Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART)

Human Reproduction Update, Vol.16, No.2 pp. 113–130, 2010

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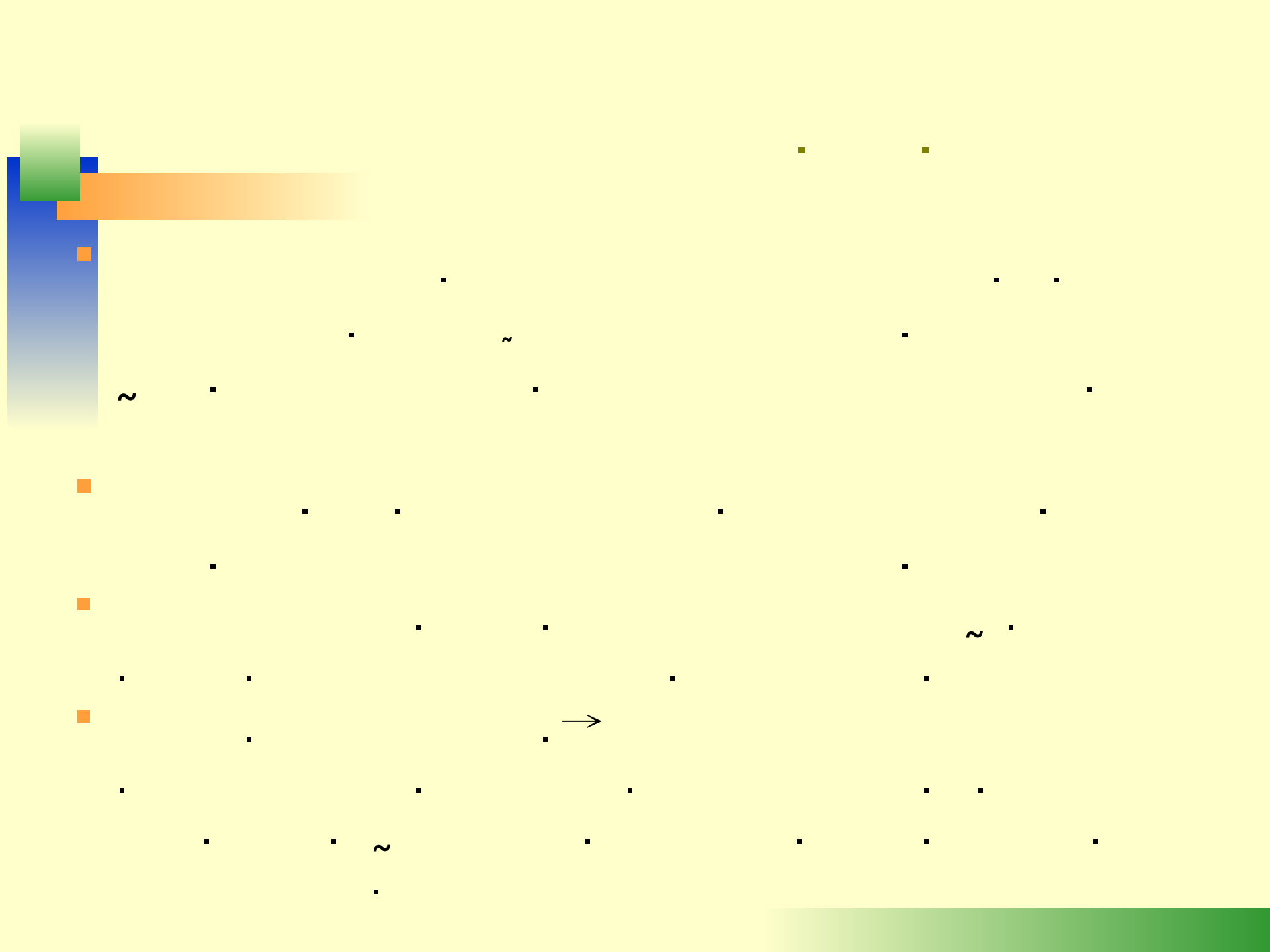
2010-6-29

- AMH on ovarian physiology
- Factors modulating AMH levels in women
- Prediction of quantative ovarian response in ART
- Prediction of qualitative ovarian response in ART
- AMH in ovarian reserve testing

- AMH in testicular physiology
- Value of AMH measurement in infertile men



- Produced by granulosa cells , from pre-antral and antral follicles
- Main role : the inhibition of the early stages of follicular development .
- Reflect the ovarian follicular pool, follicle ↓ → AMH ↓
- Clinical marker of ovarian reserve and response to gonadotrohpins
- Prediction of quantitative and qualitative ovarian response in ART

- Secreted by Sertoli cell , from GA 8wks
- Main role: the paracrine control of testicular function.
- Detected in both serum and seminal fluid →specific marker of Sertioli cell function →may obtain information on spermatogenesis
- May use to diagnostic work-up for OAT and azoospermia
- May use in prediction the successful sperm retrieval in azoospermia.

- In vitro and in vivo study, primordal →growing follicles became enhanced in absence of AMH , leading to early exhaustion of the primordial follicle pool
- More growing follicles were found in AMH null mice than in wild

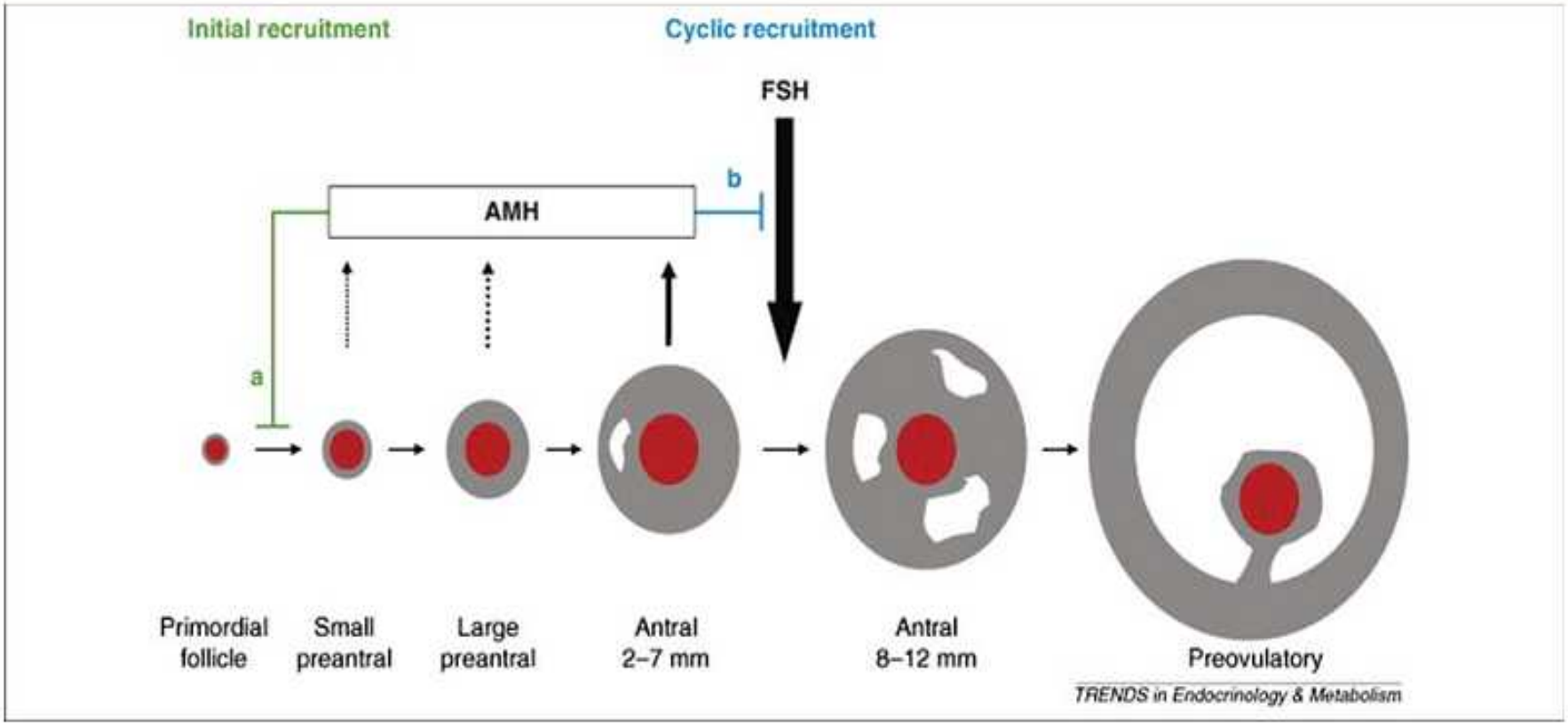


Figure 1 AMH is secreted by pre-antral and antral follicles.

It seems to inhibit initial follicle recruitment and FSH-stimulated follicle growth. The role of AMH in the two main compartments of normal ovarian follicle development (the red centre represents the oocyte, the grey area represents the granulosa cell layer and the white area represents follicle fluid in the antrum). AMH is expressed in small and large pre-antral follicles (broken arrows) and in small antral follicles (whole arrow), and the latter mainly contributes to serum levels. Initial recruitment takes place as a continuous process, whereas cyclic recruitment is driven by a rise in FSH serum levels at the end of a previous menstrual cycle. The inhibitory effects of AMH are shown (a) on the initial recruitment of primary follicles from the resting primordial follicle pool and (b) on the sensitivity of antral follicles for FSH (reproduced with permission from Broekmans *et al.*, 2008).

- Related to estradiol levels in follicular fluid

- Undetectable at birth
- Increase within 0-4y/o
- Keep stable until adult
- Decrease to undetectable at menopause
- Undetectable 3-5days after bil oophorectomy →solely of ov origin

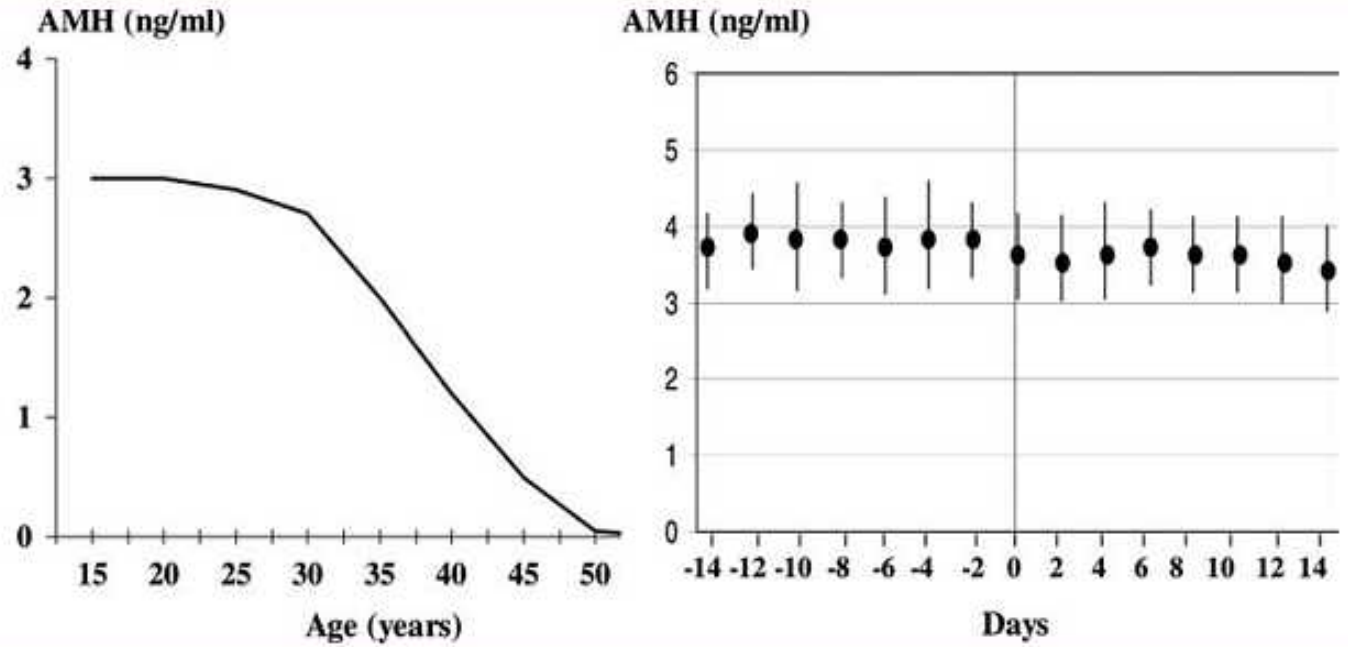
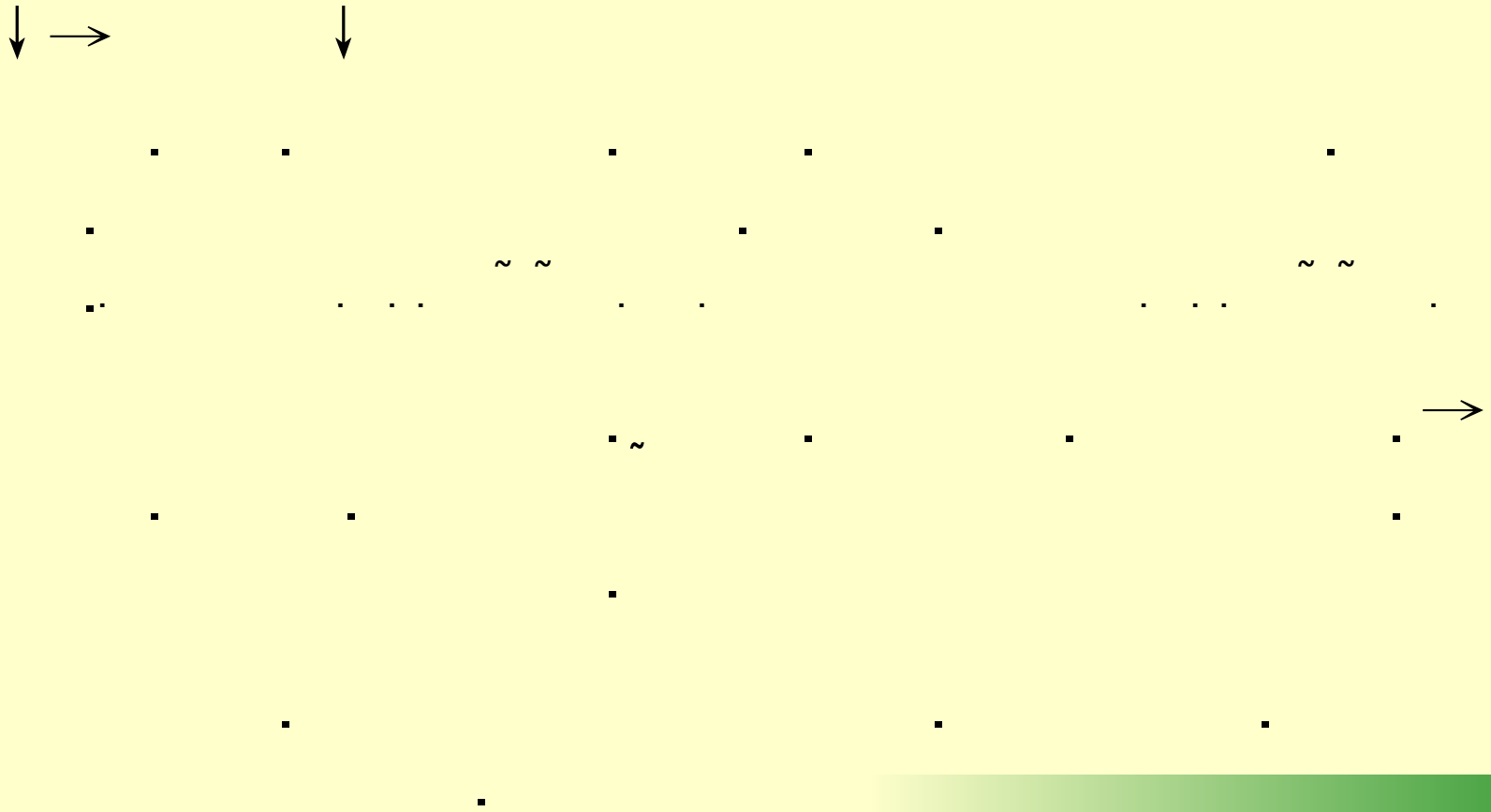


Figure 2 *Left*: Mean serum AMH levels show a reduction throughout reproductive life. Undetectable AMH levels after spontaneous menopause have been reported (constructed graphic). *Right*: Circulatory pattern of AMH during the menstrual cycle of young healthy women aged 18–24 years. Serum AMH levels have been shown to be stable throughout the menstrual cycle. Day 0 = day of LH surge (reproduced with permission from La Marca *et al.*, 2006a).



- Higher in insulin-R PCOS, and in amenorrheic PCOS
- May be more strongly related to PCO than to the full spectrum of the syndrome
  - Prospective, randomized, double blind study (Carlsen, 2009)
    - Pt had diet and lifestyle counselling and metformin
    - Randomized to either dexamethosone or placebo
    - AMH were unaffected by 6 months in all groups

- Sensitivity: 67% ; specificity : 92%
- If sono data are not available →used as a diagnostic criteria

- FSH ↑ →induces growth of follicles →lose AMH expression
- FSH ↑ →E2 ↑ → down-regulation of AMH and AMHII mRNA (Baarends , 1995)

Table I Studies on AMH as marker of ovarian response to controlled ovarian stimulation (COS)

Author	n	R with oocytes*	AMH better than					
			AFC	Ov. Vol	d3 FSH	d3 E2	d3 inhB	Age
Seifer *et al.* (2002)	107	0.48			√	√		
Van Rooij *et al.* (2002)							√	√
Fanchin *et al.* (2003a, b								
Muttukrishna *et al.* (20							√	
Hazout *et al.* (2004)					√	√	√	√
Muttukrishna *et al.* (2005)	108	0.5	=		√			
Eldar-Geva (2005)	56	0.64	X		√		√	
Silberstein *et al.* (2006)	257	0.33			√			
Fıpcioglu *et al.* (2006)	50	0.56	√		√	√		√
Lekamge *et al.* (2007)	126	0.34	=					
La Marca *et al.* (2007)	48	0.7						
Kwee *et al.* (2007)	110	0.63	X					√
Nakhuda *et al.* (2007)	77	0.63			√			
McIlveen *et al.* (2007)	84	0.78	√	√	√		=	√
Nelson *et al.* (2007)	340	0.71			√			√
Elgindy *et al.* (2008)	33	0.88	=	√	√			
Lie Fong *et al.* (2008)	125	0.47						
Jee *et al.* (2008)	59	0.53					X	
Jayaprakasan *et al.* (2008)	135	0.47	=	√	√	√		√
Wunder *et al.* (2008)	276	0.35			√		X	

Higher AMH in D3 →greater no. of retrieved oocytes .
2.5 fold higher with at least 11 oocytes than <6 oocytes

AFC and AMH perform similar power to predict ov response

Comparison with other predictors.
*R with oocytes: correlation between serum AMH levels and the number of retrieved oocytes; √, better than; X, worse than; =, equal to.

- compared value of AMH and AFC in prediction ovarian response in IVF
- No significant difference

- No. of developed follicle
- No. of retrieved ooctyes
- Cycle cancellation

Table II Sensitivity and specificity of AMH for the prediction of poor response to gonadotrophin stimulation

Author	n	Study design	Cut-off value	Sens (%)	Spec (%)	Definition of poor response	AMH assay
Van Rooij *et al.* (2002)	119	Prosp	0.3 μg/l	60	89	<4 oocytes	Immunotech–Beckman–Coulter
Muttukrishna *et al.* (2004)	69	Prosp	0.1 ng/ml	87.5*	72.2*	<4 oocytes or cancellation	Immunotech–Beckman–Coulter
Muttukrishna *et al.* (2005)	108	Retro	0.2 ng/ml	87	64	≤4 oocytes	Immunotech–Beckman–Coulter
Tremellen *et al.* (2005)	75	Prosp	8.1 pmol/l	80	85	≤4 oocytes	Immunotech–Beckman–Coulter
Peñarrubia *et al.* (2005)	80	Prosp	4.9 pmol/l	53*	96*	cancellation	Immunotech–Beckman–Coulter
Ebner *et al.* (2006)	141	Prosp	1.66 ng/ml	69	86	<4 oocytes	Immunotech–Beckman–Coulter
Fiçicioğlu *et al.* (2006)	87	Prosp	1.5 μg/l	44	100	<6 oocytes	Immunotech–Beckman–Coulter
La Marca *et al.* (2006)	80	Prosp	1.9 μg/l	97	41	≤4 oocytes	Immunotech–Beckman–Coulter
Fréour *et al.* (2007)	87	Prosp	1.5 μg/l	44	100	<6 oocytes	Immunotech–Beckman–Coulter
Smeenk *et al.* (2007)	80	Prosp	1.4 μg/l	62	73	≤4 oocytes	Immunotech–Beckman–Coulter
McIlveen *et al.* (2007)	84	Prosp	1.25 ng/ml	58	75	≤4 oocytes	Immunotech–Beckman–Coulter
Kwee *et al.* (2007)	110	Prosp	1.4 μg/l	76	86	<6 oocytes	Diagnostic System Laboratories
Nakhuda *et al.* (2007)	77	Prosp	0.35 ng/ml	90.1*	81.8*	cancellation	Diagnostic System Laboratories
Lekamge *et al.* (2007)	126	Retro	14 pmol/l	73	73	≤4 oocytes	Immunotech–Beckman–Coulter
Nelson *et al.* (2007)	340	Prosp	5 pmol/l	75†		≤2 oocytes	Diagnostic System Laboratories
Groth *et al.* (2008)	132	Prosp	1.26 ng/ml	97	41	≤4 oocytes	Diagnostic System Laboratories
Nardo *et al.* (2008)	165	Prosp	1.0 ng/ml	87	67	≤4 follicles on day 8 of COH	Diagnostic System Laboratories
Jayaprakasan *et al.* (2008)	135	Prosp	0.99 ng/ml	100	73	<4 oocytes or cancellation	Diagnostic System Laboratories

*For cycle cancellation identification; †percentage of correctly classified poor responder patients; Retro, retrospective study; Prosp, Prospective study.

- AMH in prediction of poor repsonse to FSH
  Sensitivity : 41-97 % ; specificity : 41-100%

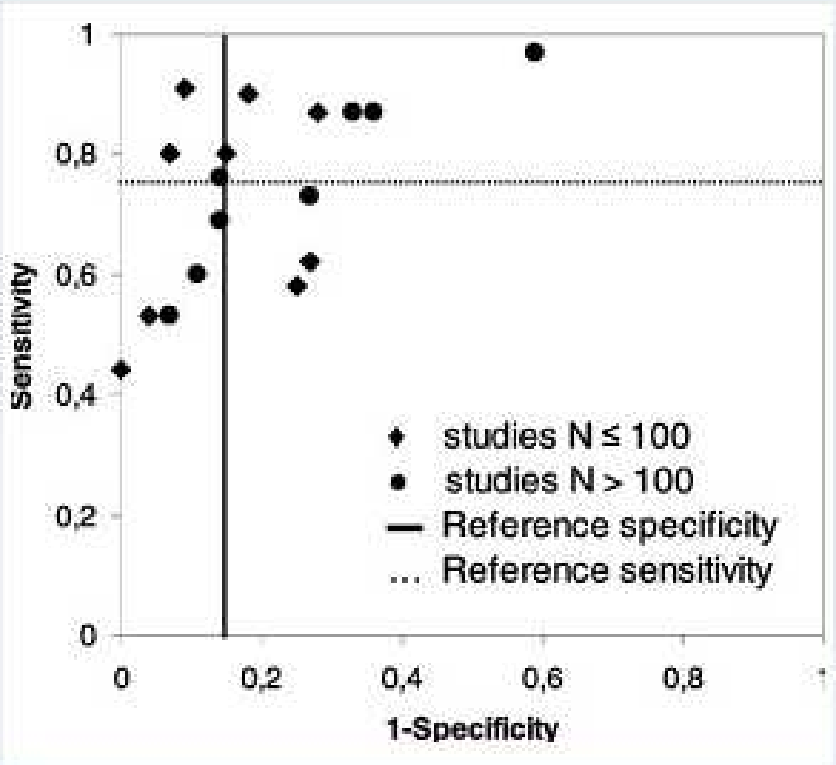


Figure 3 Sensitivity–specificity points for all studies reporting on the performance for AMH in the prediction of poor response.

Reference lines indicate a desired level for sensitivity (0.75) and specificity (0.85).

- Menstrual cycle - Independent marker
- Stable and very low inter- and intra-cycle variability

- Immunotech-Beckamn Coulter and DSL
  - Now both produced by a single company
  - Similar AMH values
- Automated system for AMH will be availabe within 2 yrs

- AMH measurement prior to gonadotrophin secretion, may be useful in the prediction of women at risk for poor-response or no response to gonadotrophins .

- AMH is menstrual-independent →a reliable marker of ovarian reserve , measured independently of the day of the cycle .

- Mild and moderate : 15-20%
- Severe :1-3%
- Specific risk factors : young age, low BMI, signs of PCOS, previous history of OHSS, high E2 on the day of hCG.
- Key to preventing OHSS: recognize the risk factors

Table III Basal AMH levels in women with normal response, hyper-response to controlled ovarian stimulation (COS) and ovarian hyperstimulation syndrome (OHSS)

Author	Design	*n*	Mean AMH levels		
			Normal response	Excessive response	OHSS
Tremellen *et al.* (2005)	Prosp	75	15.47 pmol/l	21.53 pmol/l[a]	
Eldar-Geva *et al.* (2005)	Prosp	56	14.1 pmol/l	37.8 pmol/l[b]	
Nakhuda *et al.* (2006)	Retro	30	0.63 ng/ml		3.6 ng/ml
La Marca *et al.* (2007)	Prosp	48	5.98 ng/ml	10.13 ng/ml[c]	
Nelson *et al.* (2007)	Prosp	340	10 pmol/l	27 pmol/l[c]	
Nardo *et al.* (2008)	Prosp	165	3.04 ng/ml	5.56 ng/ml[d]	

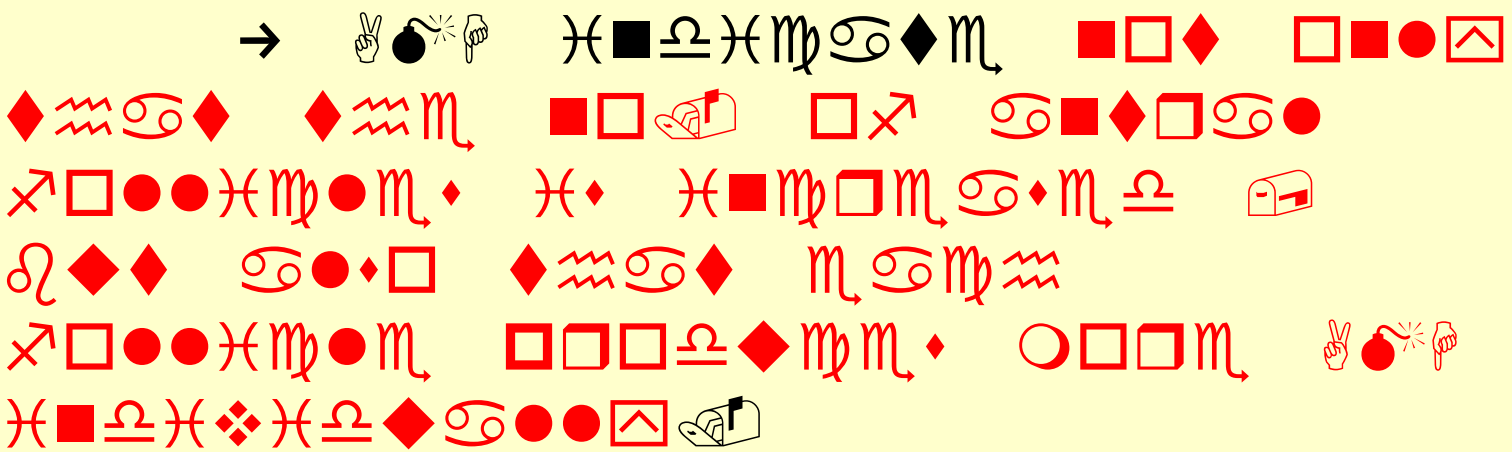
Retro: retrospective study; Prosp: prospective study.
[a]Excessive response if ≥ 18 oocytes retrieved.
[b]Excessive response if ≥ 20 oocytes retrieved.
[c]Excessive response if ≥ 16 oocytes retrieved.
[d]Excessive response if ≥ 21 oocytes retrieved.

Table IV AMH cut-off values for the prediction of hyper-response to COS and OHSS

Author	n	Study design	Cut-off value	Sensitivity (%)	Specificity (%)	Prediction of hyper-response	Prediction of OHSS
Kwee *et al.* (2007)	110	Prosp	5 mcg/l	53	91	√[a]	
Nelson *et al.* (2007)	340	Prosp	25 pmol/l	60	94.9	√[b]	
Lee *et al.* (2008)	262	Prosp	3.36 ng/ml	90.5	81.3		√
Nardo *et al.* (2008)	165	Prosp	3.5 ng/ml	88	70	√[a]	

Prosp: prospective study.
[a]Excessive response if >20 oocytes retrieved.
[b]Excessive response if ≥21 oocytes retrieve.

- AMH in follicular fluid : 3times higher in small than in large follicles
- Positive correlation with the no. of early antral follicles on D3, growing follicles on the day of hCG and oocytes retrieved



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- Embryo implantation , clinical pregnancy and ongoing pregnancy rate increase dramatically from the low to the high follicular fluid AMH groups.
- AMH in follicular fluid may be an additional factor in the selection of the oocyte .

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- AMH in follicular fluid were significantly increased who became pregnant in the respective IVF/ ICSI tx cycle.

- Implantation rate, but not pregnancy rate, was higher in the group with high basal AMH levels.
- Lack of correlation between serum AMH and embryo morphology and aneuploidy rate .

- Low serum AMH levels yielded fewer ooctyes and generated fewer embryos
- Higher pregnancy rate with high basal AMH levels

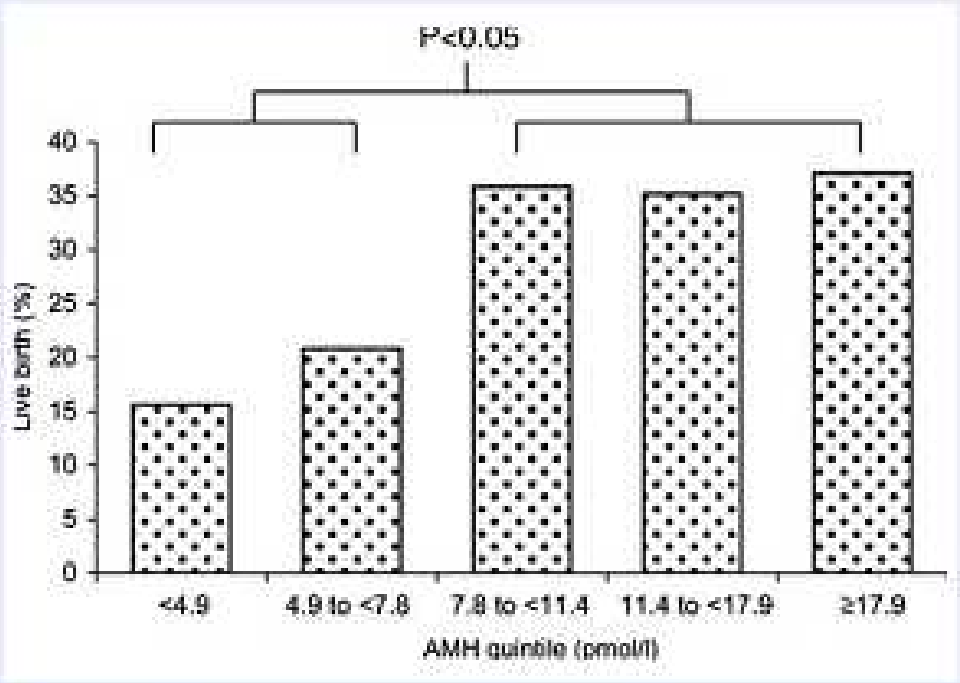


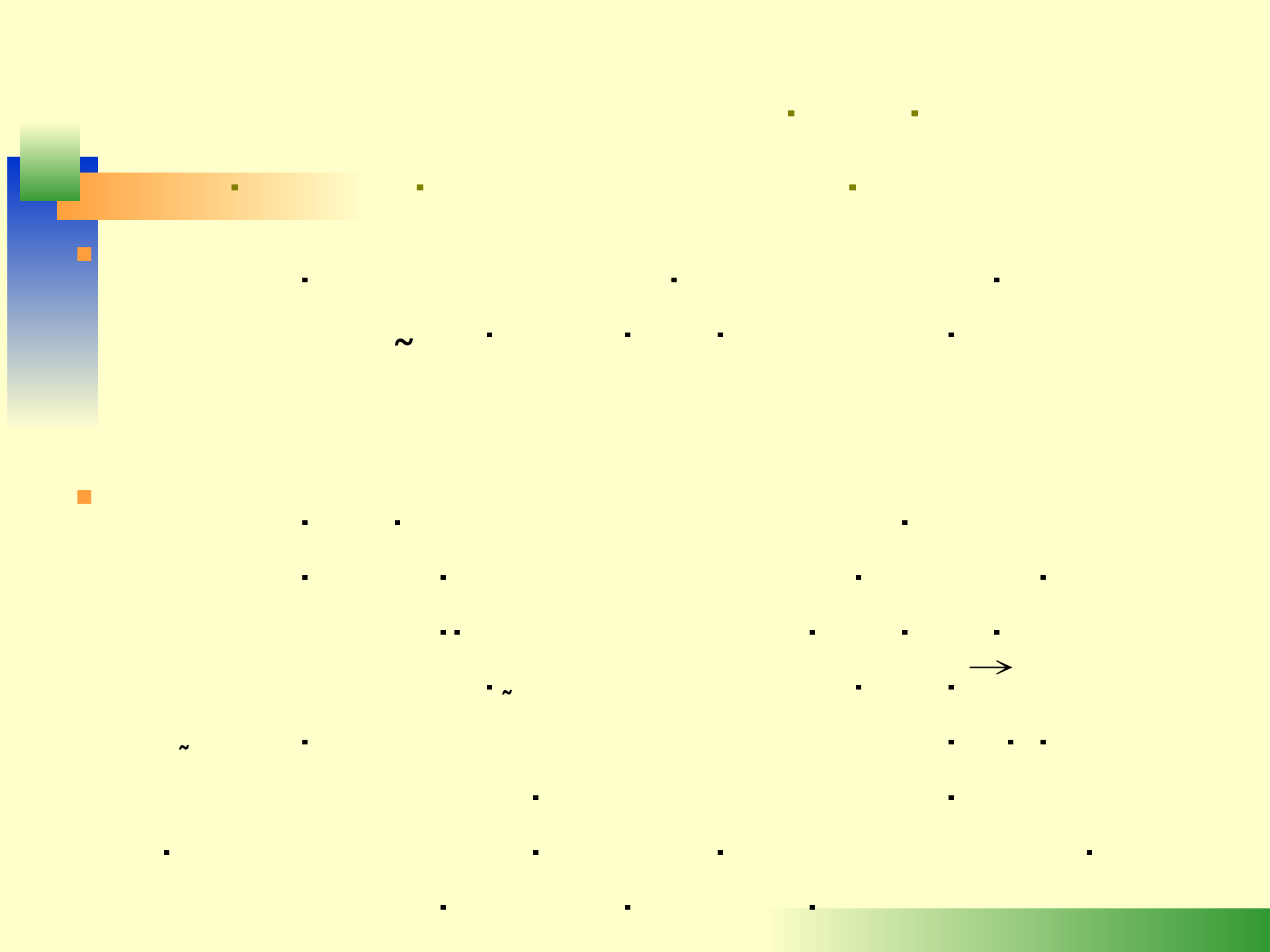
Figure 4 The mean live birth rate following IVF according to basal serum AMH levels.

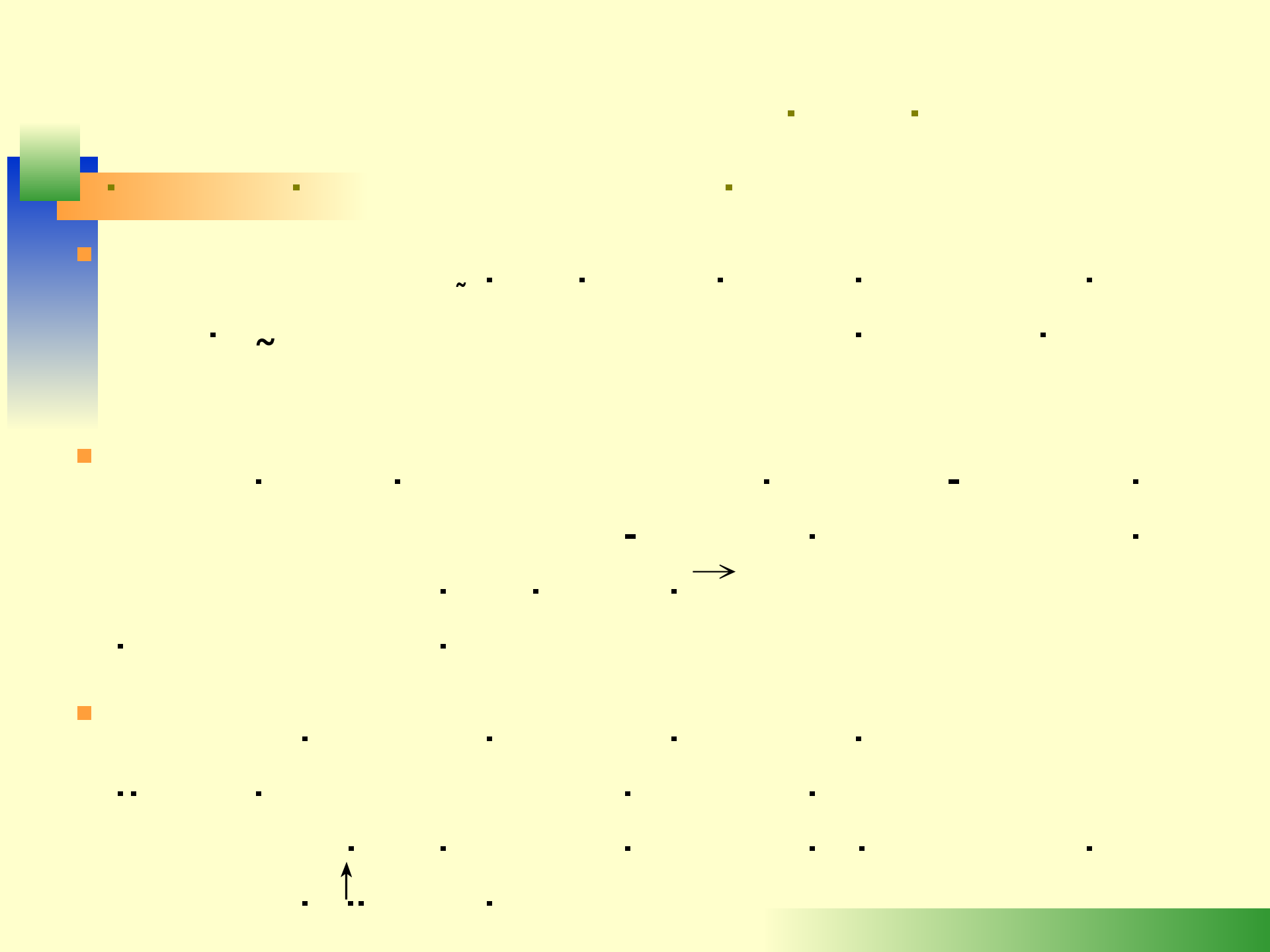
In this prospective study of 340 patients it was demonstrated that the live birth rate dramatically increased with increasing basal AMH value. However this was valid only for women with basal levels <7.8 pmol/L. Above this value there was no discrimination for the live birth (reproduced with permission from Nelson et al., 2007).

- Basal AMH does not seem to predict pregnancy or non-pregnancy , but simply enables pts to be identified as being at a low or high probability of pregnancy after IVF.

- To counsel the pts about the risk/benefit of the tx
- To reduce the cost by denying tx to bad prognosis couples
- To individualize tx strategy

- Accuracy of AMH for poor response→ false positive rate 10-20% → should not use AMH to exclude pts from IVF.
- The added value of AMH to age in the identification of poor response may be lower than expected.
- Basal AMH < 0.7ng/ml → live birth rate : 15%
- Serum AMH < 0.1-0.35ng/ml →very high risk for cycle cancellation , very poor prognosis → should refuse tx .
- Age alone would identify the majority of women who will





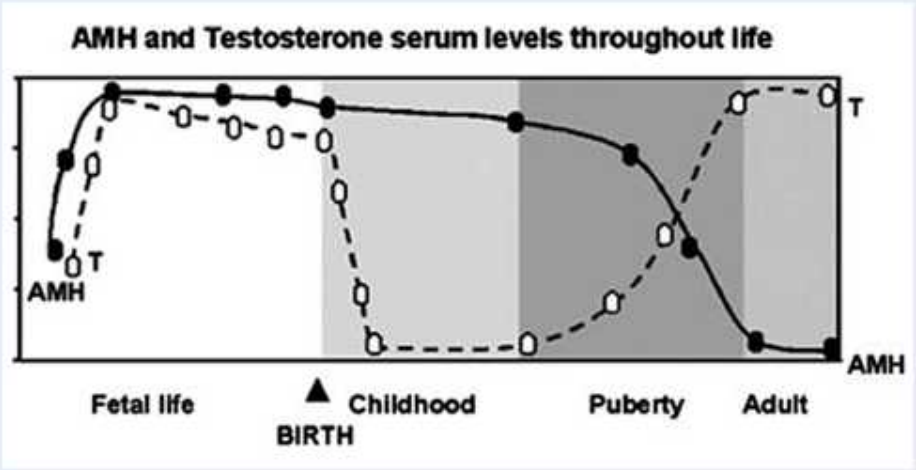
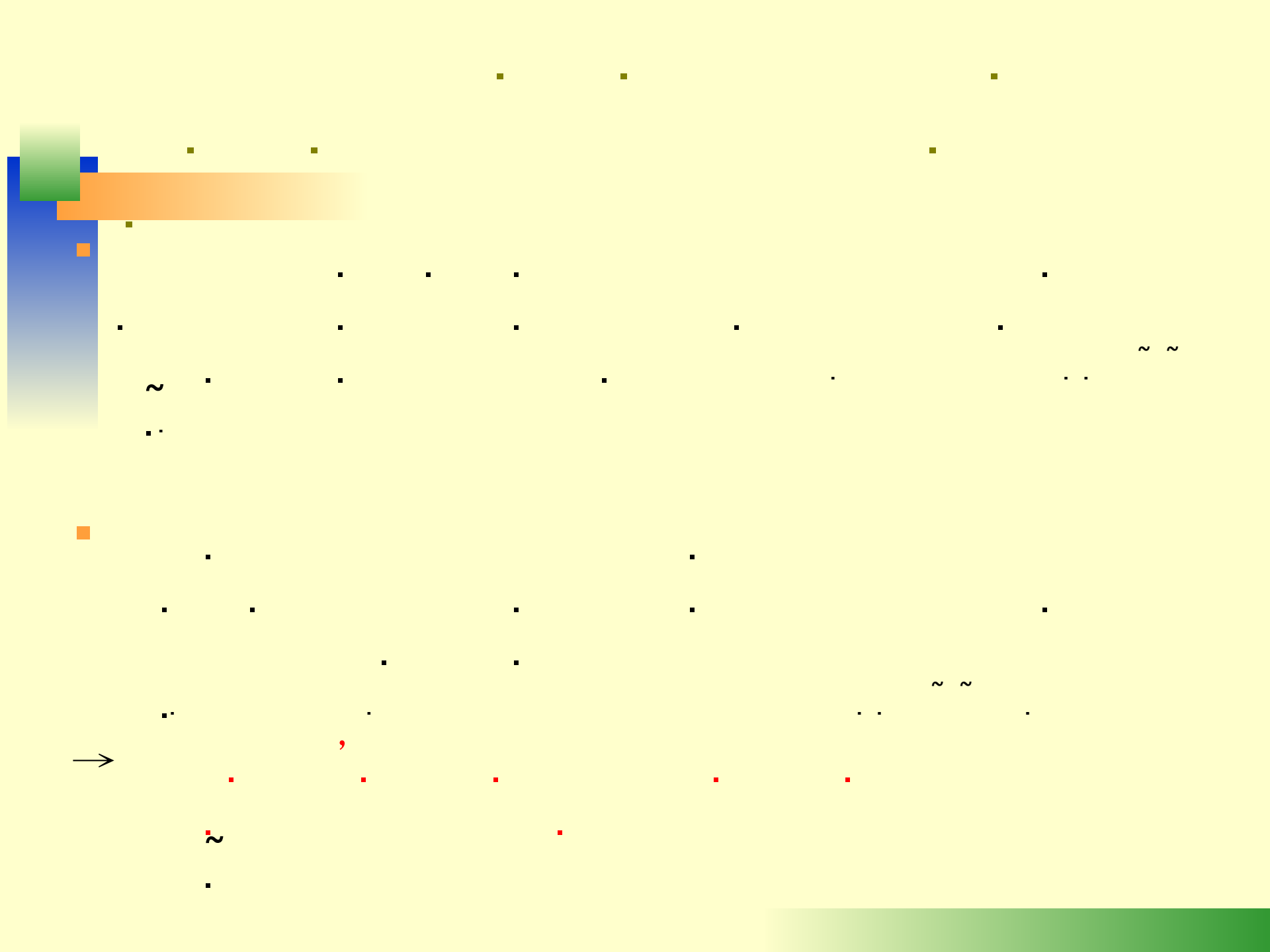


Figure 5 Profiles of serum AMH and testosterone (T) in the male.

AMH is not down-regulated by testosterone during fetal life and in the first months after birth due to the lack of expression of the androgen receptor in Sertoli cells. The physiological androgen insensitivity of fetal and neonatal Sertoli cells explains, thus, the transient coexistence of high concentrations of androgens and AMH. During puberal development AMH expression falls coincidently with the increase in androgen secretion by Leydig cells (reproduced with permission from Rey, 2005).



- Healthy men > oligozoospermic > Azoospermic

Table V 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	+++	++	−	++
Blinded to the operator	+++	+++	+++	−
Applicable to all patients (a)	+++	+++	+	+
Cheapness	+++	−	−	−

(a) FSH and antral follicle count (AFC) are not informative in patients on hormonal contraception or GnRH agonist treatment. Moreover the count of antral follicles may be difficult in women with ovarian cysts or with previous pelvic surgery.

- Most centers : FSH starting dose is based on age and BMI
- Some authors: based on AMH
- Daily FSH is tailored based on the pre-IVF AMH , and independently of the age and BMI (Nelson, 2009; Gnoth 2008)

- Predicted negligible response : AMH < 1pmol/l
- Predicted reduced response : AMH ≥ 1, <5pmol/l
- Predicted normal response : AMH ≥ 5, < 15pmol/l
- Predicted high response : AMH ≥ 15pmol/l

- Different stimulation protocols based on above categories
  - Low AMH→ high starting FSH + GnRH antagonist
  - Normal AMH → standard long protocol