



AMH: un marqueur de la fonction testiculaire tout au long de la vie?

Pr Pascal Pigny

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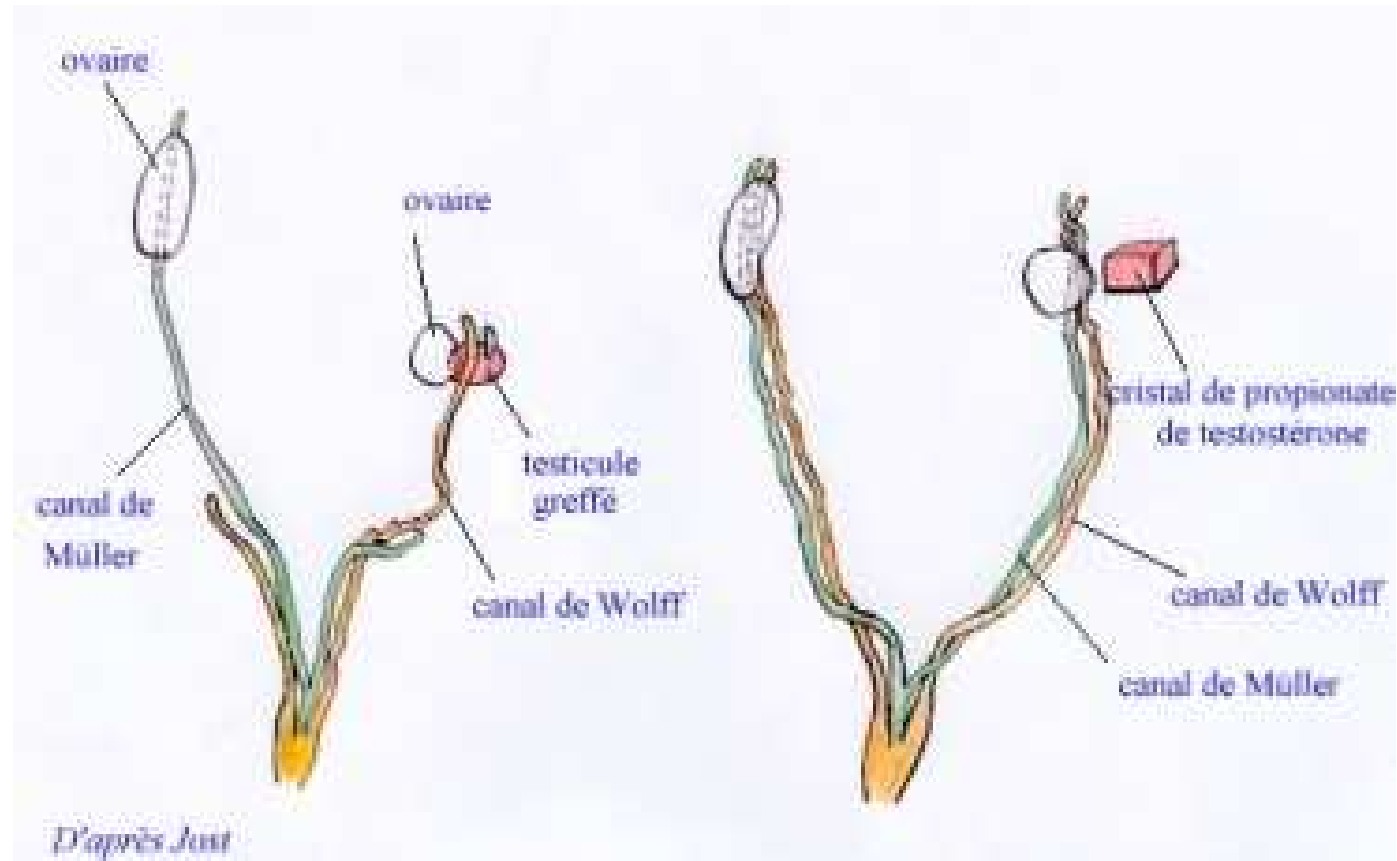
Centre Hospitalier Régional
Universitaire de Lille

Dr Ingrid Plotton

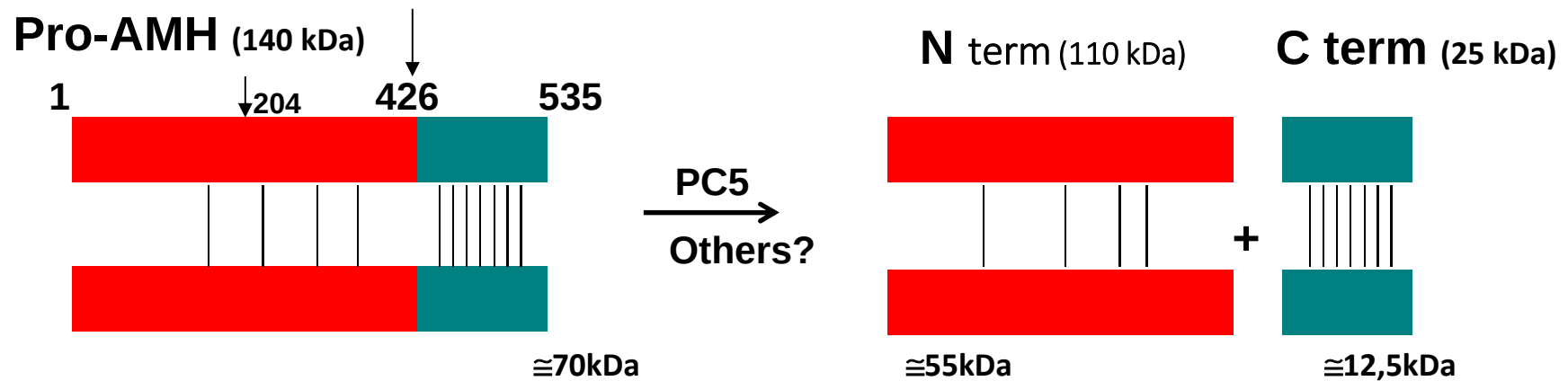
Service d'Hormonologie-
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Rares
Centre de Biologie et Pathologie Est
CHU Lyon



Découverte de l'AMH

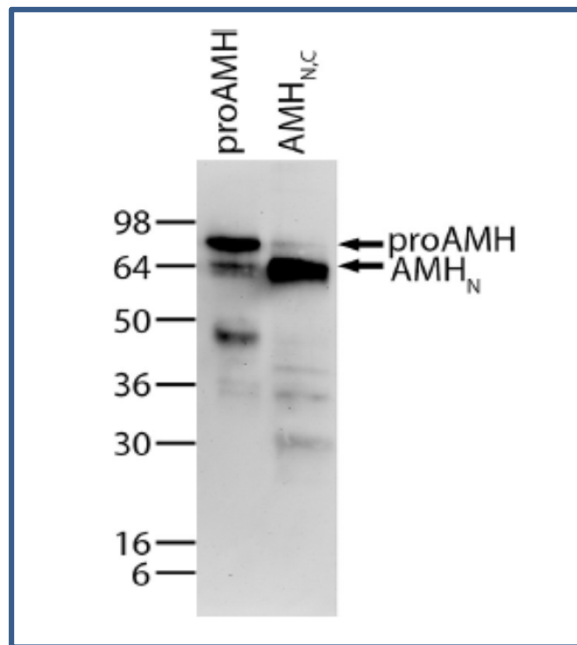


Intracellular AMH processing gives several molecular forms

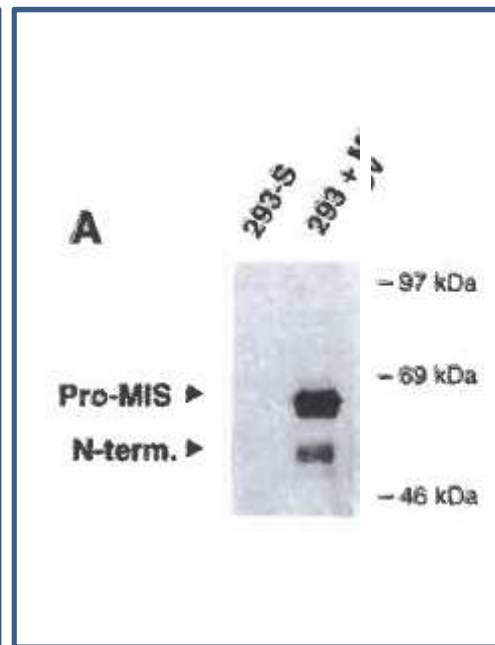


Uncleaved form: pro-AMH

Nachtigal et al. *PNAS* 1996: studies on embryonic testes in culture



Analysis of purified preparations

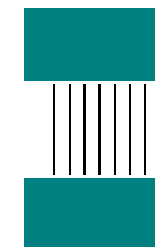


Analysis of culture media from Embryonic testes

Cleaved Associated Form: AMH_{N,C}



Mature (25 kDa)



Isolated C-term form: AMH_C

Proteolytic processing of anti-Müllerian hormone differs between human fetal testes and adult ovaries

L.S. Mamsen^{1,*}, T.S. Petersen¹, J.V. Jeppesen¹, K. Møllgård²,
M.L. Grøndahl³, A. Larsen⁴, E. Ernst⁵, C. Oxvig⁶, A. Kumar⁷, B. Kalra⁷,
and C.Y. Andersen¹

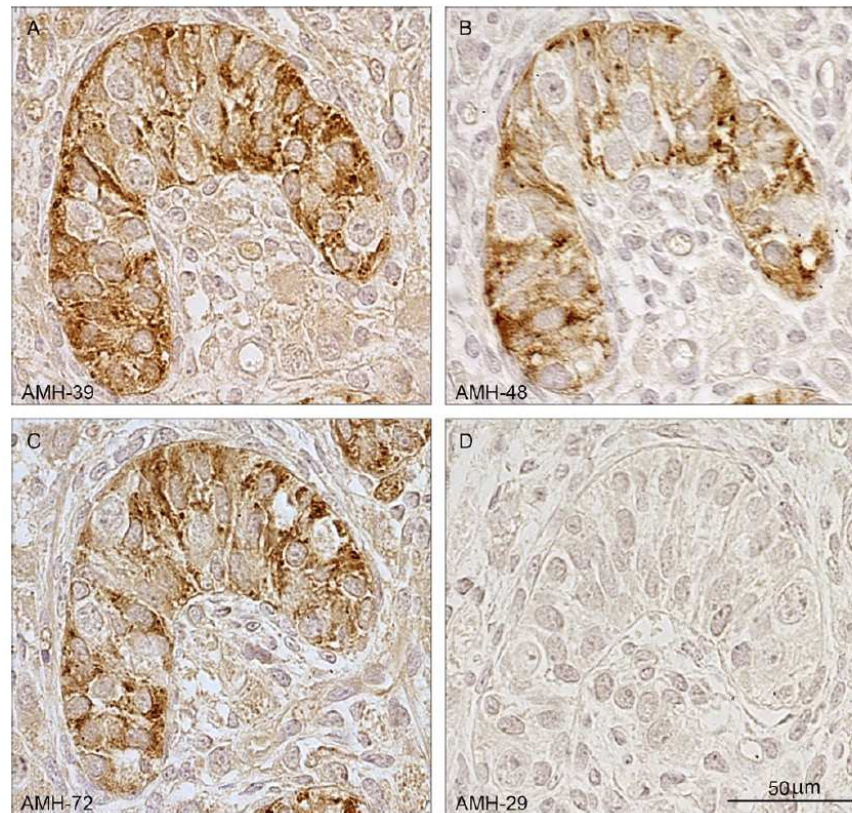


Table 1 Characteristics of monoclonal AMH antibodies used.

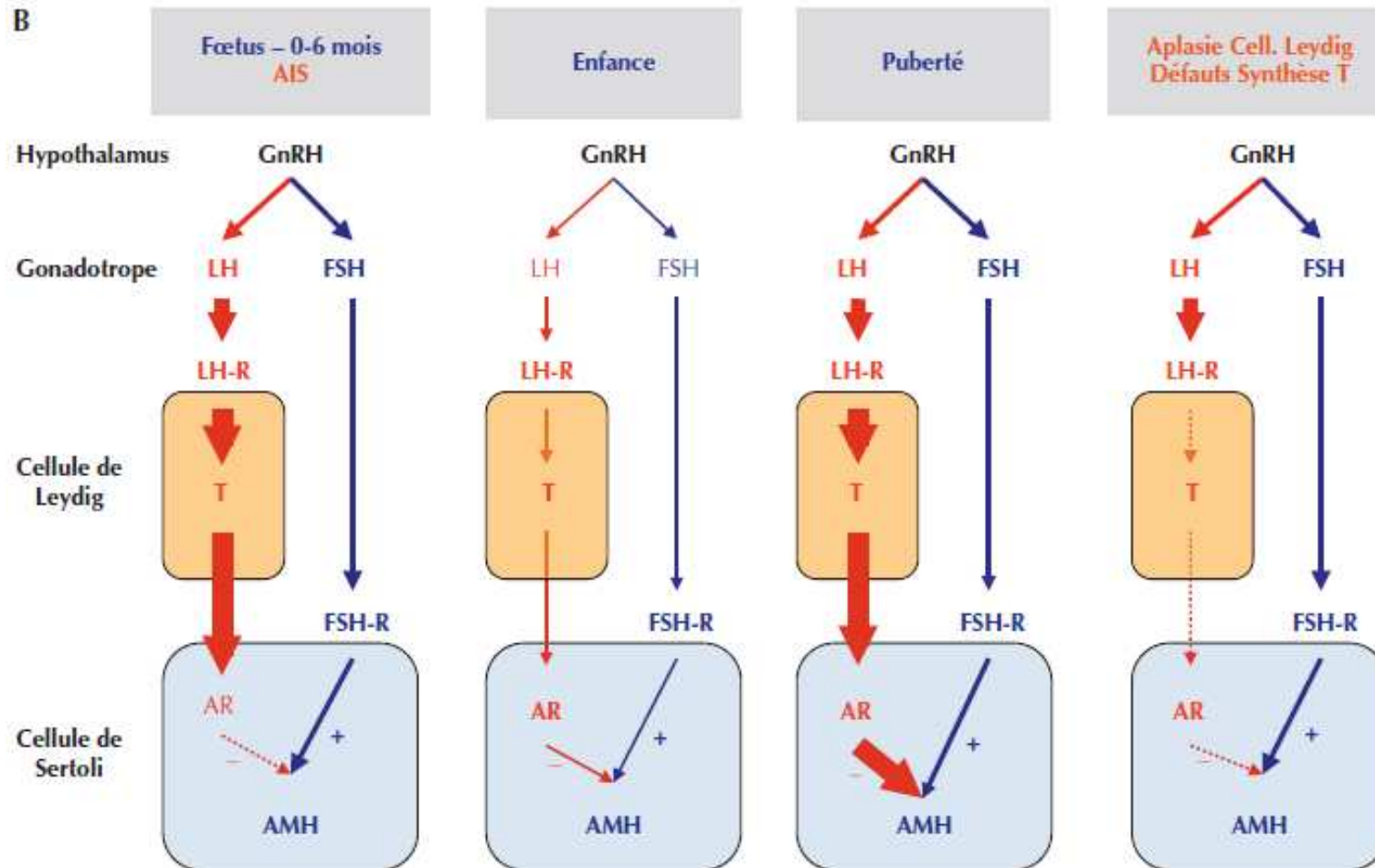
Antibody number	AMH epitope region (aa)	AMH region
AMH-1	491–502 (+ weak recognition: 435–446)	Mature C-terminal
AMH-29	491–502	Mature C-terminal
AMH-39	330–341	Pro-mature
AMH-48	260–271	Pro-mature
AMH-72	337–348	Pro-mature

AMH_C

AMH_{N,C}

Figure 2 Human fetal testis aged 10 weeks pc. Antibody AMH-39/48/72 binding pro-mature AMH were expressed in Sertoli cell cytoplasm (**A–C**). Antibody AMH-29, binding the mature C-terminal AMH was not detected (**D**). Scale bar: 50 µm.

Régulation de la biosynthèse d'AMH chez le ♂

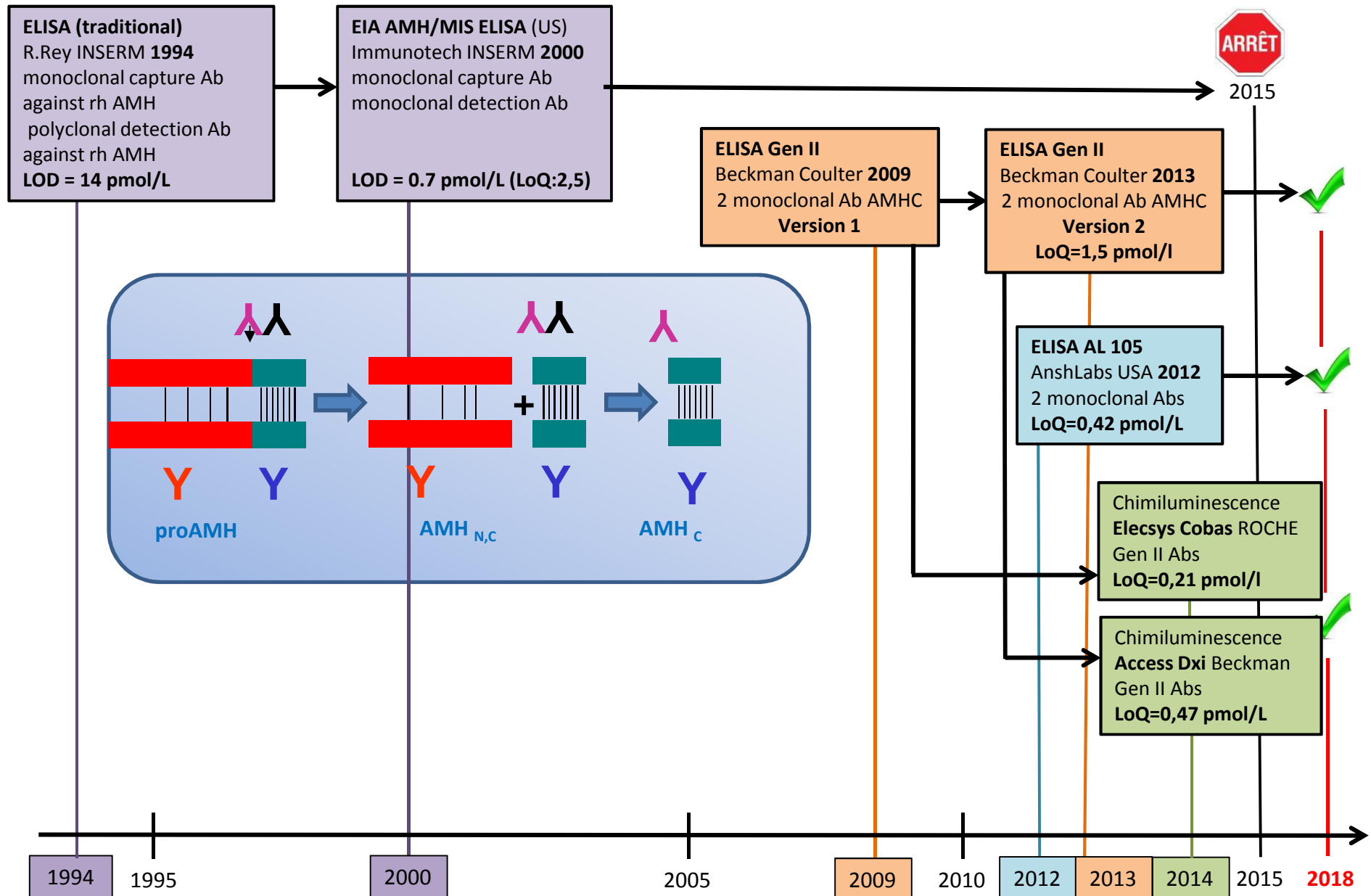


Mini-revue

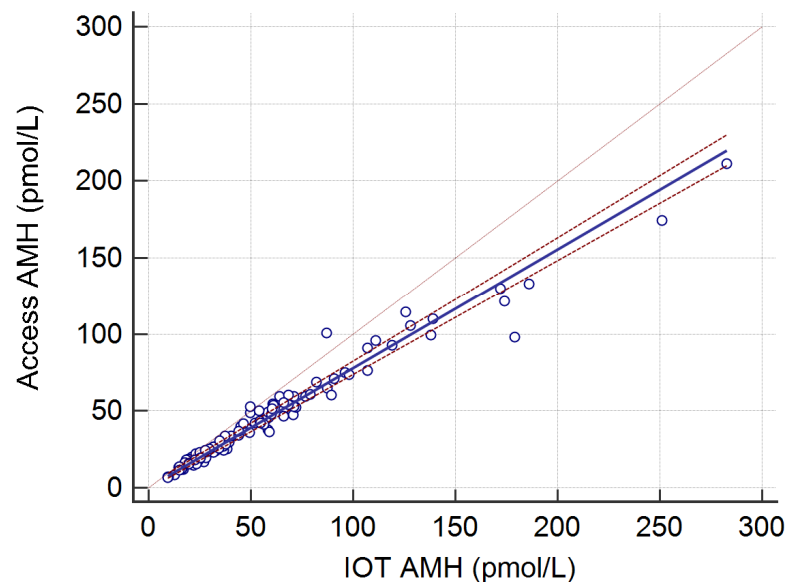
nt Médecine de la Reproduction, Gynécologie Endocrinologie 2014 ; 16 (1) : 56-68

Rodolfo A. Rey^{1,2}
Jean-Yves Picard³
Nathalie Josso³

AMH assays measure total AMH



Comparative assessment of five serum antimüllerian hormone assays for the diagnosis of polycystic ovary syndrome



$$\text{Access} = 0.77 \text{ EIA IOT AMH} + 0.12$$

(A) Correlation between Gen II ELISA, AL-105i EUSA, or Access DxI automatic assay and EIA AMH/MIS ELISA was evaluated by the Passing-Bablok regression procedure. The blue line shows the regression curve; the dashed red lines show the 95% CI around the regression curve; the pink line shows the line of perfect correlation. (B) Bland-Altman plots showing the distribution of the differences of AMH concentration between two

Pascal Pigny, Ph.D.,^a Elisse Gorisse, M.D.,^b Amjad Ghulam, M.D.,^a Geoffroy Robin, M.D.,^b Sophie Catteau-Jonard, M.D., Ph.D.,^b Alain Duhamel, M.D., Ph.D.,^c and Didier Dewailly, M.D.^b

Non-equivalence of anti-Müllerian hormone automated assays—clinical implications for use as a companion diagnostic for individualised gonadotrophin dosing

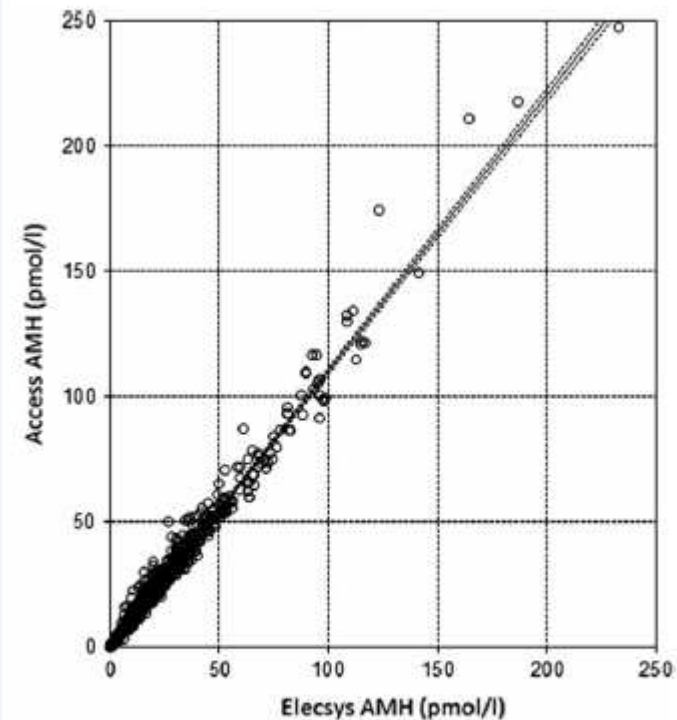
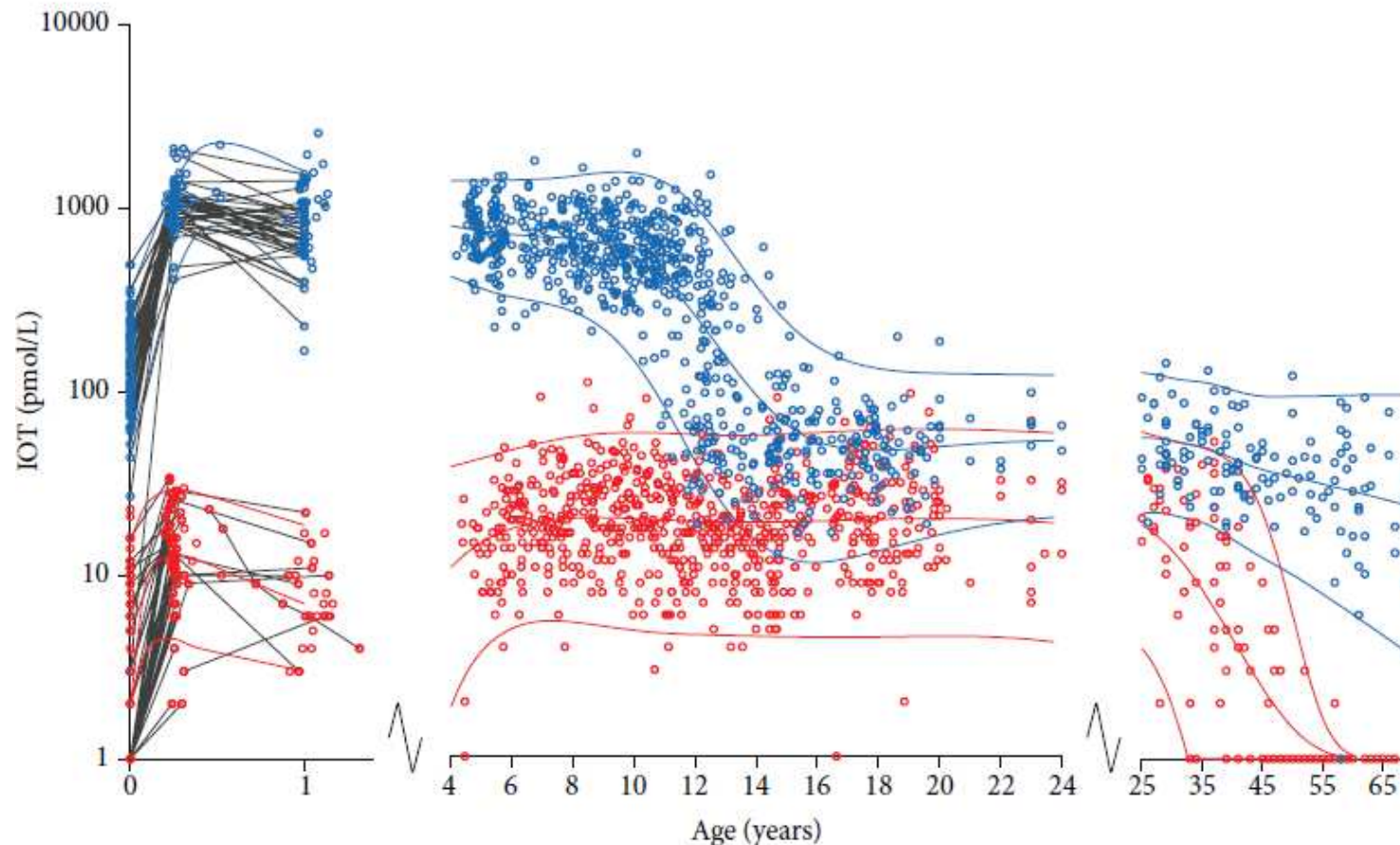


Figure 2 Passing-Bablok regression line with 95% confidence intervals of Elecsys and Access Assays. Passing-Bablok regression line with 95% confidence intervals $\text{Access} = -0.05 + 1.10$ (95% CI: 1.09–1.12) $\times$ Elecsys. The dots represent the paired values of AMH.

Stamatina Iliodromiti^{1,*}, Barbara Salje¹, Didier Dewailly², Craig Fairburn³, Renato Fanchin⁴, Richard Fleming³, Hang Wun Raymond Li⁵, Krzysztof Lukaszuk^{6,7,8}, Ernest Hung Yu Ng⁵, Pascal Pigny⁹, Teddy Tadros⁴, Joseph van Helden¹⁰, Ralf Weiskirchen¹¹, and Scott M. Nelson¹

Anti-Müllerian Hormone and Its Clinical Use in Pediatrics with Special Emphasis on Disorders of Sex Development

Marie Lindhardt Johansen,¹ Casper P. Hagen,¹
Trine Holm Johannsen,¹ Katharina M. Main,¹ Jean-Yves Picard,² Anne Jørgensen,¹
Ewa Rajpert-De Meyts,¹ and Anders Juul¹



ELISA AMH/MIS Immunotech

Intervalle de référence de l'AMH chez l'enfant <4 ans

Comparaison des trousse

Tableau 1 présente la comparaison des valeurs d'AMH obtenues pour les mêmes échantillons entre les techniques AMH Roche, AMH Gen II et AMH IOT: pour chacune des tranches d'âge étudiée entre la naissance et 4 ans.

Age	13-20j	2.8-5.1 mois	8.5 à 9.8 mois	4 ans
	Moy +ET* (min-max) n=29	Moy +ET* (min-max) n=51	Moy +ET* (min-max) n=47	Moy +ET* (min-max) n=22
AMH ROCHE	693 +/-395 228-2320	1541 +/-505 618-3213	1570 +/-705 517-4280	1044 +/-441 498-2352
AMH Gen II	1190 +/-881.8 413.1-4509	2507 +/-980 811-5320	2609 +/-1259 917-6676	1599 +/-574 809-2738
AMH IOT	923.5 +/-419.2 286.2-2119	1833 +/-552 8111-2986	1809 +/-680 741-4365	1331 +/-513 712-2356

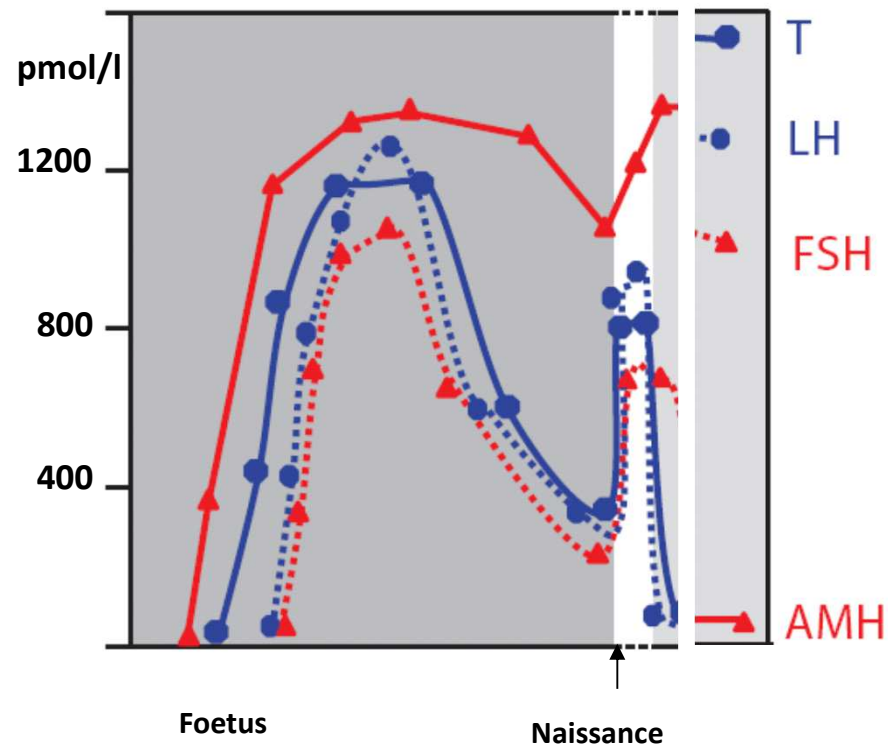
* moy. (moyenne), ET (écart type), min-max (valeurs extrêmes)

Plotton et al. 2015

AMH en période pré-natale

Profil de sécrétion de l'AMH

Taux plasmatiques



AMH :

- Haut niveau de la vie foétale à la période prépubertaire
- Diminution de l'AMH dès le stade II III de la puberté

Profil de sécrétion de l'AMH en pré- et péri-natal

Table 1

Usual values for AMH in fetuses, newborns and infants under 1 year

	Female			Male		
	<i>n</i>	Median	5th–95th percentile	<i>n</i>	Median	5th–95th percentile
Fetal blood	25	<0.4	<0.4–0.5	23	44.4	13.4–86.2
Cord blood	53	<0.4	<0.4–1.1	48	30.7	7.4–610.7
1–30 days	70	<0.4	<0.4–1.3	73	57.2	23.8–124.0
31–120 days	31	0.8	<0.4–6.4	39	88.4	46.8–173.0
4–12 months	13	1.0	<0.4–2.7*	53	124.7	67.4–197.0

The results are expressed in ng/ml as medians and 5th–95th percentiles and as minimum and maximum values (* in females over 4 months). When undetectable (i.e. below the 0.3 ng/ml limit of assay sensitivity), AMH was assigned a concentration equivalent to the first concentration of the standard curve (0.4 ng/ml).

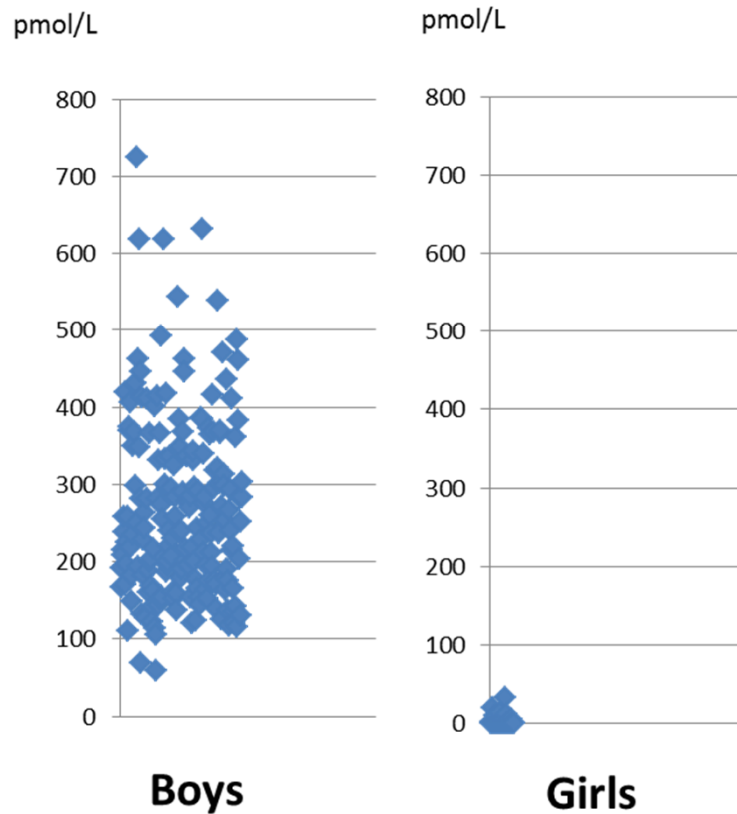
The immunoenzymatic AMH assay is a sandwich method with two immunological steps (AMH/MIS enzyme immunoassay kit cat 2368, Immunotech-Beckman Coulter, France). In the first step, AMH is captured by a murine mon-

Me G

- ATCD 1^{er} enfant Retard mental+ micropenis
- 2^e enfant anomalies cérébrales, échographie+ micropenis ITG
- 3^e grossesse en cours micropenis
- Ponction de sang foetal à 34 SA
- Caryotype 46,XY
- AMH 26.37 pmol/L

AMH en période néo-natale

Graph repartition of AMH values in boys and girls neonate



230 sang de cordons ombilical

Prélevé à la naissance

192 garçons

38 filles

AMH Cobas, Roche

pmol/L	n	Median	1st Quartil	3d Quartil	min-max
Boys	192	249.95	178.7	338	58.5-724.3
Girls	38	1.03	0.56	2.59	0.28-32.5

pmol/L	n	Mean	min-max
DA	3	544	292-777
TD	4	98.8	72.4-140

DA mutation récepteur aux androgènes

TD dysgénésie testiculaire

Vasseur et al, Poster ESPE 2016

Maurice, né à 36 SA+2 jrs, pèse 2400 g, taille 44 cm, PC=33 cm, présente une **cryptorchidie bilatérale** à la naissance. Pas de notion d'exposition aux pesticides ou solvants. Grossesse spontanée. 1^{er} enfant du couple.

<u>Bilan hormonal réalisé à J2:</u>		valeurs références
Testostérone (CLIA-Architect):	2,11 ng/ml	0,09-2,99
AMH (Access-Dxi):	478 pmol/L	221-914

Caryotype en cours

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Caryotype en cours.

Présence de tissu testiculaire fonctionnel. L'échographie confirmera la présence d'un testicule en position inguinale et le second en abdominal.

Aurélien, né à 41 SA par césarienne, pèse 3440 g, taille 51 cm, PC=33 cm. Il a 2 sœurs âgées de 7 et 1 an. Echographies normales durant la grossesse. Sexe annoncé: garçon. Aurélien présente un **hypospade postérieur** avec courbure de la verge . Les testicules sont en place et bien trophiques.
Un diagnostic de DSD est évoqué.

<u>Bilan hormonal réalisé</u>	à J16	à J52	valeurs références
Testostérone (CLIA-Architect):	9,05 ng/ml	6,24 ng/ml	0,09-2,99
AMH (Access-Dxi):	1597 pmol/L	1998 pmol/L	278-1885
LH (CLIA-Architect)	5,8 UI/L	8,1 UI/L	0,2-3,0
FSH (CLIA-Architect)	0,8 UI/L	0,9 UI/L	<2,4

Caryotype revient 46, XY

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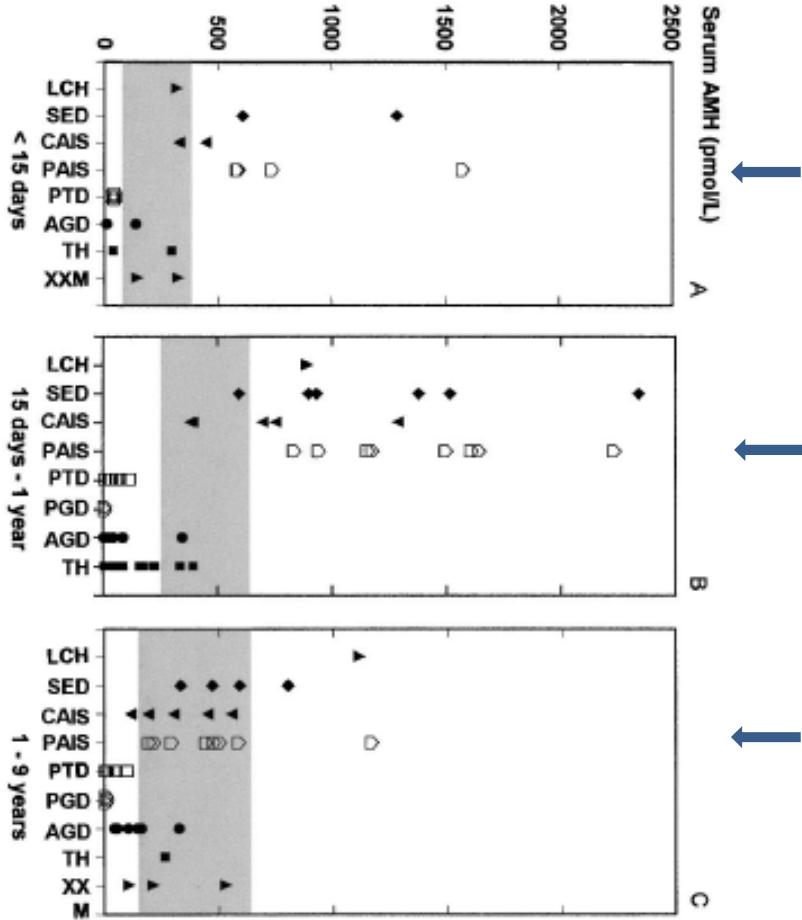
Caryotype revient 46, XY

Résistance partielle aux androgènes.

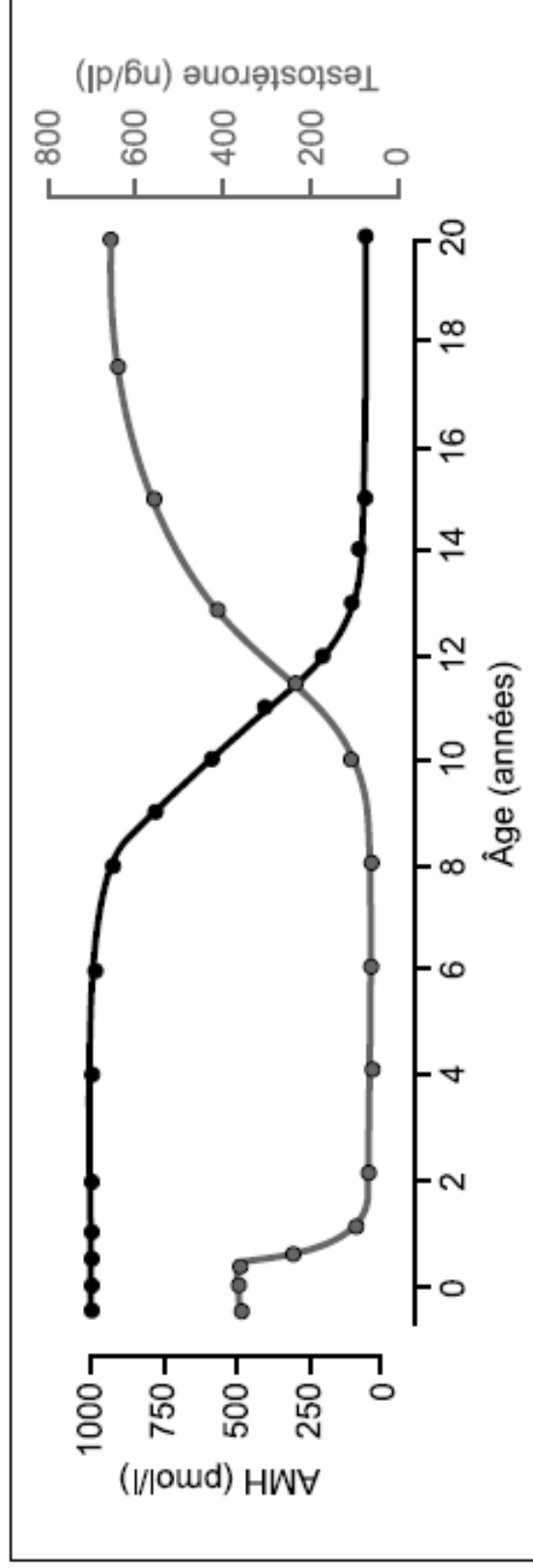
Evaluation of Gonadal Function in 107 Intersex Patients
by Means of Serum Antimüllerian Hormone
Measurement*

RODOLFO A. REY, et al.

ELISA AMH/MIS Immunotech



AMH en période péri-pubertaire



Le chassé-croisé entre AMH et testostérone chez le sujet masculin : en noir, la courbe de l'AMH ; en gris, celle de la testostérone (© Rodolfo Rey).

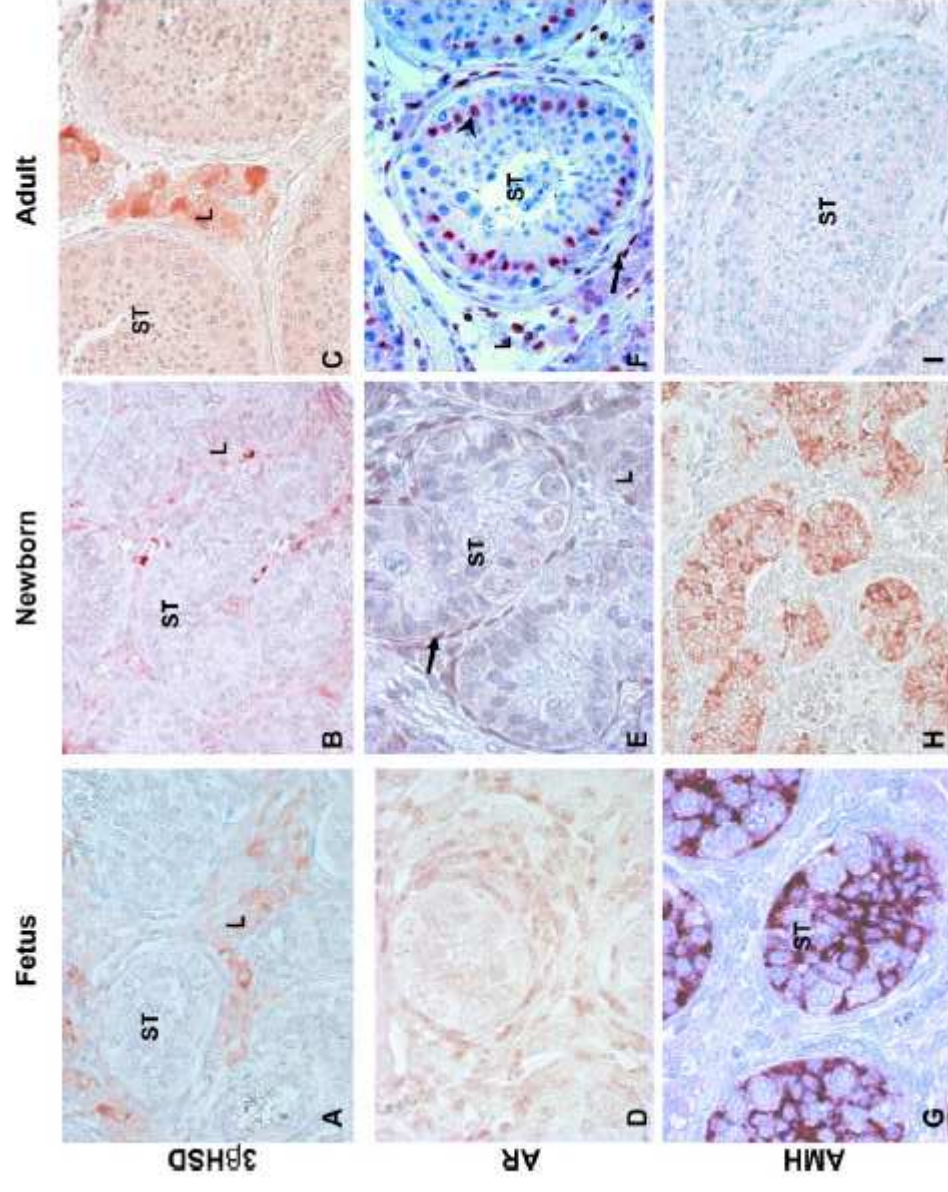


FIG. 1. Immunoreactivity of 3 β HSD, AR, and AMH in human testis: representative illustrations of immunoreactivity in fetal, neonatal, and adult testis. A–C, Immunoreactivity of 3 β HSD in fetal (A), neonatal (B), and adult (C) testis. Enzyme expression is localized to the cytoplasm of Leydig cells in fetal, newborn, and adult testis. SC and germ cells are negative. D–F, Immunoreactivity of AR in fetal (D), newborn (E), and adult (F) testis. In fetal (D) and newborn testis (E), nuclear AR expression is restricted to Leydig cells and myoid peritubular cells (arrows). In adult testis (F), AR expression is present in the nucleus of Leydig cells, myoid peritubular cells (arrows), and also SC (arrowhead). G–I, Immunoreactivity of AMH in fetal (G), newborn (H), and adult (I) testis. AMH is strongly expressed in SC cytoplasm in fetal (G) and newborn (H) testis. AMH is undetectable in adult human testis. L, Leydig cells; ST, seminiferous tubule. Original magnification, $\times 20$.

AMH et retard pubertaire

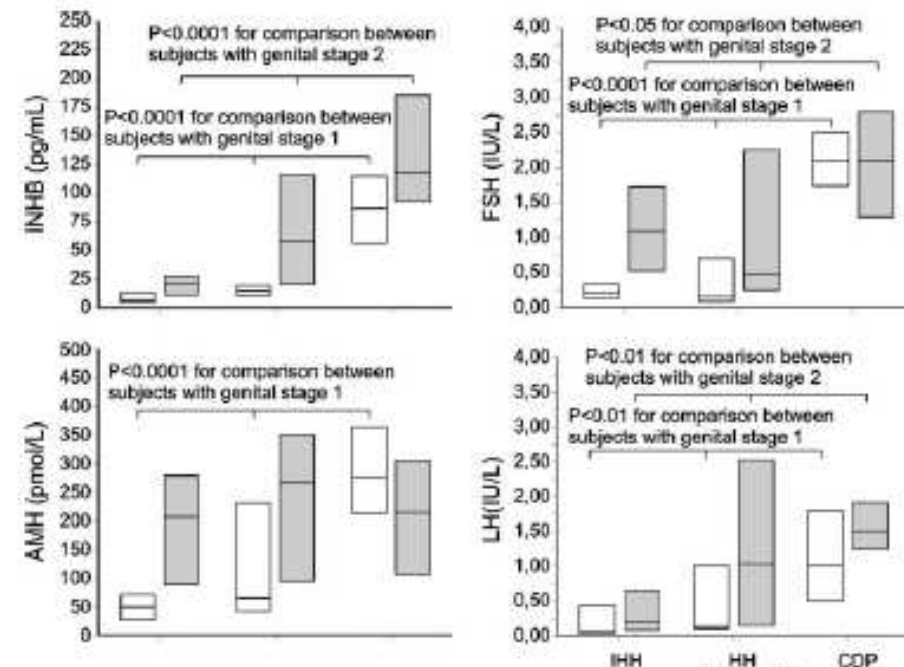


TABLE 1. Hormonal characteristics of the subjects with IHH, HH within CPHD, and CDP

	IHH	CPHD	CDP	P
n	16	15	51	
Age (yr)	16.0 (14.3; 17.0)	15.0 (14.6; 15.1)	15.5 (15.0; 16.0)	N.S.
Stage 1/stage 2	9/7	7/8	23/28	N.S.
T (nmol/liter)	0.6 (0.4; 1.3)	0.2 (0.1; 0.6)	1.1 (0.9; 2.1)	<0.0001
INHB (pg/ml)	9 (5; 22)	20 (15; 65)	108 (68; 168)	<0.0001
AMH (pmol/liter)	66 (37; 198)	225 (65; 300)	245 (110; 364)	<0.01
FSH (IU/liter)	0.4 (0.2; 1.4)	0.5 (0.2; 0.7)	2.0 (1.4; 2.6)	<0.0001
LH (IU/liter)	0.2 (0.0; 0.4)	0.5 (0.1; 1.4)	1.3 (0.7; 1.8)	<0.0001

Data are expressed as median (25th; 75th percentiles). The three groups were compared using the Kruskal-Wallis test. Genital stage 1, testis volume less than 3 ml. Genital stage 2, testis volume 3–6 ml. N.S., Not significant. For T, to convert to ng/ml, divide by 3.47; for AMH, to convert to ng/ml, divide by 7.14.

AMH chez l'homme adulte

Decrease of anti-Mullerian hormone in genetic spermatogenic failure

I. Plotton¹, L. Garby², Y. Morel¹ & H. Lejeune²

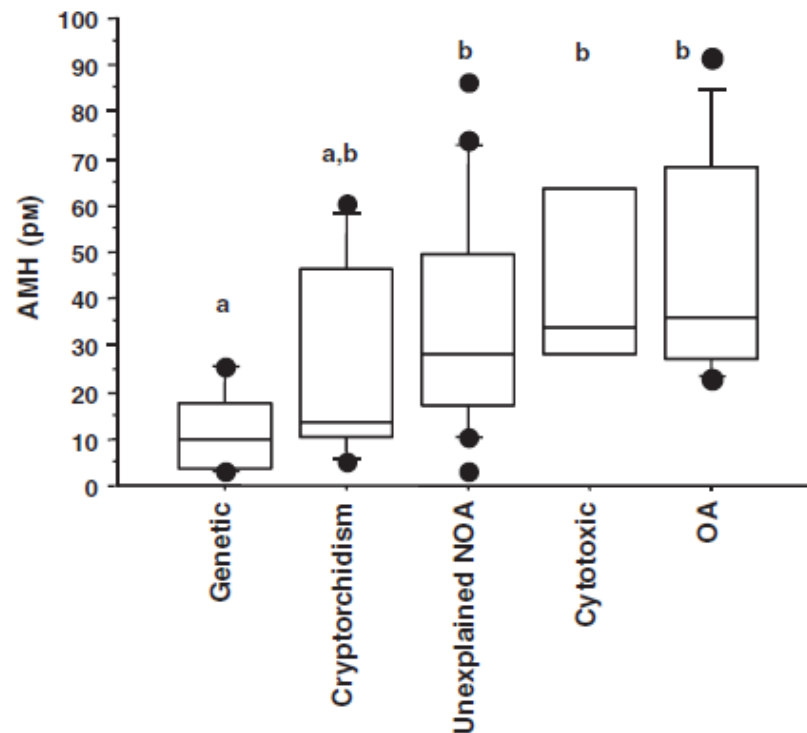


Fig. 1 Anti-Mullerian Hormone (AMH) plasma levels in azoospermia according to aetiology: AMH plasma levels between genetic spermatogenic failure (Genetic), cryptorchidism related spermatogenic failure (Cryptorchidism), unexplained nonobstructive azoospermia (unexplained NOA), cytotoxic spermatogenic failure (Cytotoxic) and obstructive azoospermia (OA) were significantly different by Kruskal–Wallis' test ($P = 0.0021$). According to 2 by 2 comparisons by Mann–Whitney's U -test, conditions labelled with the same letter were not significantly different (Genetic versus unexplained NOA: $P = 0.0051$; Genetic versus Cytotoxic: $P = 0.0055$; Genetic versus OA: $P = 0.0003$).

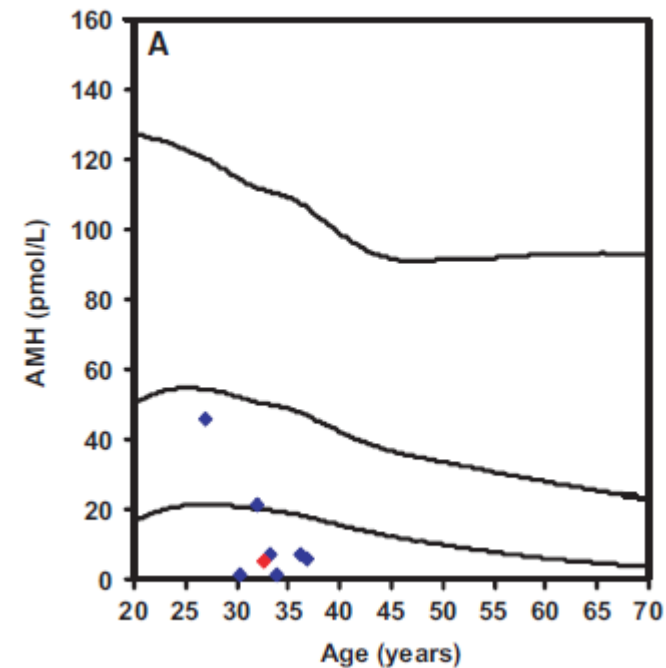
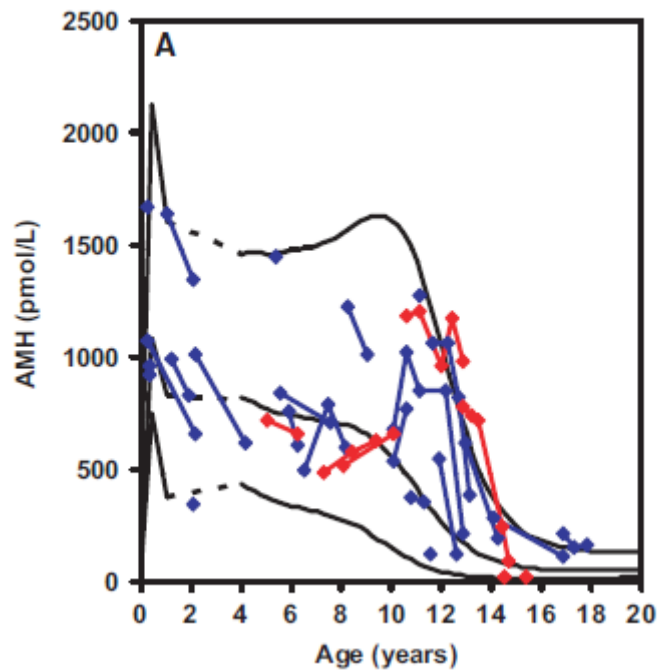
Âge >24 ans

ELISA AMH/MIS Immunotech

Serum concentrations of Anti-Müllerian Hormone (AMH) in 95 patients with Klinefelter syndrome with or without cryptorchidism

Lise Aksglaede (lise.aksglaede@rh.regionh.dk)¹, Peter Christiansen¹, Kaspar Sørensen¹, Malene Boas¹, Allan Linneberg², Katharina M Main¹, Anna-Maria Andersson¹, Niels E Skakkebaek¹, Anders Juul¹

Acta Paediatrica 2011 **100**, pp. 839–845



ELISA AMH/MIS Immunotech

- ◆ Sans histoire de cryptorchidie
- ◆ Avec histoire de cryptorchidie

L'AMH sérique pourrait elle prédire le résultat de la TESE?

Klinefelter Syndrome in Adolescence: Onset of Puberty Is Associated with Accelerated Germ Cell Depletion

ANNE M. WIKSTRÖM, TANELI RAIVIO, FARUK HADZISELIMOVIC, SAKARI WIKSTRÖM, TIMO TUURI, AND LEO DUNKEL

J Clin Endocrinol Metab, May 2004, 89(5):2263–2270

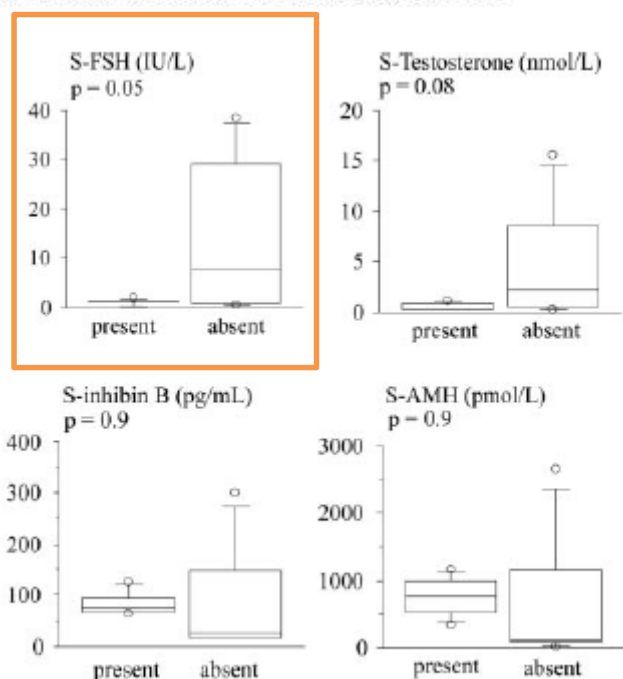


FIG. 3. A, Testicular volumes and serum FSH, testosterone, inhibin B, and AMH levels in 14 boys with KS with spermatogonia present or absent in the biopsy specimen ($n = 7$, both groups). Horizontal lines, 10th, 25th, 50th, 75th, and 90th percentiles, and extreme values are shown separately. B, Longitudinal changes in individual testis volumes. Black circles, group I, white circles, group II (see Table 1).

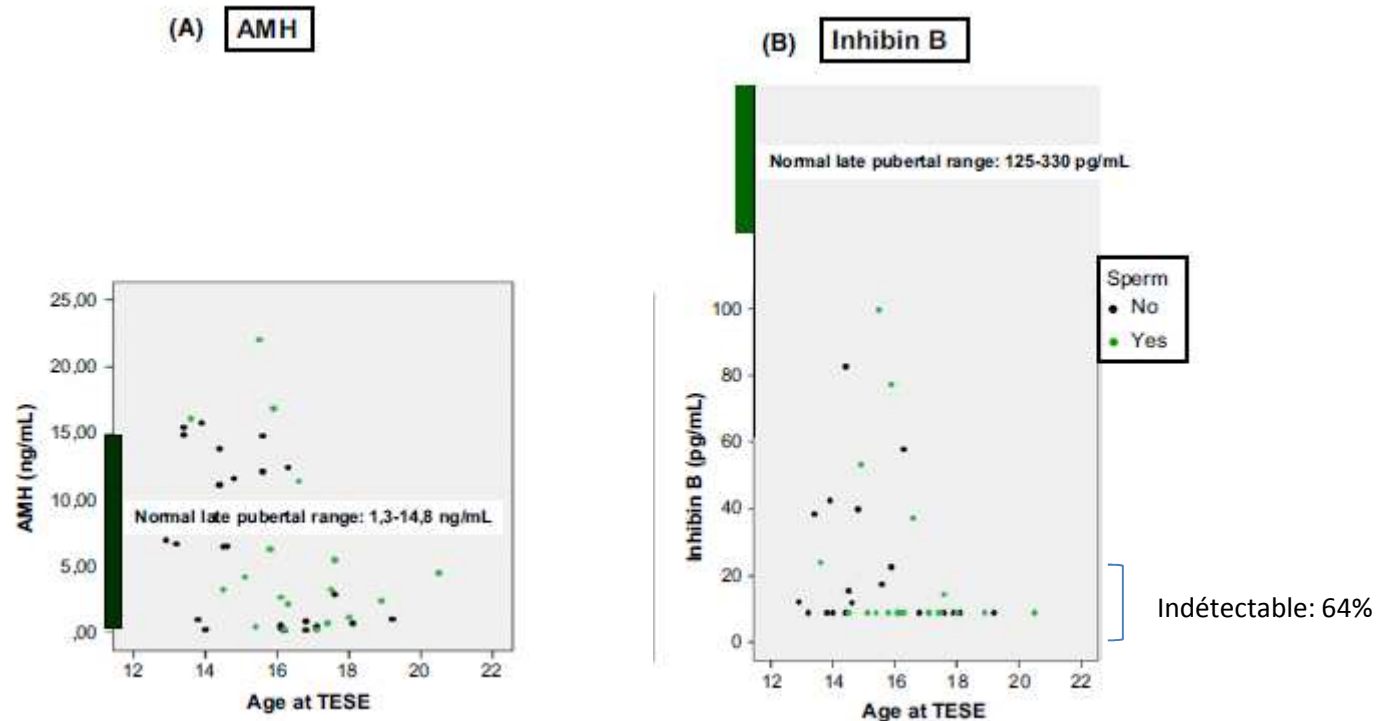
ELISA Serotec Oxford
BioInnovation,

ELISA AMH/MIS Immunotech

Age and markers of Leydig cell function, but not of Sertoli cell function predict the success of sperm retrieval in adolescents and adults with Klinefelter's syndrome

L'AMH sérique pourrait elle prédire le résultat de la TESE?

¹J. Rohayem, ¹R. Fricke, ¹K. Czeloth, ²C. Mallidis, ²J. Wistuba, ¹C. Krallmann, ¹M. Zitzmann and ¹S. Kliesch



ELISA DRG, Germany

N=50 adolescents 13-19 yrs

Anti-Müllerian Hormone Is a Marker for Chemotherapy-Induced Testicular Toxicity

Mattan Levi, Noa Hasky, Salomon M. Stemmer, Ruth Shalgi, and Irit Ben-Aharon

Department of Cell and Developmental Biology (M.L., N.H., R.S.), Sackler Faculty of Medicine, Tel Aviv University, Israel and Institute of Oncology (S.M.S., I.B.-A.), Davidoff Center, Rabin Medical Center, Beilinson Campus, Petah-Tiqva 49100, and Sackler Faculty of Medicine, Tel-Aviv University, Israel

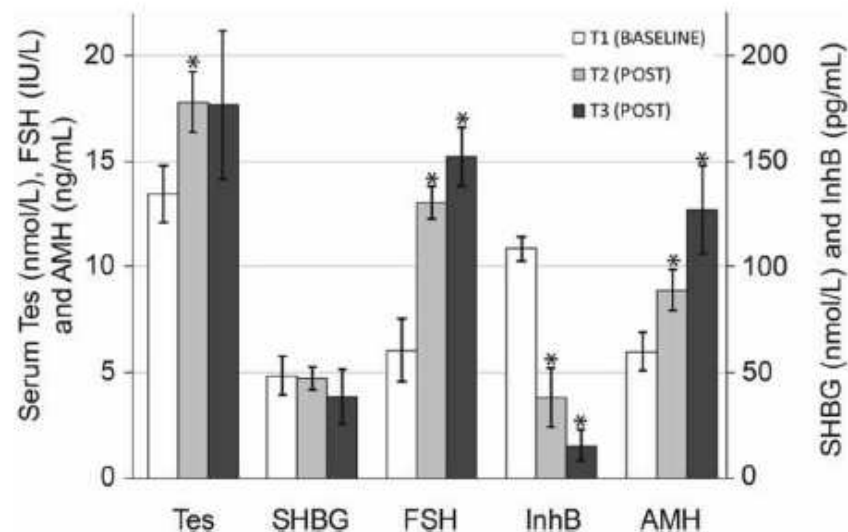
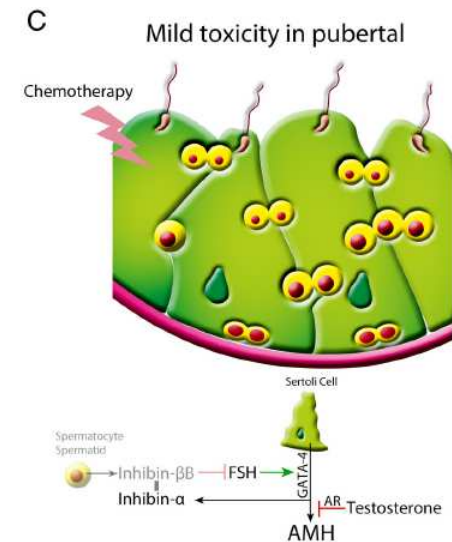
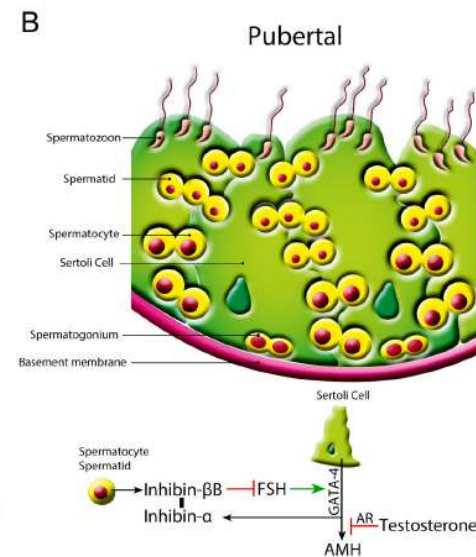


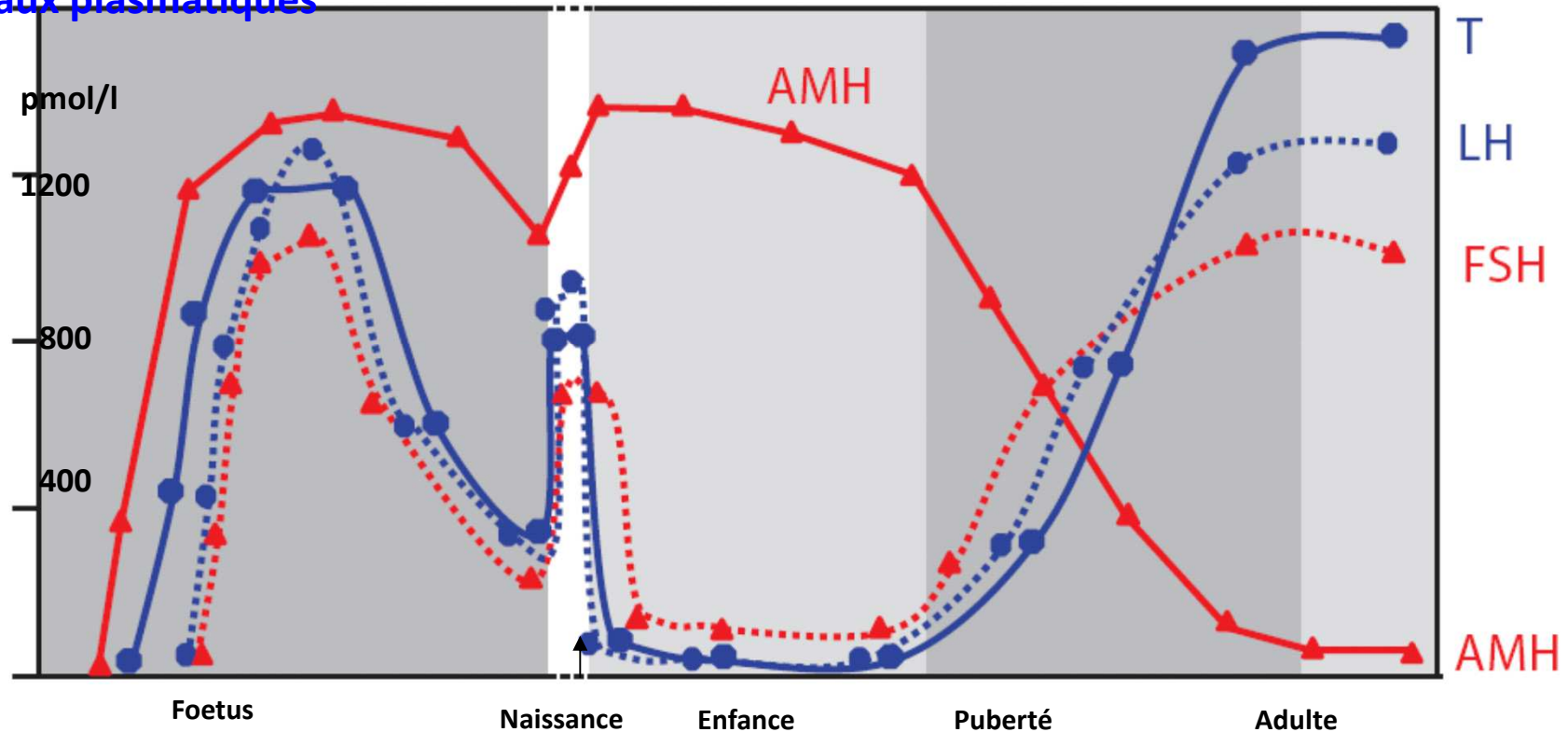
Figure 1. Serum hormones level in men. Hormone levels were measured in the serum of 19 patients 1 week before the first chemotherapeutic treatment (baseline; T1), 3–4 days (T2), and 6 months (T3) after the last course of chemotherapy. InhB, inhibin-B; Tes, T. Each bar is mean $\pm$ SEM. *, Significantly different from T1 value (*t* test; $P < .05$).



AMH GenII, ELISA, Beckman Coulter

Profil de sécrétion de l'AMH

Taux plasmatiques



AMH :

- Haut niveau de la vie foétale à la période prépubertaire
- Diminution de l'AMH dès le stade II III de la puberté

Conclusion

- AMH
- Rôle essentiel au cours du développement embryonnaire
- Marqueur utile à la naissance et dans l'enfance pour évaluer la fonction gonadique
- Homme adulte : une valeur basse sera en faveur d'une atteinte profonde et sévère de la Sertoli
- Nécessite d'établir des valeurs de références et de standardiser les méthodes pour comparer les résultats