Procréation médicalement assistée et surveillance biologique

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PMA et surveillance biologique

- But des analyses hormonales
  - → Avant la PMA
  - → Suivi de la PMA

- Aspects analytiques des immunoessais
  - → Hormones stéroïdales
  - → Prolactine
  - → Gonadotrophines

- AMH

Classification d' anovulation

→ *Traitement spécifique pour induction d' ovulation*

Hypogonadisme Hypogonadotrope (OMS I, 5-10%)

Pompe à GnRH si origine hypothalamique **LH,FSH ↓; E2 ↓**

Anovulation normogonadotrope (OMS II, surtout OMPK: 70-85%)

LH > FSH; SHBG ↓

Citrate de clomifène **androgènes ↑**

Régime/Metformine (obésité, résistance à l' insuline)

Hypogonadisme Hypergonadotrope (OMS III, 10-30%)

Gonadotrophines **E2 ↓ LH,FSH ↑**

Hyperprolactinémie (5-10%)

Agonists de la dopamine **Prolactine ↑**

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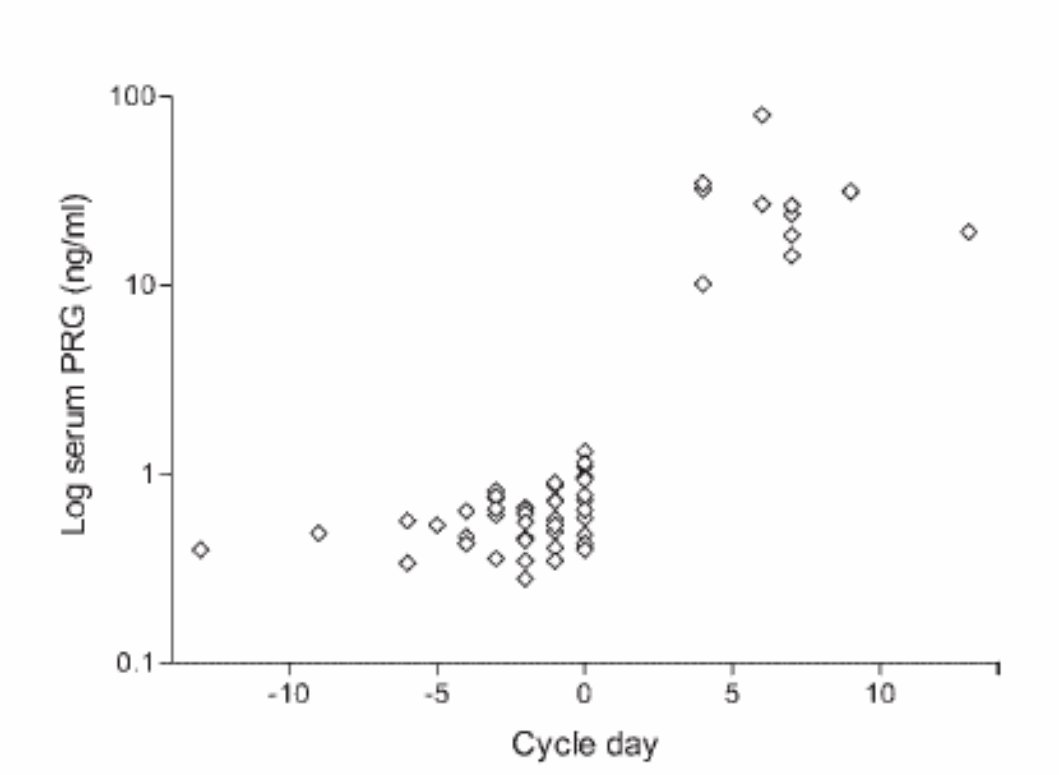
Evaluation de la phase lutéale

P midlutéal ≥ 3 µg/L ovulation

≥ 6 µg/L corps jaune adéquate



n = 16

Classification des patients avant stimulation pour PMA

⟹ choix de la dose d'FSH

- 'Normo répondeuses' — **FSH J3 < 10-15 IU/L**
  - 200 IU/J d' FSH
- 'Mauvaises répondeuses' — **FSH J3 > 10-15 IU/L**
  - 300 IU/J d' FSH
- 'Hyper répondeuses' (OMPK) — **FSH N, LH/FSH ↑**
  - 100 IU/J d' FSH — **Androgènes ↑**
  - *Eviter le Risque de SHO!* — **SHBG ↓**

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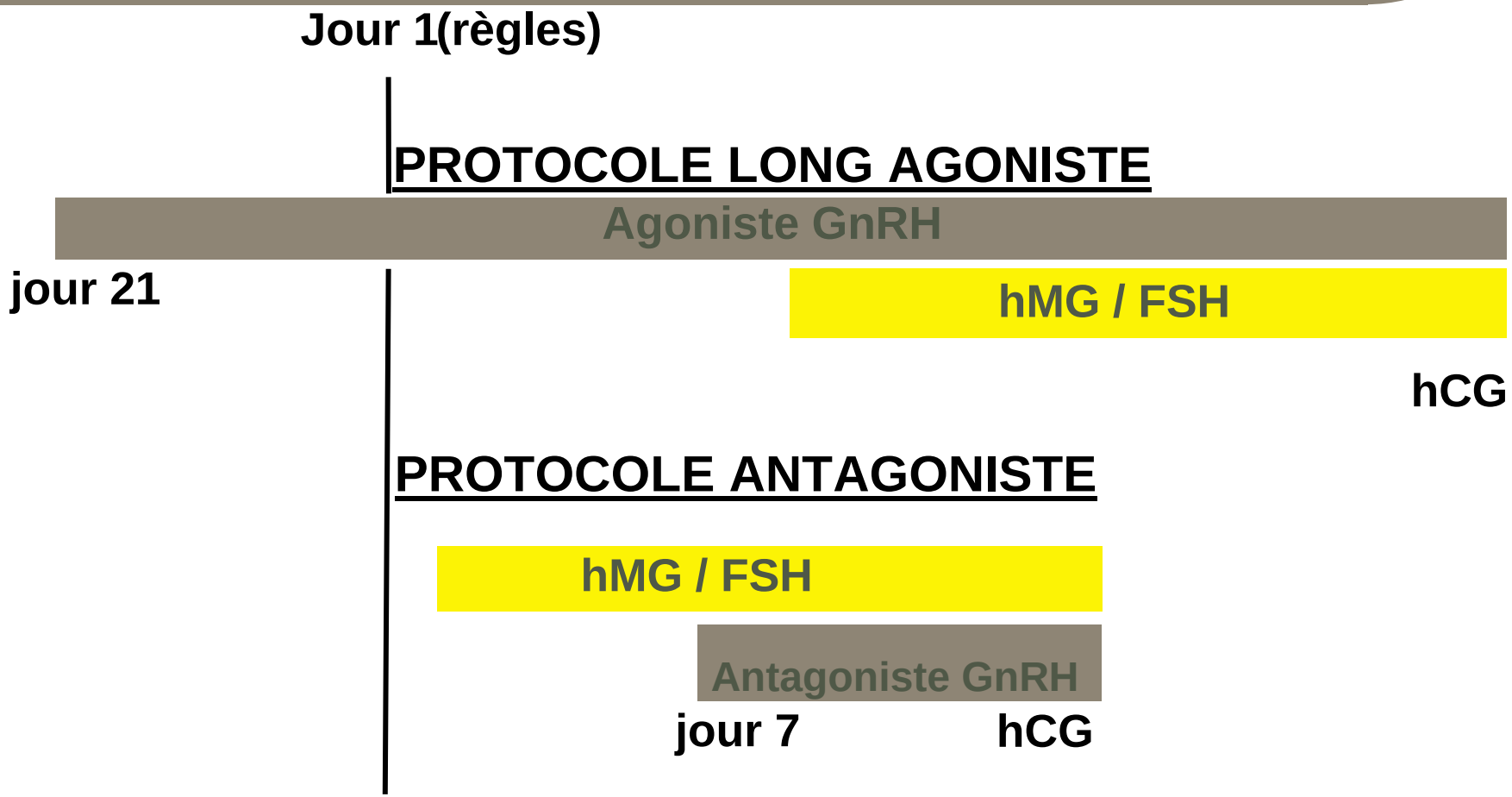
Suivi biologique de la superovulation



INITIER | **CROISSANCE FOLLICULAIRE** | **hCG**

Superovulation pour IVF/ICSI

DEMARRER STIMULATION PAR hMG / FSH

UNIQUEMENT SI:

1. **LH < 2 IU/L**
2. **E_2 < 50 ng/L**
3. **P_4 ≤ 1,5 µg/L**
4. **Absence de cystes ≥ 20 mm de diamètre**

SI NON : augmenter dose et durée de GnRHa

& attendre de démarrer

Superovulation pour IVF/ICSI

ADAPTATION DES DOSES DE GONADOTROPHINES:

après 4 jours: **E_2 doit être $\geq$ 100 ng/L**

E_2 40-50 % augmentation journalière

L' augmentation journalière d'E2 de 40-50 % doit durer de 6 à 8 jours

P_4 doit rester $\leq$ 1.5 µg/L

Superovulation pour IVF/ICSI

DECISION HCG

ECHOGRAPHIE: DIAMETRE FOLLICULE ≥ 17 mm

= OVOCYTE apte d' atteindre le stade M II après HCG

E 2 / follicule large (≥15 mm) : 200-300 ng/L

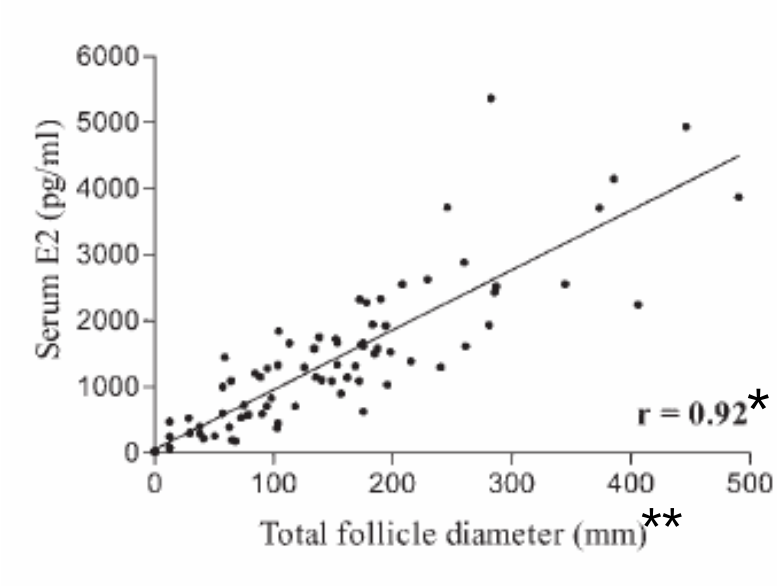
= follicule mature

Busereline/hMG:

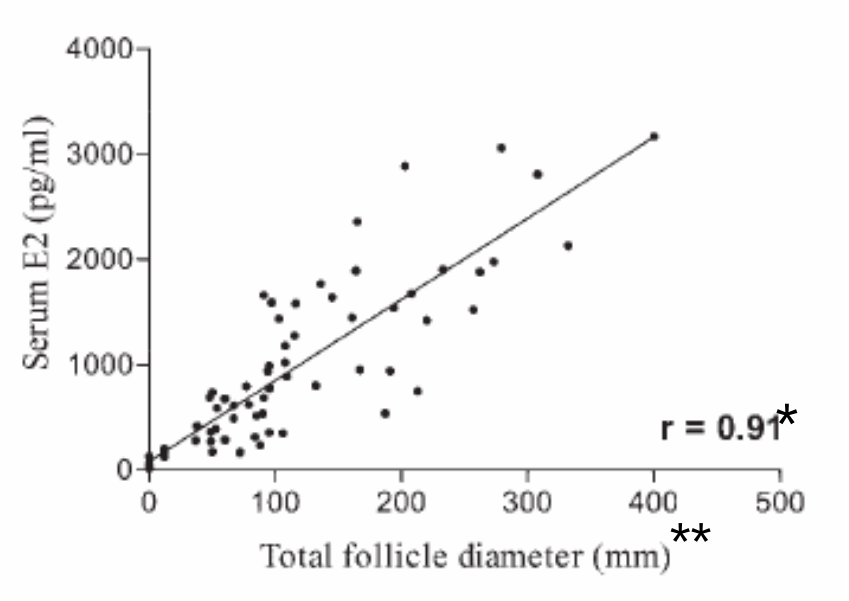
Taux normal de maturation 36h après hCG (10.000 U)

= 85 % des ovocytes sont métaphase II

E2 sérique et la superovulation



Long GnRH agonist cycles



GnRH antagonist cycles

*** p<0.001**

**** follicles ≥ 10 mm**

Anckaert, CCLM 2002

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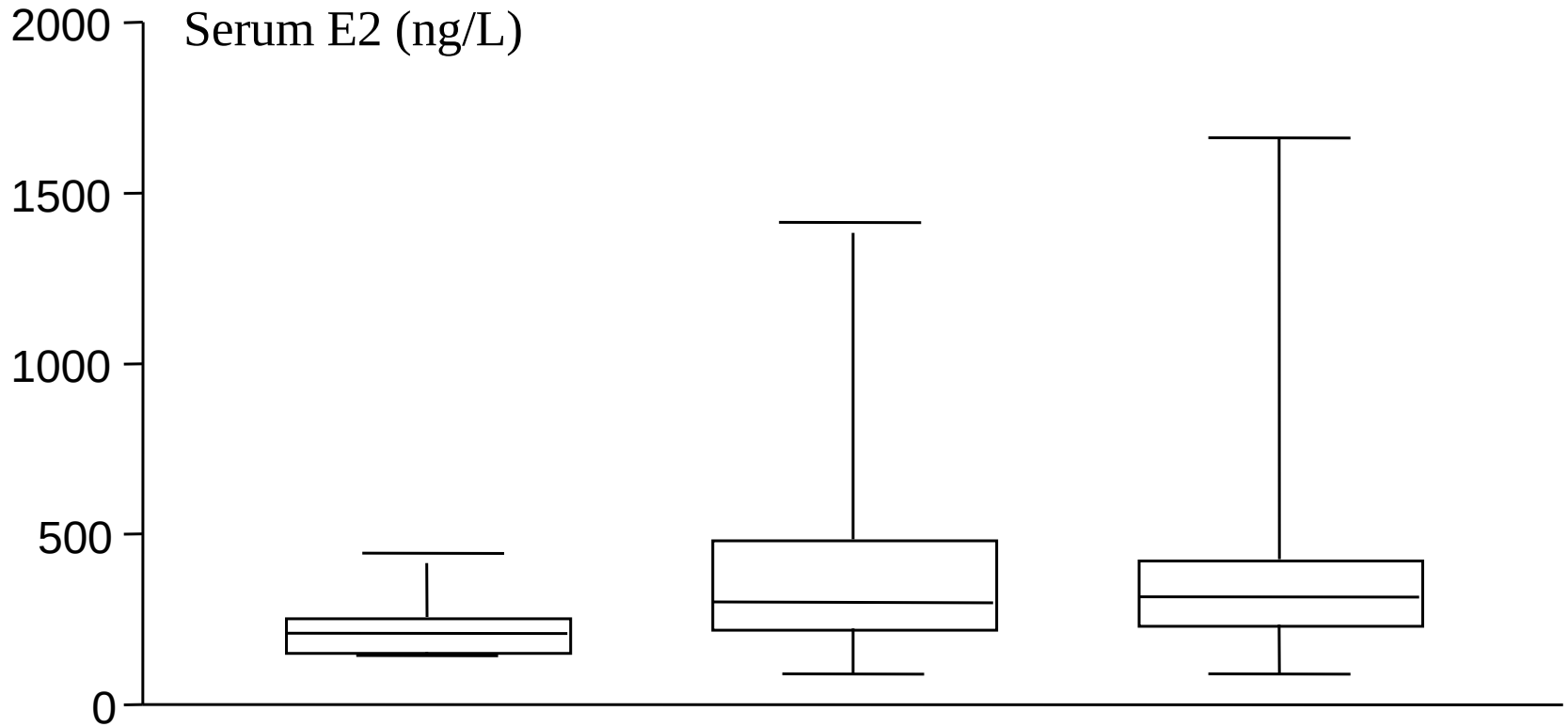
Serum E2 par follicule ≥ 15 mm: 200 à 300 ng/L



	Cycles naturels	Agonist + hMG	Antagonist + FSH
médiane	**211^{ab}**	**301^{a}**	**317^{b}**

^{a} $p < 0.01$, ^{b} $p < 0.05$

Anckaert, CCLM 2002

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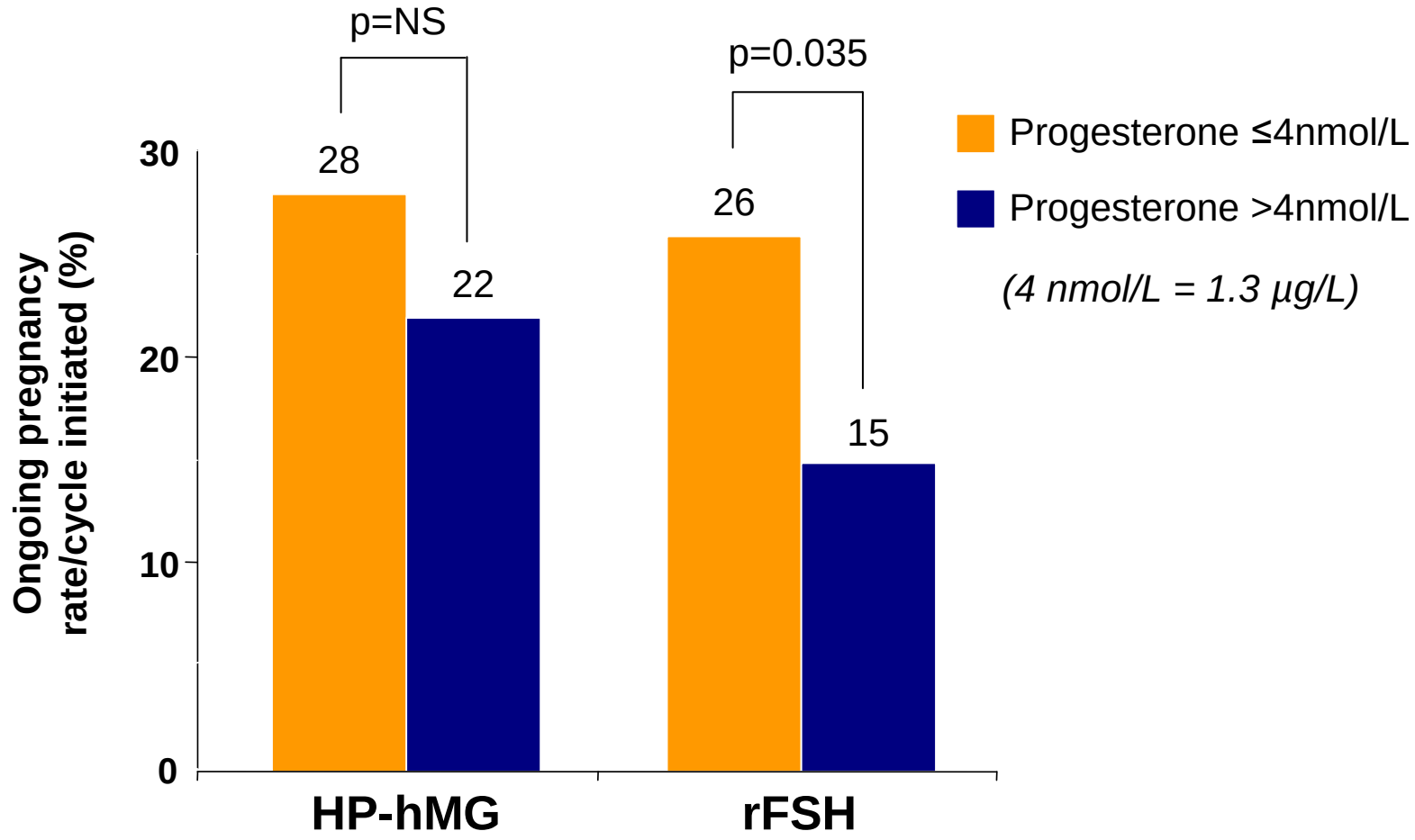
Significantly lower ongoing pregnancy rate in rFSH patients with higher progesterone levels at the end of stimulation



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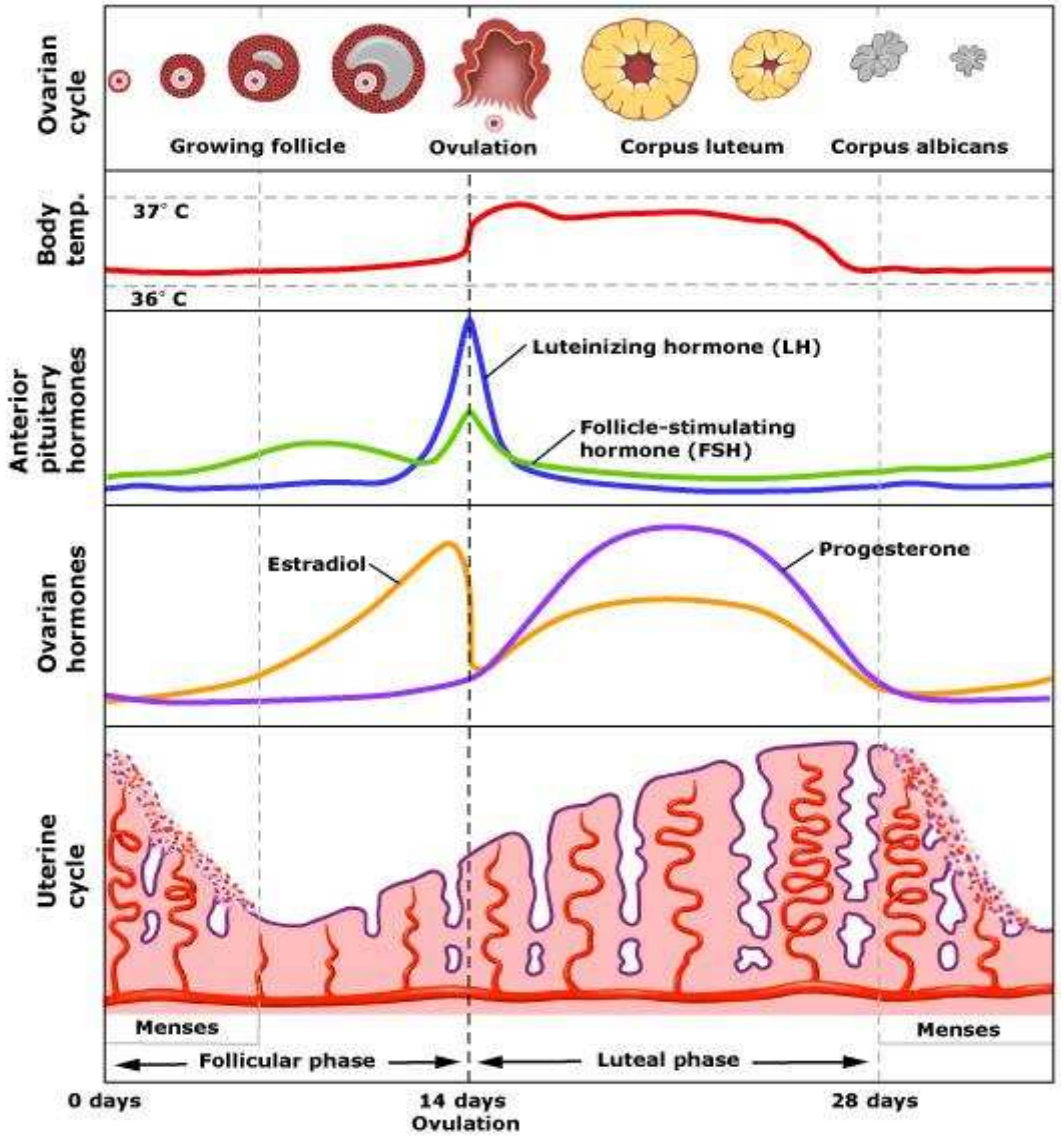
Paramètres hormonaux importants



E2: Reflet de la croissance folliculaire

P: Reflet de la présence de follicule
large mature *(≤ 1.5 µg/L)*
follicule post-ovulatoire *(> 3 µg/L)*

LH, P: Si élevé: = pré-ovulation
→ planifier l' insémination *(le lendemain)*

P: Evaluation de la période post-implantation

FSH (J3): Réserve ovarienne

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Evolution steroid hormone assays

Extraction/chromatography RIA

↑specificity, ↓precision

Direct RIA

- *Monoclonal Abs with high specificity*
- *Blocking binding proteins*

Non-isotopic automated immunoassay

↑precision , high troughput, speed

BUT *↑between-method CV*

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Indications E2 in ART

- Monitoring follicular growth
  - → Ovulation induction
  - → COH for IVF/ICSI

Optimalisation assays for:

speed, high troughput, good precision at high concentration level

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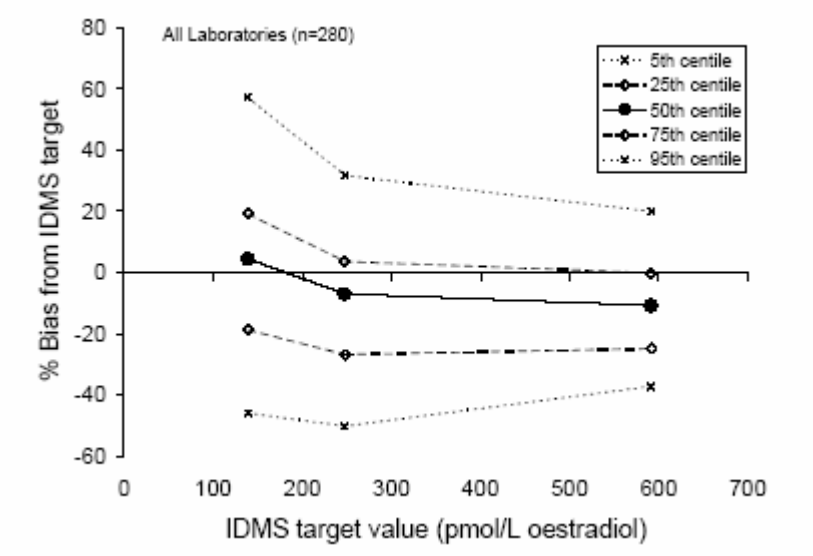
- Cycle irregularity / Anovulation
- Monitoring down-regulatie GnRH analogues

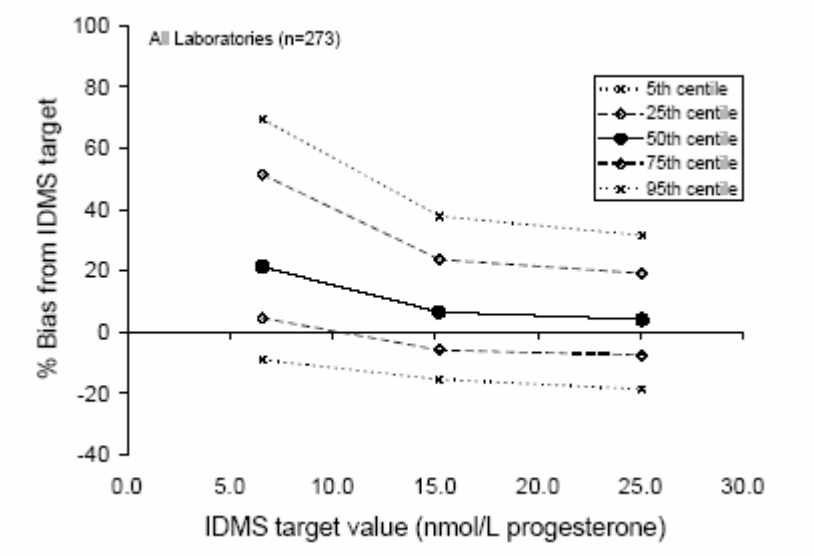
Demand high sensitivity assays

Direct E2 and P immunoassays vs ID-GC/MS (UKNEQAS 2005)

All methods



100 pmol/L = 27 ng/L



5 nmol/L = 1.6 µg/L

http://www.ukneqas.org.uk/directory/CC/steroid.htm

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Accuracy and precision of automated E2 and P assays using native serum samples

- Belgian External Quality Assessment (Institut scientifique de Santé publique)

- Fresh frozen serum samples
  - → without additives and preservatives → no matrix effects
  - → from single donors
  - → pooled serum from pregnant women
  - → target value determined with reference method (ID-GCMS)

- 6 most frequently used automated methods

Coucke W, Hum Reprod, in press

Imprecision and bias of P immunoassays

All concentrations are in nmol/l

	Target value	Advia Centaur (n=13)	DPC Immulite (n=25)	Elecsys (n=66)	Access (n=7)	Vitros (n=11)	Vidas (n=18)
CV %	0.8	*58%*	*43%*	*23%*	*84%*	*33%*	*74%*
	6.2	*16%*	*11%*	*6%*	*33%*	*9%*	*10%*
	22.5	*8%*	*10%*	*7%*	*18%*	*9%*	*12%*
BIAS %	0.8	*175 %*	*54 %*	*49 %*	*202 %*	*103 %*	*145 %*
	6.2	*64 %*	*22 %*	*-23 %*	*81 %*	*-10 %*	*21 %*
	22.5	*35 %*	*15 %*	*12 %*	*63 %*	*30 %*	*47 %*

6.2 nmol/L = 1.9 µg/L

Coucke W, Hum Reprod, in press

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Imprecision and bias of E2 immunoassays

All concentrations are in pmol/l

CV %

Target value	Advia Centaur (n=13)	DPC Immulite (n=25)	Elecsys (n=66)	Access (n=7)	Vitros (n=11)	Vidas (n=18)
198	*24%*	*21%*	*11%*	*23%*	*24%*	*15%*
209	*24%*	*14%*	*11%*	*49%*	*22%*	*16%*
598	*14%*	*11%*	*7%*	*18%*	*11%*	*7%*
778	*22%*	*11%*	*8%*	*12%*	*13%*	*12%*
1841	*21%*	*12%*	*5%*	*18%*	*8%*	*11%*

E2 precision goals: 150-1000 pmol/L: < 25%; 1000-10.000 pmol/L: <10%, *Thienpont L, Clin Chem 1996*

BIAS %

198	*7 %*	*-5 %*	*5 %*	*30 %*	*15 %*	*9 %*
209	*-12%*	*-4%*	*15%*	*22%*	*18%*	*20%*
598	*9 %*	*-17 %*	*7 %*	*36 %*	*-26%*	*0 %*
778	*14 %*	*-3 %*	*22 %*	*16 %*	*-12 %*	*10 %*
1841	*-4%*	*-6%*	*18%*	*-10%*	*2%*	*43%*

198 pmol/L = 54 ng/L

Coucke W, Hum Reprod, in press

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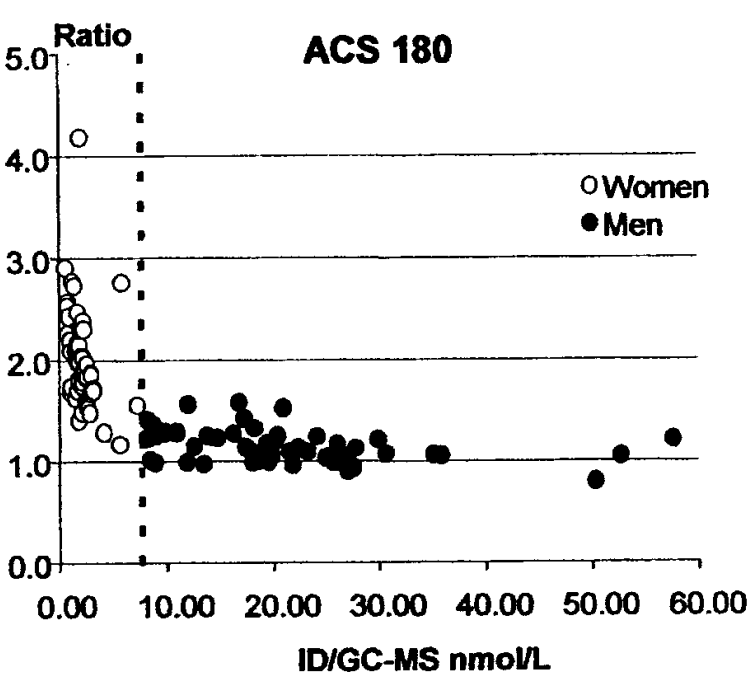
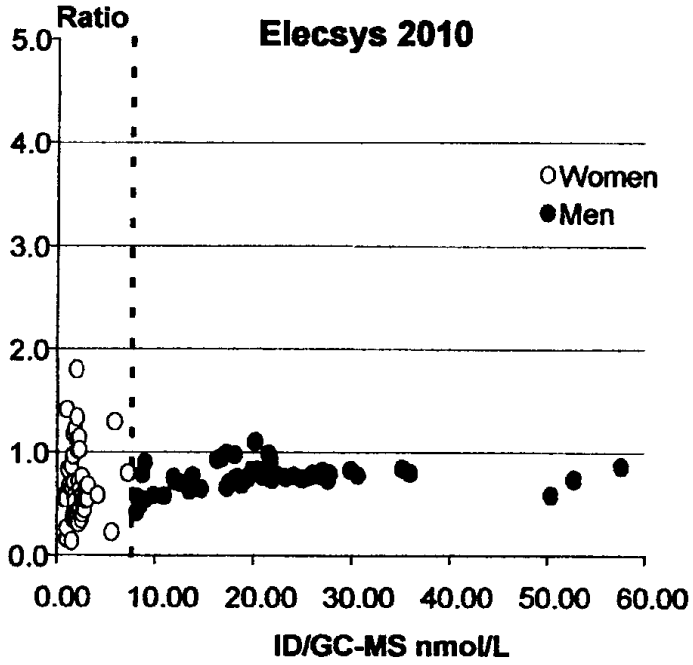
Testosteron automated immunoassay bias





No method acceptable for women/children: ***7/10 immunoassays overestimate (mean: 46% higher than ID-GCMS)***

Most acceptable in men: ***some underestimation (mean: 12% lower than ID-GCMS)***

Taieb J, Clin Chem 2003

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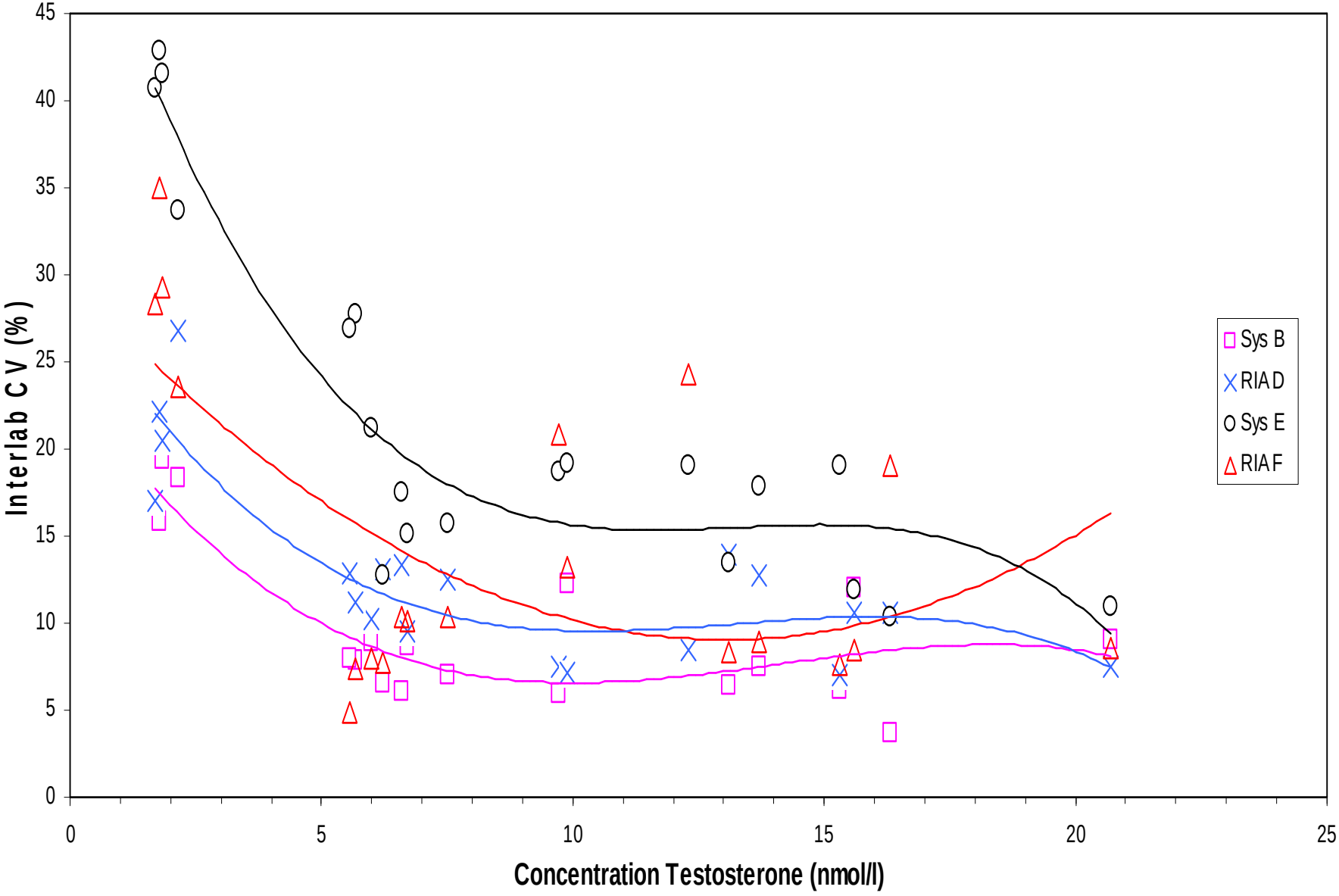
Precion profileTestosterone (LWBA)



Testosteron reference values from proven fertile young men

Serum total T (nanomoles per liter) reference intervals for each platform reported

Method	Expected values	Geometric scale	
		Lower	Upper
GC/MS			
A	5.8–28.2	10.4	29.8
B	9.1–55.3	12.2 (11.3–12.8)	33.7 (32.7–35.3)
C	8.4–28.7	12.7 (11.8–13.2)	34.4 (33.8–35.8)
D	9.9–27.8	8.6 (8.3–8.7)	32.5 (31.7–34.2)
E	4.6–28.2	12.0 (10.9–12.7)	31.9 (30.8–32.6)
F	8.4–28.7	7.5 (6.7–8.0)	25.8 (24.1–27.8)
G	6.1–27.3	11.3 (10.9–11.2)	33.5 (32.2–38.4)
		10.0 (9.8–10.0)	27.6 (27.1–29.0)

n = 124, well-defined group of healthy young men with normal reproductive function explicitly verified

Sikaris, JCEM 2005

Conclusion direct steroid immunoassays

- Large inter-method CV caused by
  - → *≠ calibration*
  - → *≠ antibody specificity*
  - → *≠ effect binding proteins*
  - → *optimalisation assays for ≠ concentration range*
- Insufficient sensitivity (not appropriate for low values) for some systems for E2 and P and for all systems for testosterone
- Poor method robustness for some methods_____*(high between-user within-method variation)*
- **Some systems are superior to others!**

Testosteron: organic solvent extraction

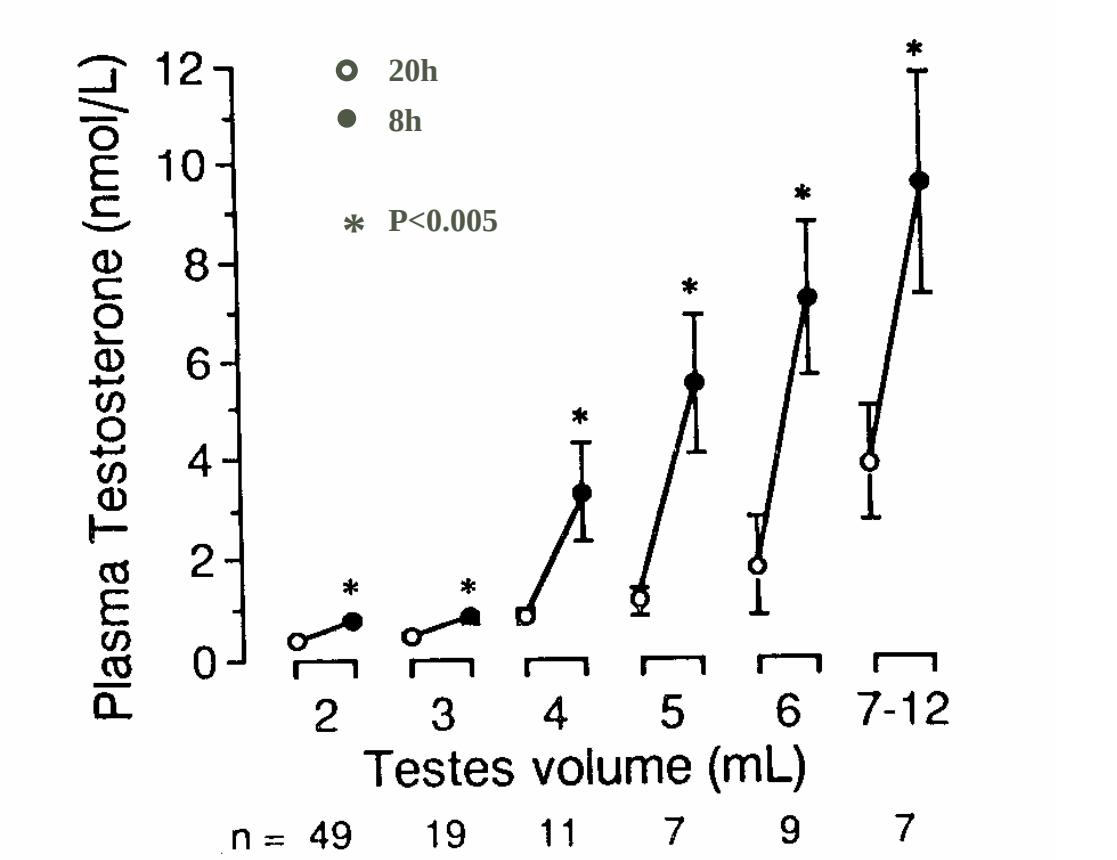
Extraction

- Protein denaturation
- Release of testosteron from SHBG
- Elimination of (water-soluble) conjugated metabolites

RIA

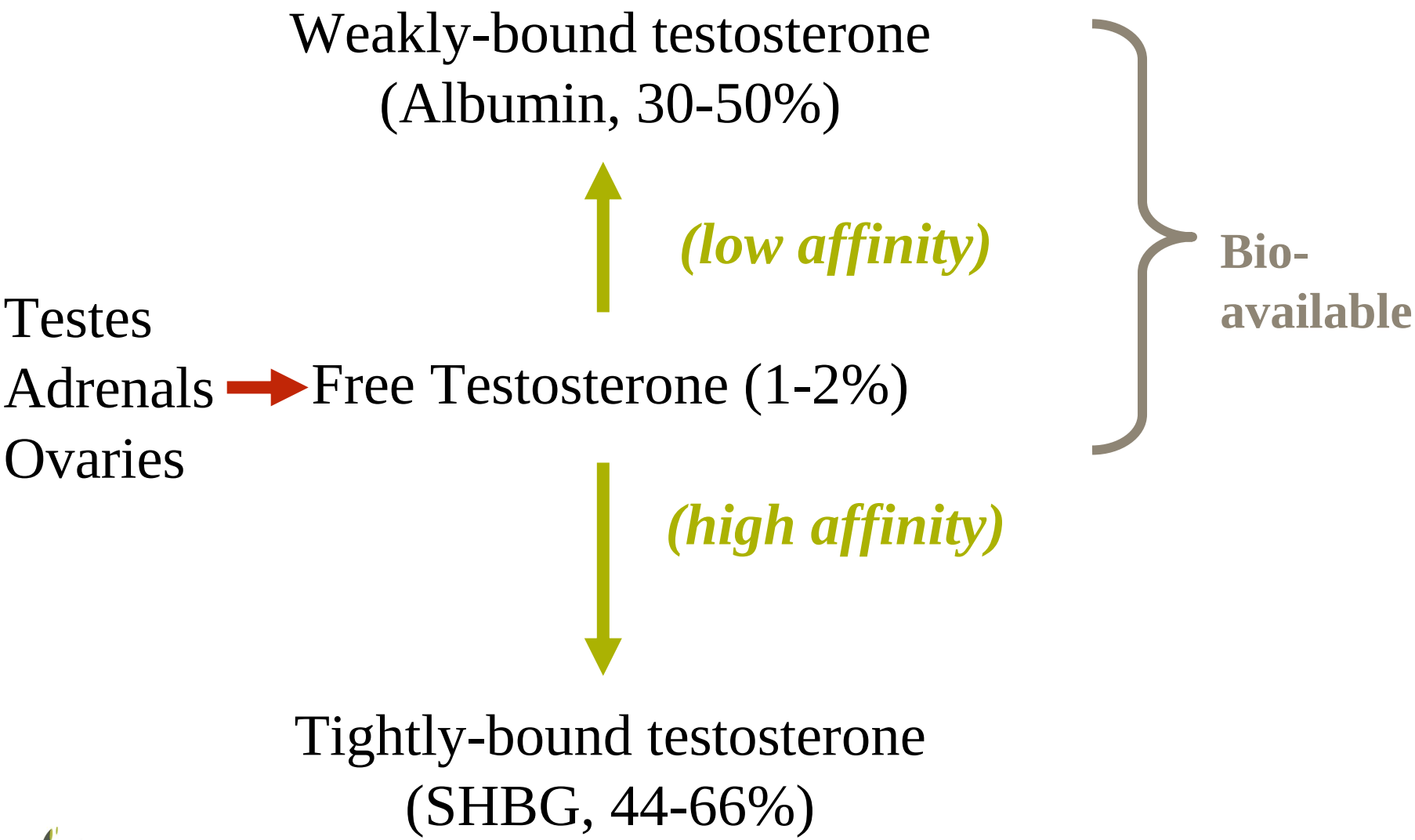
- **↑specificity**
- **↑sensitivity (women, children)**

Serum Testosteron: diurnal rythm



Wu, Clin Chem 1993

Circulating Testosteron



SHBG alterations

- → Hyperthyroidism
- → Estrogen
- → Anticonvulsive drugs, dexamethasone
- → Liver cirrhosis
- → Pregnancy
- → Malnutrition
- → Aging men

- → Obesity
- → Insulin resistance
- → Hypothyroidism
- → Androgens, glucocorticoids, progestin
- → Nefrotic syndrome
- → Hirsutism / virilisation
- → GH excess

Serum total testosteron is not a reflection of biologically active testosteron

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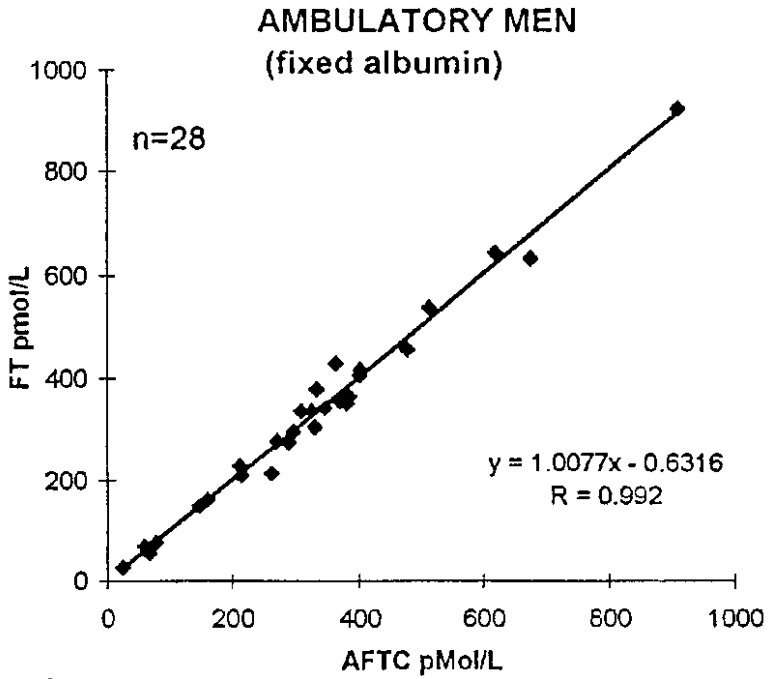
Calculated free testosteron based on measurement of total testosterone and SHBG



http://www.issam.ch/freetesto.htm

Good correlation with equilibrium dialysis, except in case of:
- hormone treatment interfering with SHBG binding
- pregnancy
- abnormal albumine

Vermeulen A, JCEM 1999

E2 immunoassay interference

TABLE 1

Serum E_2 values as measured with the Elecsys assay, with radioimmunoassay, and with the Elecsys assay after ether extraction of the sample.

Sample condition	Elecsys E_2 (pg/mL)	RIA E_2 (pg/mL)	Ether-extraction Elecsys E_2 (pg/mL)
Follicular phase natural cycle	757	82	ND
1 wk of GnRH agonist	428	ND	ND
2 wk of GnRH agonist	418	<15	<15
3 wk of GnRH agonist	388	<15	<15

Note: ND = not done (insufficient sample volume left).

Anckaert. Cancellation of an IVF cycle as a result of E_2 assay interference. Fertil Steril 2006.

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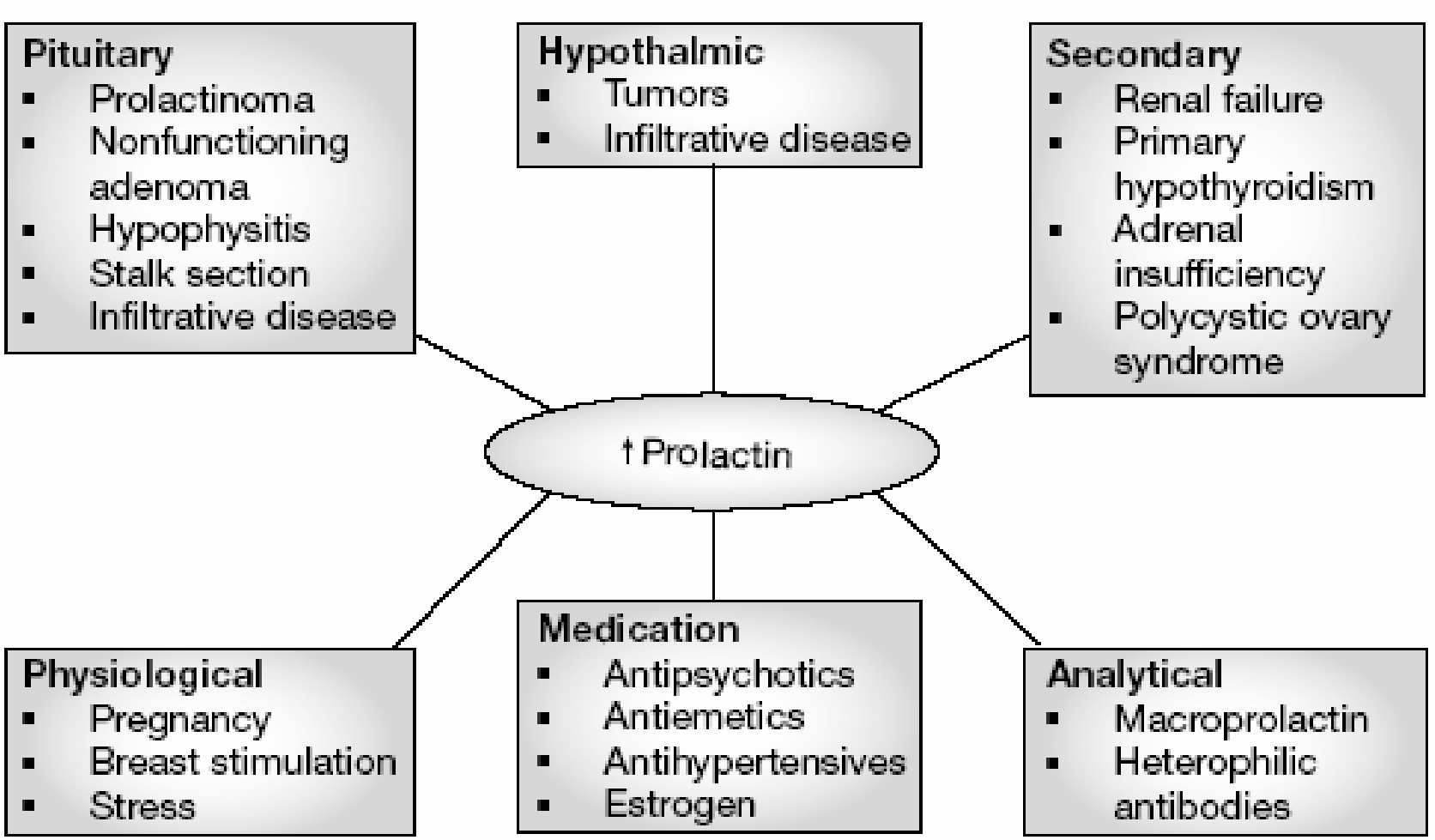
Causes of hyperprolactinaemia



Major forms of PRL in serum in basal state

Variant	**MW**	**%**
Monomeric hPRL	23 kDa	80-90
BigPRL	50 kDa	8 - 20
BigBig PRL = MacroPRL	> 170 kDa	1 - 5
Fragments *Glycosylated forms*	*16, 8 kDa,…*	*?*

Macroprolactinaemia

- Definition: Hyperprolactinemia where
  - → an important fraction of circulating PRL consists of (in-vivo) biologically inactive macroprolactin
  - → and monomeric PRL is within the reference values
- Gel Filtration Chromatography: > 30-60% macroPRL
- > 90% of cases: macroPRL = PRL-IgG complex
- Accounts for up to 26% of cases of hyperPRL
- Can cause unnecessary work-up and treatment

Immunoreactivity for macroPRL is assay- and sample- dependent

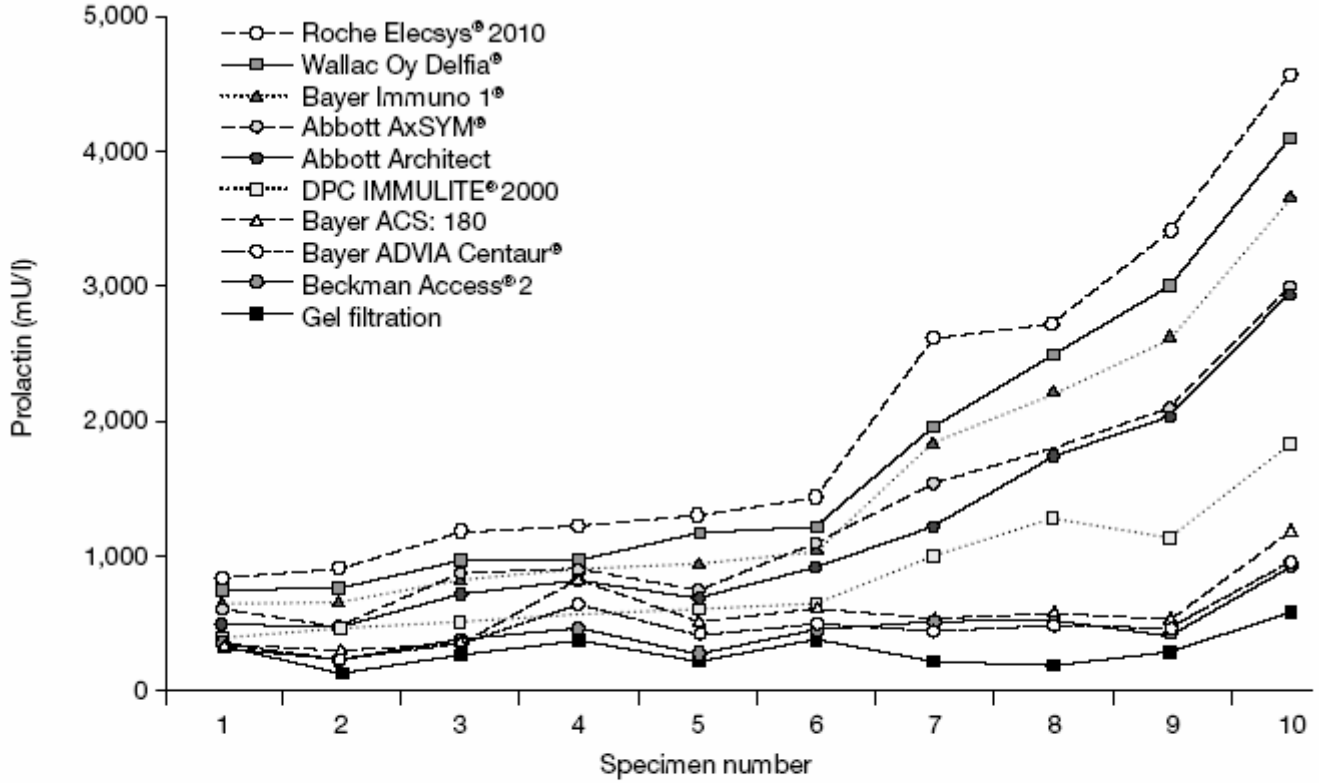


Figure 5 Mean serum prolactin levels reported by nine different immunoanalyzer user groups in specimens collected from 10 subjects who had macroprolactinemia. For comparative purposes, the monomeric prolactin level in each specimen following removal of macroprolactin by gel filtration is shown. Permission obtained from The Endocrine Society © Smith TP *et al.* (2002) Gross variability in the detection of prolactin in sera containing big big prolactin (macroprolactin) by commercial immunoassays. *J Clin Endocrinol Metabol* **87:** 5410–5415. Copyright 2002, The Endocrine Society.

PEG precipitation

- First choice method for detection of macroPRL if no interference in immunoassay

- If serum PRL > 1000 mIU/l
  - → 200 µL serum + 200 µL PEG 6000 25% (g/v) in PBS buffer in conical tube (room temperature), vortex 1 min
  - → 30 min centrifugation at 3000 RPM
  - → measurement of PRL in serum and in supernatant after PEG treatment with the Elecsys PRL II assay

- Calculation

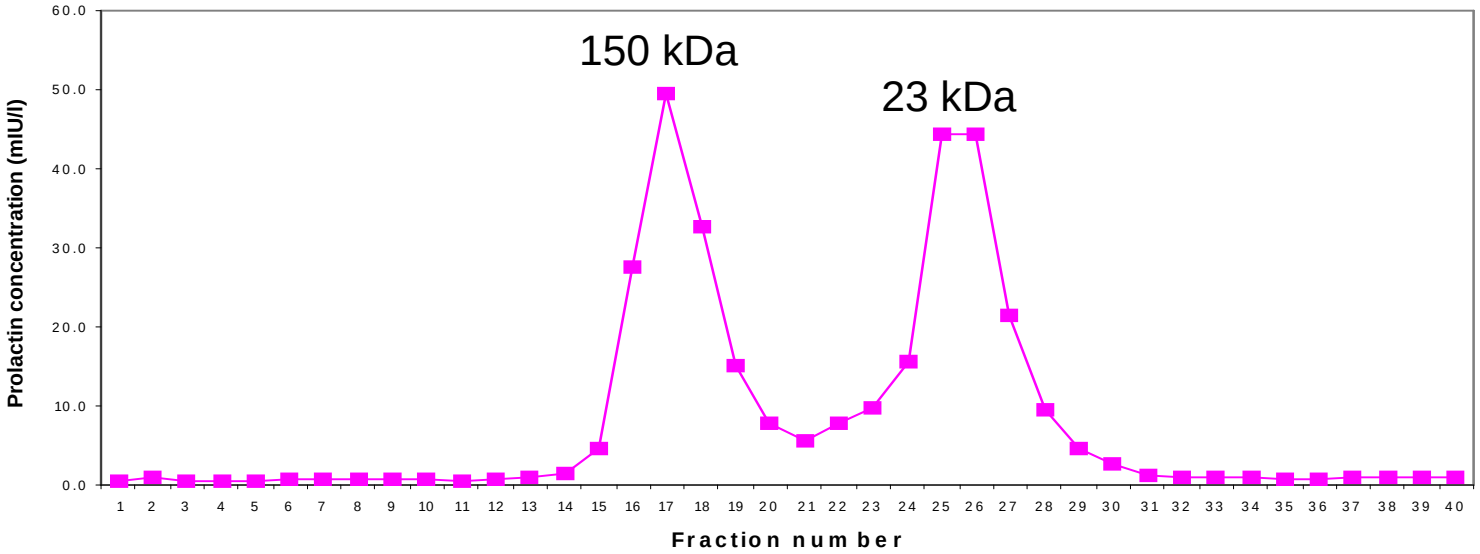
  % Recovery = (PRL supernatant * 2)/PRL serum *100

  Interpretation: ≥ 60%: no macroPRL

  < 60%: macroPRL

MacroPRL and monomeric hyperPRL

Serum PRL (Elecsys PRL I assay): 2822 mIU/l
% R-PEG: 38% indicative of macroPRL
Monomeric PRL: 1890 mIU/l (↑)



→ **Monomeric PRL** = PRL serum after PEG precipitation

(use adapted reference intervals <u>after</u> PEG precipitation)

Fahie-Wilson Clin Lab 2007

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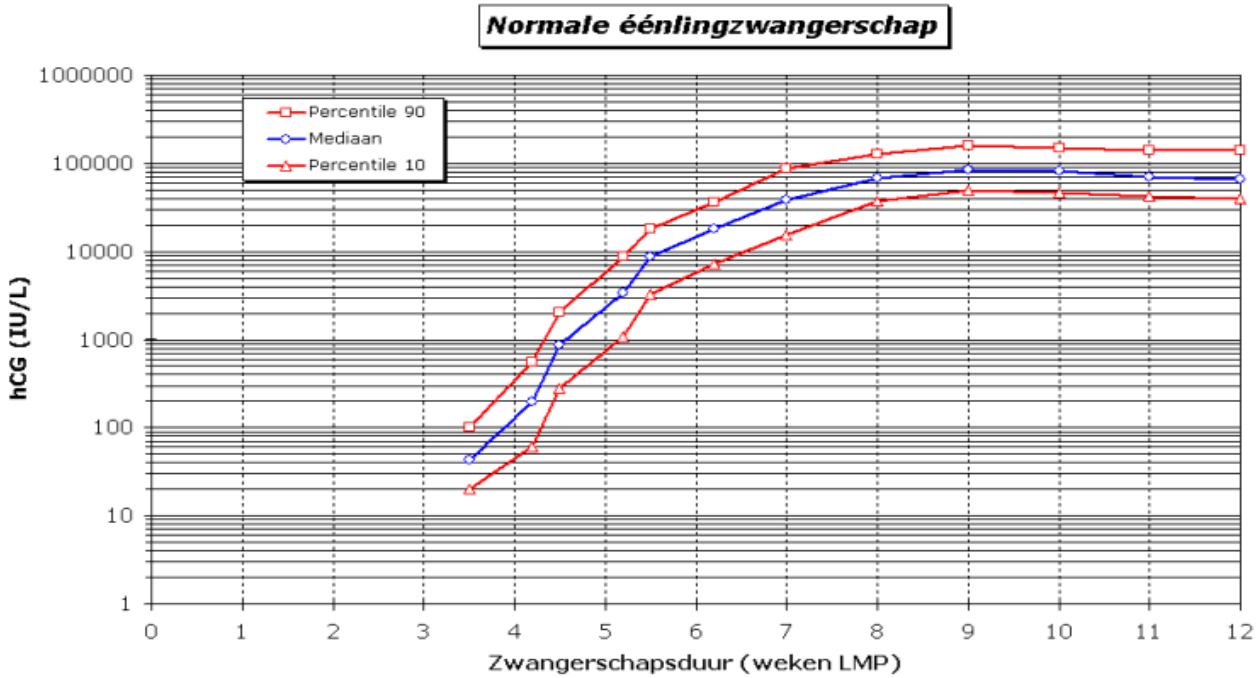
Serum hCG in singleton pregnancy



hCG appears in serum 7-10 days after LH surge

hCG doubles: - every 1.5 days up to 5 - 6 weeks (LMP)

- then every 3.5 days (from 7 weeks LMP or when hCG > 10.000 IU/l)

Serum hCG in ectopic pregnancy

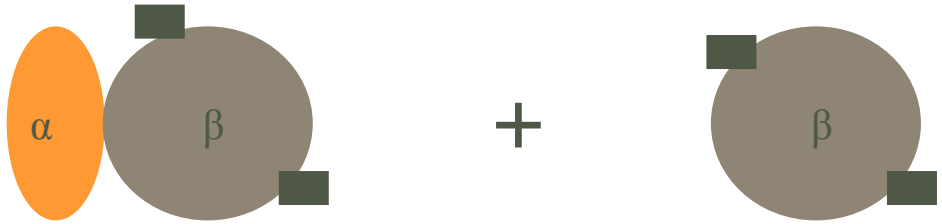
- hCG discriminatory zone: ectopic pregnancy to be excluded if (D&C)
  - → transvaginal US: absence of demonstrable intra-uterine pregnancy
  - → and hCG > 1000 à 2000 IU/L

- hCG increase rate is often abnormal, but it is normal in 1/3 ectopic pregnancies

- Serum progesterone: good discriminative capacity between viable and non-viable, but NOT for discrimination between ectopic and non-ectopic.
  - → > 20-25 ng/mL: viable pregnancy
  - → 5-20 ng/mL: grey zone
  - → < 5 ng/mL: non-viable (0.3% viable pregnancy)

hCG immunoassay format

<u>TOTAL</u> hCG IMMUNOASSAY (hCG+beta)



<u>INTACT</u> hCG IMMUNOASSAY

Serum hCG isoforms

% hCG forms in normal pregnancy, trophoblastic disease and testicular cancer

hCG form	Pregnancy *3-4 weeks*	Pregnancy *5-40 weeks*	Mole	Chorioca	Testis non-seminoma
Intact hCG	0-30	**70-100**	0-97	0	0
Hyperglyc. hCG	**65-100**	0.5-5	2-20	Up to 100	Up to 100
Free β-hCG	3-15	0.5-1	2-100	10-100	10-100
Nicked hCG		0.5-30	5-100	5-100	5-100
hCG minus ßCTP			(↑)*	(↑)*	(↑)*

* May be predominately elevated

Variability automated gonadotrophin assays

- Different specificity of monoclonal antibodies for circulating isoforms
  - → ≠ Glycosylation
  - → Micro-heterogeneity in polypeptide chain (LH, FSH)
  - → Fragments, nicked forms, aggregates

- Calibration differences
  - Primary international standards
    - LH 80/552 = gepurified pituitary extract
    - FSH 78/549 = pituitary extract
    - FSH92/510 = recombinant human FSH
  - Secundary kit standards

- Matrix effects

Between-lab variability

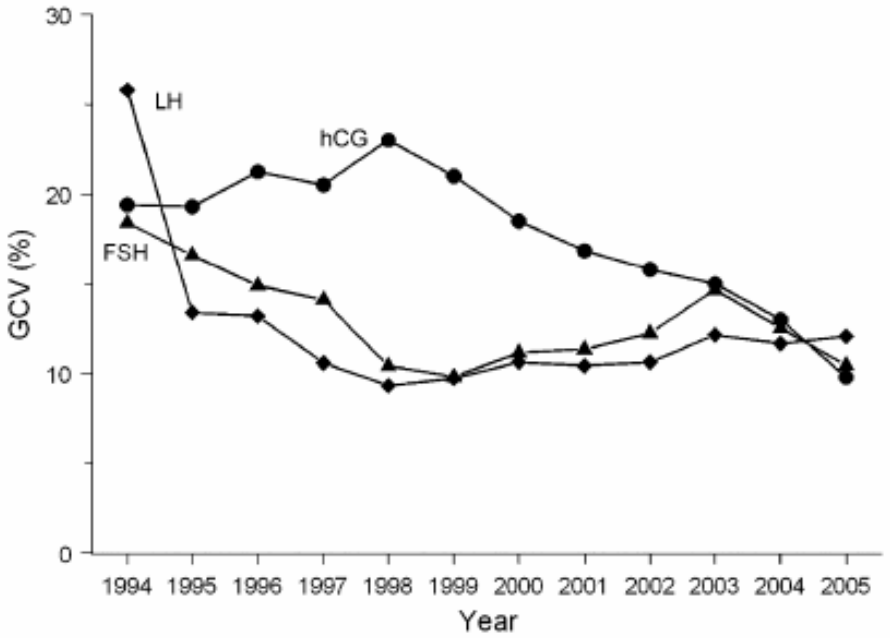


Fig. 1. Within-sample between-laboratory geometric coefficients of variation (mean and range) in the UK NEQAS for LH, FSH and hCG (1995–2004). Results (mean and range) are shown for endogenous specimens prepared from unadulterated normal donor sera (LH or FSH) or normal donor sera containing diluted sera from pregnant women (hCG). [Data from the UK NEQAS (Edinburgh) *Annual Review for 2004*.]

General conclusion hCG/LH/FSH 2005/2006: good precision and robustness (= within-method between-lab CV) and acceptable bias for most methods

Luteinising Hormone (LH)
Follicle Stimulating Hormone (FSH)

- Secretion by adenohypophyse
- Glycoprotein hormones (MW 28 – 33 kDa)
- Heterodimers
  - → α-chain = identical LH, FSH, TSH, hCG
  - → β-chain → biological and immunological specificity
    → LH and hCG: 80% homology

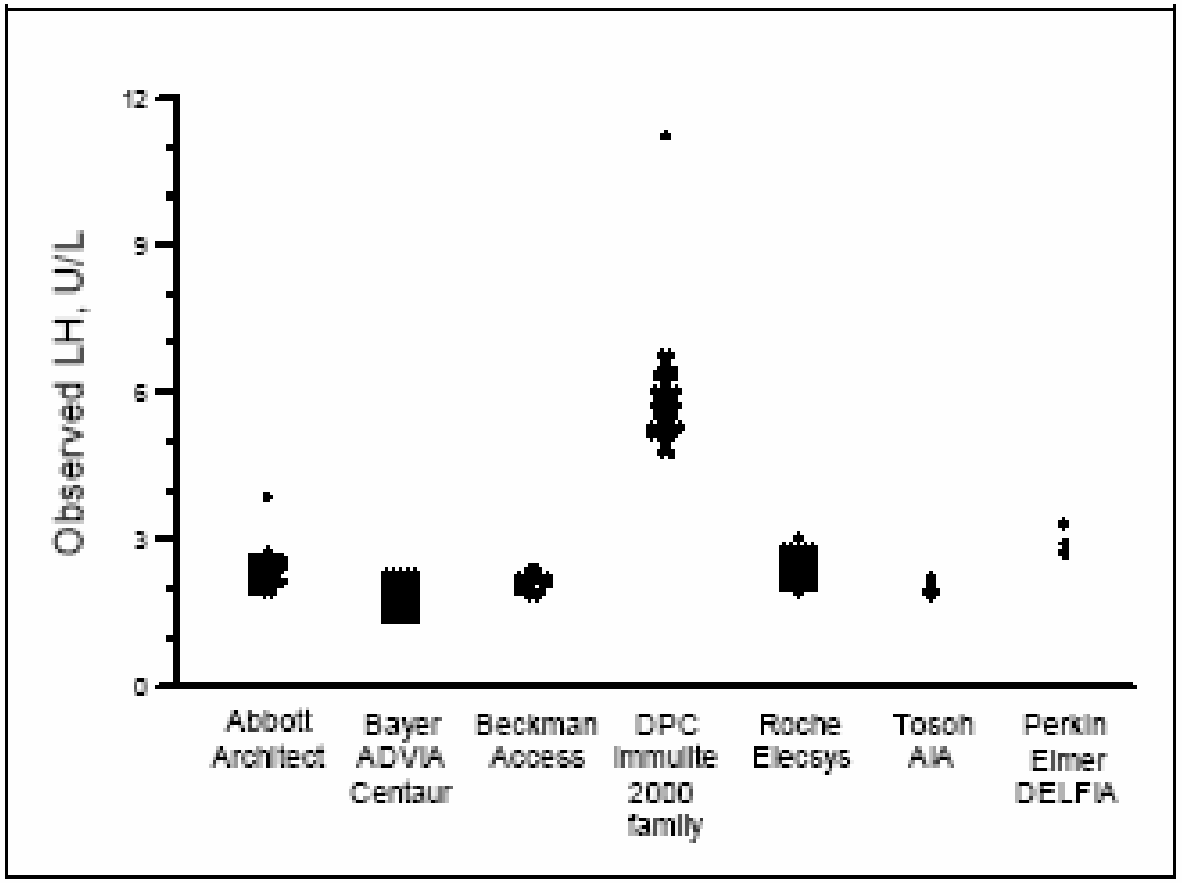
↓

Specific monoclonal Abs to avoid crossreactivity

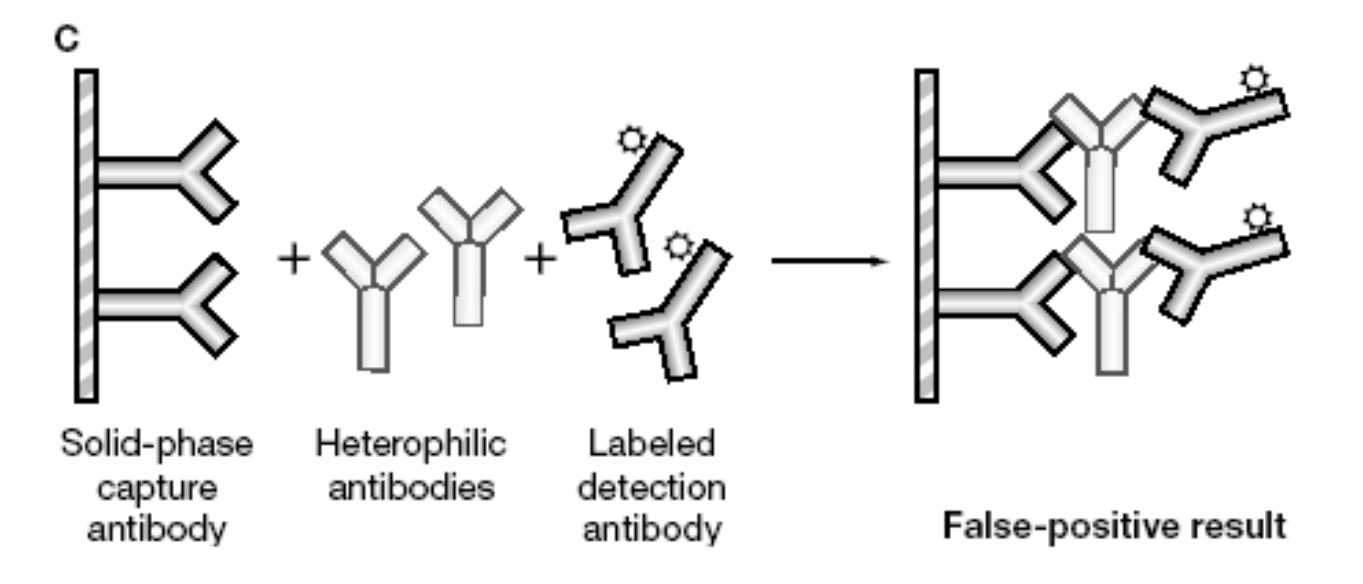
UK NEQAS 2006
Crossreactivity of LH assays with hCG

Second trimester pregnancy serum pool (hCG 17.200 IU/L)

Figure 2. 34 LH - Cross-reactivity with hCG.



Heterophilic antibody interference



<u>If interference is suspected:</u>

-measure with alternative method

-sample dilution in analyte-free serum or assay diluent

-eliminate interfering antibodies

LH and FSH biological functions

- Men
  - → LH ⇒ Leydig cell: testosteron secretion
  - → FSH ⇒ Spermatogenesis

- Women
  - → LH/FSH ⇒ Theca and granulosa cells: secretion androgens and oestrogens
  - → FSH ⇒ Growth and selection of follicles
  - → LH ⇒ Ovulation and Corpus Luteum

FSH reference values from proven fertile young men

Serum FSH (IU/liter) reference intervals for each platform

Method	Expected values	Geometric scale	
		Lower	Upper
O	1.4–13.6	1.3 (1.3–1.4)	8.3 (7.9–9.0)
P	0.7–11.1	1.1 (1.0–1.2)	9.1 (8.1–10.8)
Q	1.4–18.1	1.0 (0.9–1.3)	7.7 (7.5–7.7)
R	4.6–12.4	1.2 (1.2–1.4)	7.7 (7.7–9.4)
S	1.6–9.8	1.4 (1.3–1.4)	6.6 (6.6–7.0)
T	1.4–13.6	1.5 (1.3–1.6)	8.4 (7.8–8.9)
U	1.0–8.0	1.3 (1.2–1.4)	10.0 (8.9–11.4)

Consensus ref interval: 1.3-8.4 IU/l

Sikaris, JCEM 2005

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PMA et surveillance biologique

- But des analyses hormonales
  - → Avant la PMA
  - → Suivi de la PMA

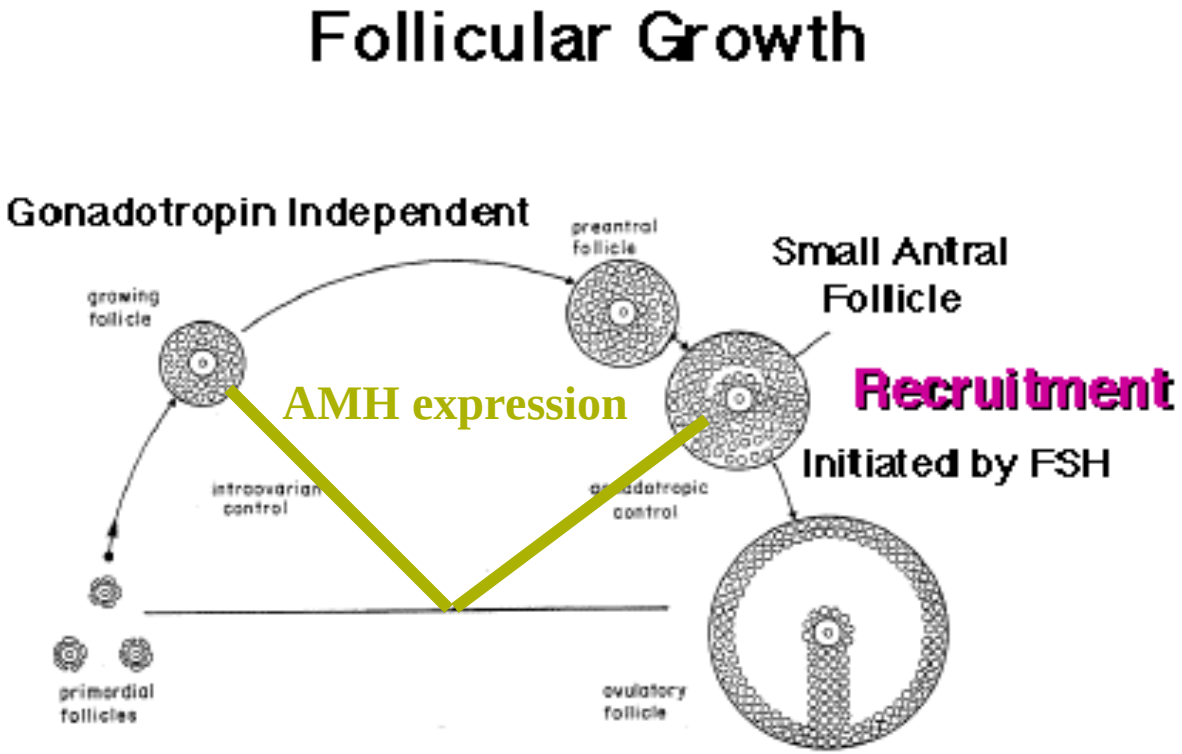
- Aspects analytiques des immunoessais
  - → Hormones stéroïdales
  - → Prolactine
  - → Gonadotrophines

- AMH

AMH

- Glycoprotein, 140kDa homodimer
- TGFβ family of growth and differentiation factors (inhibins, activins,…
- Anti-Mullerian Hormone: causes regression of Mullerian duct in male foetus
- Secretion:
  - → Men: Immature Sertoli cells
    Fetal and postnatal
  - → Women: Granulosa cells ovaria
    Adult

Granulosa cells: AMH expression



- Expression in pre-antral follicles and small antral follicles
- No expression in pre-ovulatory follicle and corpus luteum
- Role of AMH = control of follicle growth
  - → inhibition recrutement primordial follicles
  - → reduction of FSH sensitivity of large pre-antral and small antral follicles

Serum AMH predicts number of oocytes retrieved after COH and live birth rate

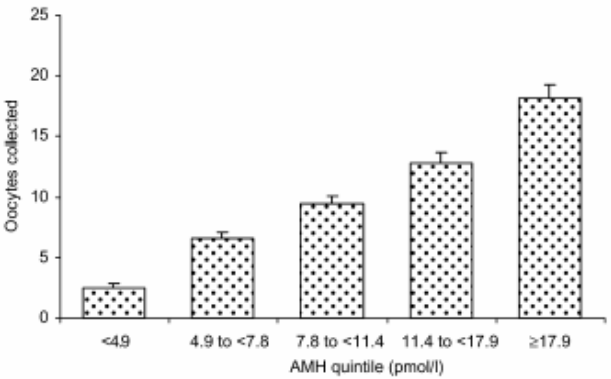


Figure 4: Mean oocyte yield per AMH and FSH quintile
Values are mean ± SEM

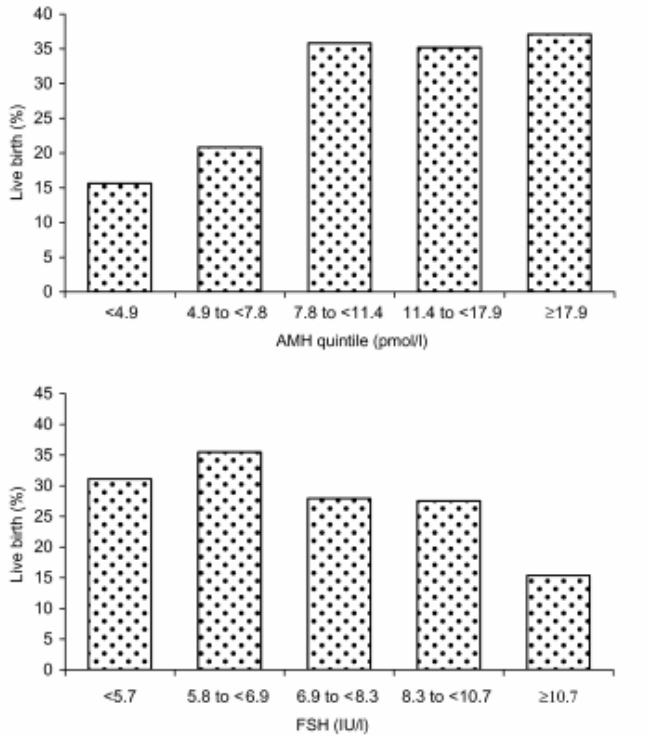


Figure 1: Live birth rate per AMH and FSH quintile
Values are live birth rates for all embryos derived from a single cycle, including fresh and frozen embryo transfers

Strong correlation between AMH and oocyte yield, AMH is a better predictor than FSH and age

ROC analysis: AMH is superior to FSH and age

Nelson S, Hum Reprod 2007

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Serum AMH predicts excessive ovarian response to FSH during COH

AMH is a better predictor than FSH and age

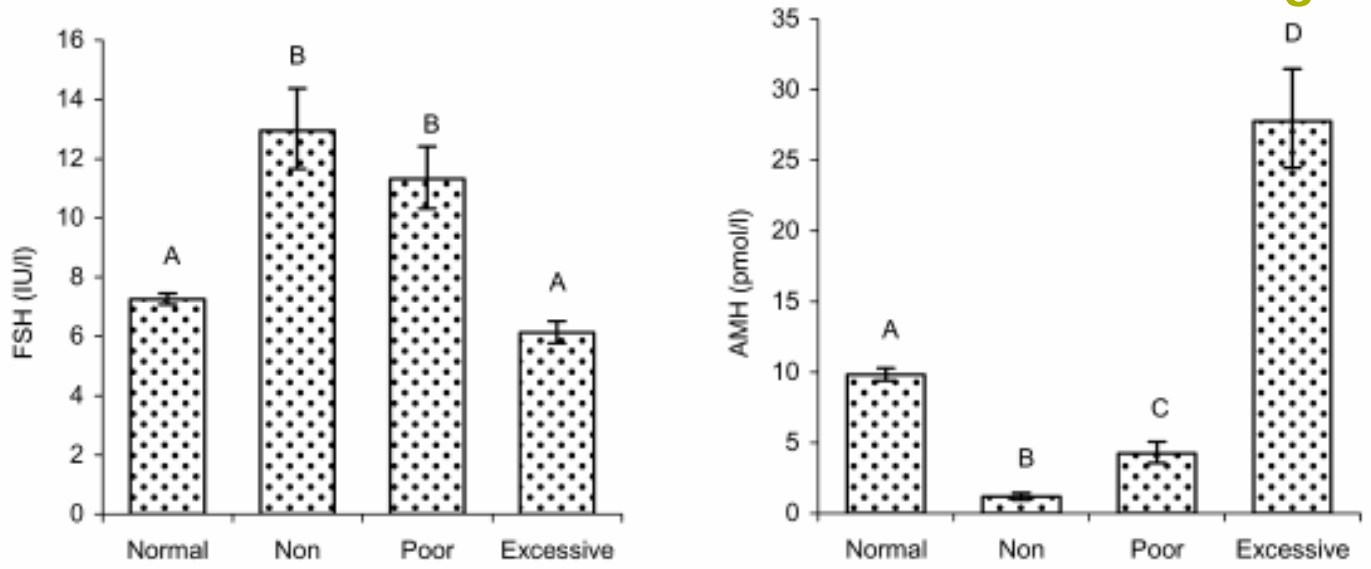

Figure 2: FSH and AMH concentrations relative to category of ovarian response
Values are geometric mean ± SEM of geometric mean. Groups with a letter in common do not differ significantly at $P < 0.01$

General conclusion: prospective studies are necessary on the concept of individualized and optimized treatment based on AMH prior to first cycle

Nelson S, Hum Reprod 2007

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Serum AMH is increased in PCOS

- PCOS: two- to threefold increase in serum AMH

  → Defective selection mechanism of dominant follicle results in anovulation and accumulation of small antral follicles (mainly 2-5 mm), which contribute to AMH secretion

  → Increased AMH production per follicle occurs and the increased granulosa cell AMH production may contribute to anovulation (AMH lowers the sensitivity of follicles to FSH)

- Serum AMH cut-off 8.4 ng/ml for diagnosis of PCOS offers
  → high specificity (92%)
  → and sensitivity (67%)

  (Pigny, JCEM 2006)

Advantages of serum AMH measurement

- Ovarian aging: serum AMH decreases before the increase of FSH and the decrease of inhibin B
- On cycle day 3: Intercycle reproducibility for AMH is higher than for inhibin B, E2, FSH and antral follicle count $\Rightarrow$ one single AMH measurement is sufficient
  Fanchin R, Hum Reprod 2005
- AMH levels remain relatively constant during the menstrual cycle $\Rightarrow$ AMH measurement can be done during the entire menstrual cycle