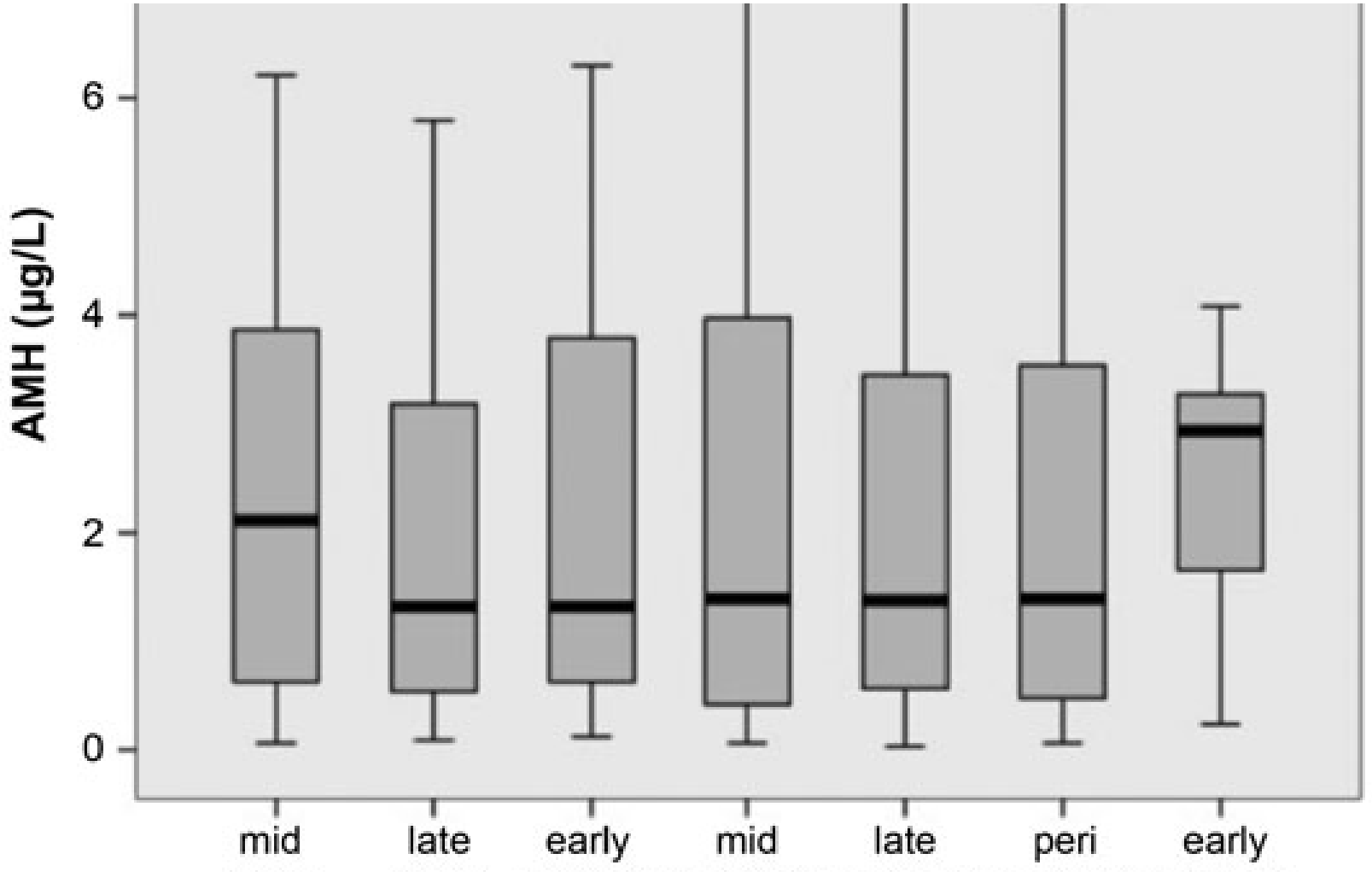
B. If the largest follicle is > 10 mm in diameter:

i. Further ascertain the size range of the follicles by measuring each sequentially smaller follicle, in turn, until a follicle with a diameter of ≤10 mm is found
ii. Perform a total count (as described) regardless of follicle diameter
iii. Subtract the number of follicles of > 10 mm from the total follicle count

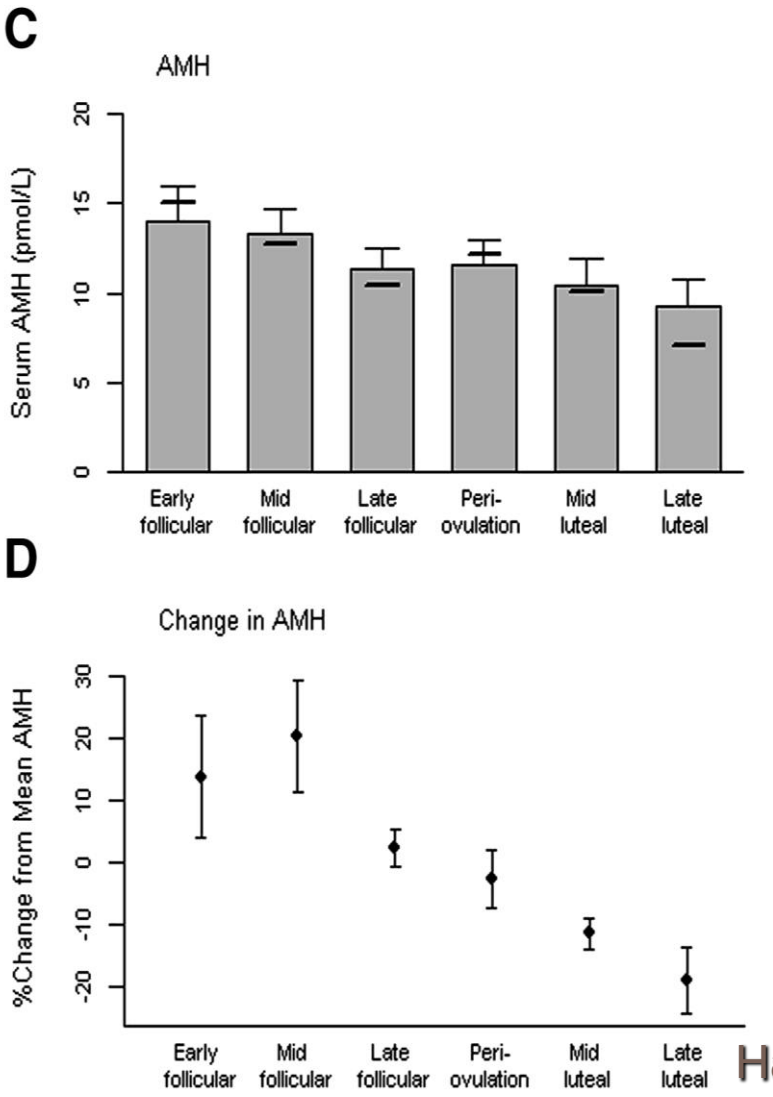
The AMH test

- Variability throughout the menstrual cycle
- Assay availability and variability

The AMH variability throughout the menstrual cycle



AMH: menstrual cycle variability



- AMH levels in the follicular phase appear to be 20-30% greater than in the luteal phase

Hadlow et al, Fertil Steril 99:1791, 2013

Human Reproduction, Vol.25, No.1 pp. 221–227, 2010
Advanced Access publication on October 19, 2009 doi:10.1093/humrep/dep366

human reproduction

ORIGINAL ARTICLE *Reproductive endocrinology*

Comparison of inter- and intra-cycle variability of anti-Müllerian hormone and antral follicle counts

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BACKGROUND: The antral follicle count (AFC) and anti-Müllerian hormone (AMH) both represent age-related follicular decline quite accurately, although long-term follow-up studies are still lacking. The best ovarian reserve test would need only a single, cycle-independent measurement to be representative.

METHODS: To compare the inter- and intra-cycle stability of AFC and AMH, we used age-adjusted intra-class correlation coefficients (ICCs). To measure inter-cycle stability across a number of up to four menstrual cycles, we used data, prospectively collected for the purpose of an other study, from 77 regularly cycling, infertile women aged 24–40 years. AMH and AFC values were measured on cycle day 3. To study intra-cycle variability, we used data from a prospective cohort study of 44 regularly cycling volunteers, aged 25–46 years and measured AMH and assessed the AFC (2–10 mm) every 1–3 cycle days.

RESULTS: Between menstrual cycles, AFC and AMH varied between 0 and 25 follicles (median 10), and 0.3 and 27.1 ng/ml (median 4.64). The difference in age-adjusted ICC between AMH [ICC, 0.89 (95% CI, 0.84–0.94)] and AFC [ICC, 0.71 (95% CI, 0.63–0.77)] was 0.18 (95% CI, 0.12–0.27). For the intra-cycle variation, 0–43 antral follicles (median 7) were counted per volunteer. The difference in age-adjusted ICC between AMH [ICC, 0.87 (95% CI, 0.82–0.91)] and AFC [ICC, 0.69 (95% CI, 0.46–0.82)] was 0.18 (95% CI, 0.034–0.42).

CONCLUSIONS: Serum AMH demonstrated less individual intra- and inter-cycle variation than AFCs and may therefore be considered a more reliable and robust means of assessing ovarian reserve in subfertile women.

AMH Assays

Diagnostic Systems Limited (DSL) ABD

Immunotech Limited (IOT) France

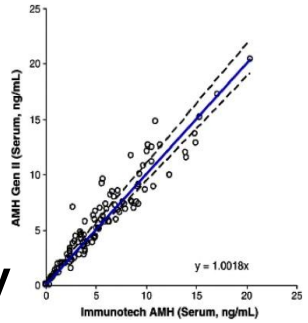
- The European and US assays were developed with different antibodies and reported out very different results, using different units.
- That problem has now been resolved by the manufacture of both ELISAs by the same company and the development of a new assay that combines the best features of both . Thus, currently there is only one assay

BECKMAN COULTER GEN II

Novel approach for AMH measurement (ELISA)

Beckman Coulter AMH Generation II (AMH Gen II)

- still a manual system (not automated)
  - employs the DSL antibody
  - calibrated to the IOT standard
- values *(in ng/mL)* comparable to the IOT assay and correlated to the DSL assay *(values > 40%)*
  - sensitivity *(limit of quantitation)* = 0.16 ng/mL
- hormone stability
  - whole blood
    - at room temperature: increments up to 31% after 4 days
    - at 4°C: lesser increments
  - serum & plasma
    - stable at room temperature and at 4°C up to 5 days



AMH dictated COH protocols

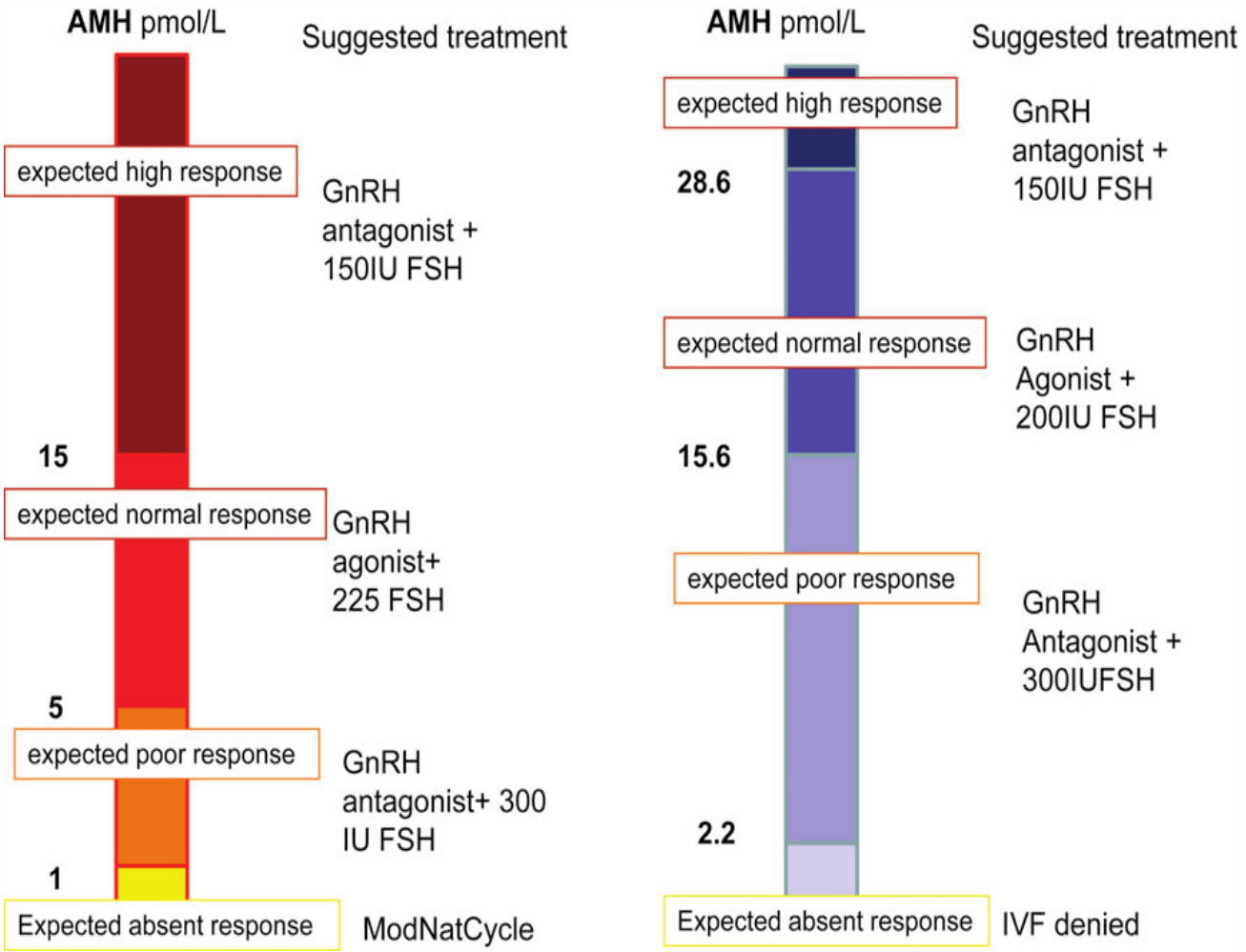


Figure 4 Strategic modelling of controlled ovarian stimulation on the basis of ovarian reserve markers. The introduction of individualized AMH-tailored controlled ovarian stimulation utilizing agonist and antagonist protocols has been reported as associated with improved IVF cycle, i.e. increased pregnancy rate. Similarly a reduction in the incidence of adverse outcomes, such as OHSS, has been reported (modified with permission from Nelson *et al.* (2009) and Yates *et al.* (2011). (AMH was measured with the DSL assay). AMH; anti-Mullerian Hormone.

Is there a low AMH cut off value to refuse IVF treatment ?

- However, AMH measurements are not suitable for denying access to IVF treatment, as women with very low, even undetectable levels, still have a chance of pregnancy.

Human Reproduction, Vol.26, No.7 pp. 1905–1909, 2011
Advanced Access publication on April 30, 2011 doi:10.1093/humrep/der134

human reproduction

ORIGINAL ARTICLE *Reproductive endocrinology*

Live birth chances in women with extremely low-serum anti-Mullerian hormone levels

Andrea Weghofer[1,2,*,†], Wolf Dietrich[3,†], David H. Barad[2,4], and Norbert Gleicher[2,5]

Table II Pregnancy outcomes in 128 IVF patients with extremely low AMH levels (0.1–0.4 ng/ml).

	All patients (n = 128/254)[a]	95% CI	≤Age 42 years (n = 70/145)[a]	95% CI	>Age 42 years (n = 58/109)[a]	95% CI	P
Clinical pregnancies per cycle	20 (7.9%)	[4.9%–11.9%]	16 (11.0%)	[6.4%–17.3%]	4 (3.7%)	[1.0%–9.1%]	0.031
Clinical pregnancies per patient	20 (15.6%)	[9.8%–23.1%]	16 (22.9%)	[13.7%–34.5%]	4 (6.9%)	[1.9%–16.7%]	0.013
Deliveries after 1st IVF cycle	8 (6.3%)	[2.7%–11.9%]	7 (10.0%)	[4.1%–19.5%]	1 (1.7%)	[0.04%–9.2%]	0.055
Deliveries per patient	12 (9.4%)	[4.9%–15.8%]	10 (14.3%)	[7.1%–24.7%]	2 (3.4%)	[0.4%–11.9%]	0.036

[a]Patients/ART cycles.

Individualized Gn-dosing algorithms

- **Popovic-Todorovic et al-2003**
  - RCT; Standart patients
  - 150 IU vs calculated Dose; Agonist
  - AFC, Ovarian v; Doppler score; Famele age; Smoking habit
- **Olivennes et al-2009**
  - CONSORT; Prospective uncontrolled
  - Calculated Dose; Agonist
  - Basal FSH; BMI; Female age and AFC
- **La Marca et al-2012, 2013**
- **OPTIMIST -Enrolling**

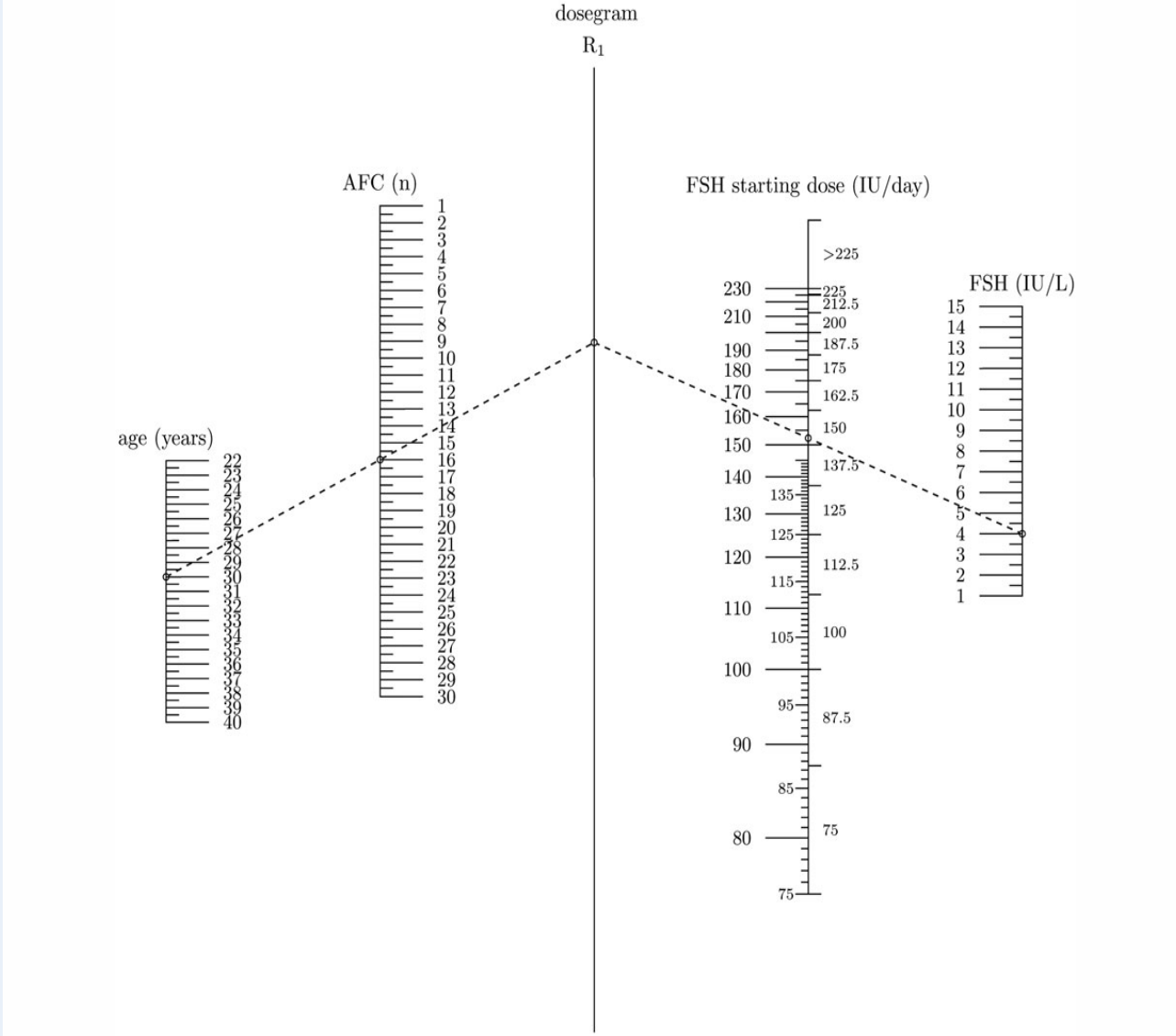


Figure 5 Nomogram for calculation of the FSH starting dose based on age, AFC and Day 3 serum FSH. In the example, for a 30-year-old woman with AFC = 16 and d3FSH = 4 IU/l, the FSH starting dose is 152 IU/day. Since the new FSH delivery system will have the dosage dial based on doses of FSH of 12.5 IU, on the right side of the FSH starting dose column, the FSH dose as selected for the delivery system is reported (150 IU/day, for example). (from La Marca *et al.* (2013) with permission). AFC; antral follicle count.

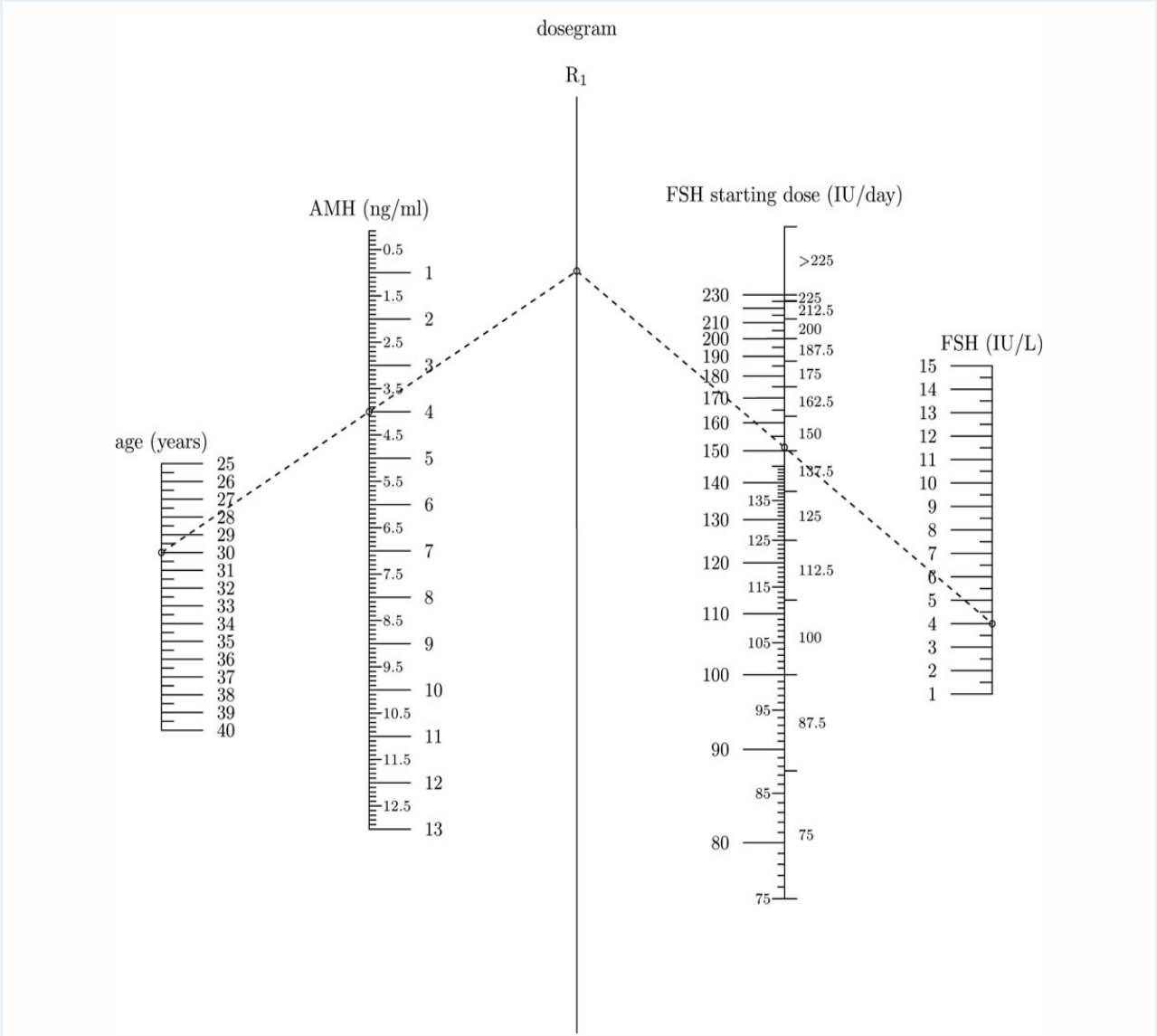


Figure 6 The nomogram for the calculation of the FSH starting dose based on age, serum AMH and FSH. In the example, for a 30-year-old woman with serum AMH level of 4 ng/ml and FSH level of 4 IU/l, the FSH starting dose is 152 IU/day. Since the new upcoming FSH delivery system will have the dosage dial based on doses of FSH of 12.5 IU, on the right side of the FSH starting dose column, the FSH dose as selected for the delivery system is reported (150 IU/day for the example). (AMH was measured with the IBC assay. AMH conversion factor: 1 ng/ml = 7.143 pmol/l) (from La Marca *et al.* (2012b), with permission). AMH, anti-Mullerian Hormone.

CONCLUSION

- Accurate prediction of ovarian reserve has several advantages and can help to improve female reproductive health
- Age
- Counting antral follicles is “operator dependent”
- Relative cycle stability and operator independency make AMH a very appealing marker of ovarian reserve

CONCLUSION

- **AMH is the most useful serum method of**
  - determining ovarian reserve
  - pretreatment counseling
  - selecting choice of infertility treatment
  - avoidance od ovarian hyperstimulation
- **No marker is perfect, and AMH is no exception**
  - Antimüllerian hormone is certainly a good predictor of egg supply, but it may not predict egg quality
  - Automated methodology should become available

CONCLUSION

- For the first time in female reproductive biology, it is possible to measure the submerged part of the iceberg of follicle growth, i.e. the intrinsic, so-called ‘acyclic’ ovarian activity
- Further research is needed to establish whether individualized treatment protocols based on basal AMH serum concentrations will result in improved clinical outcomes by
  - reducing poor response rates
  - lowering the incidence of OHSS
  - increasing live birth rates

Thanks for your patience …