

Intérêt du dosage de l'AMH en endocrinologie de la reproduction : de l'enfant à l'adulte

Isabelle Demeestere

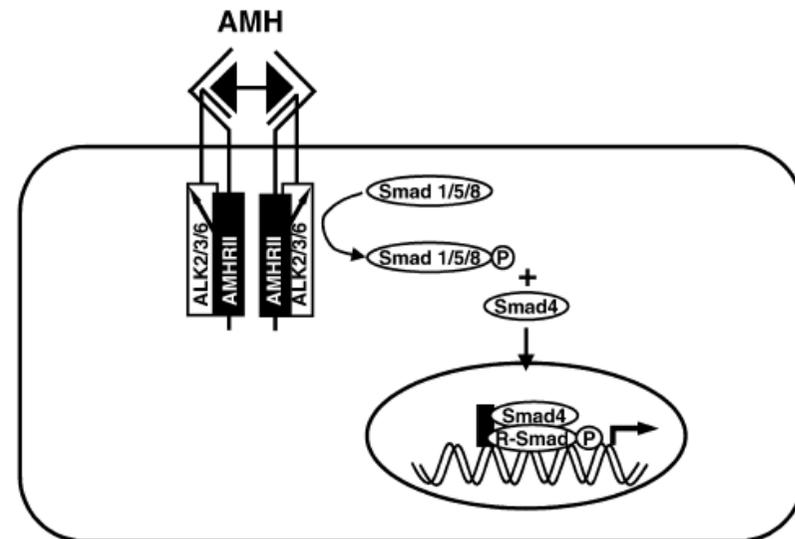
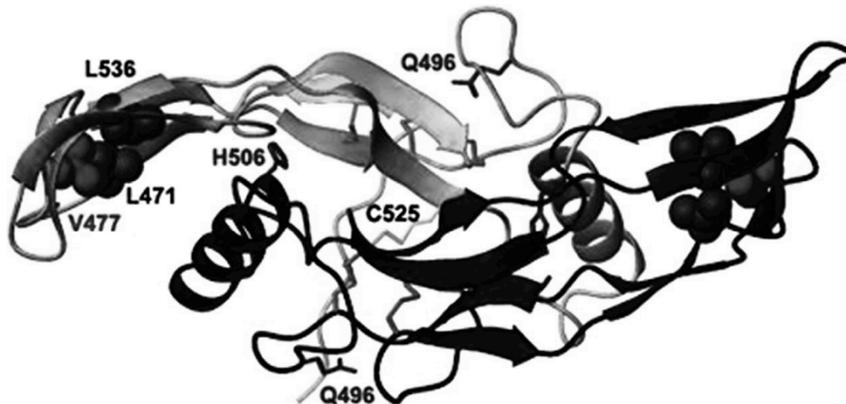
Laboratoire de Recherche en Reproduction Humaine, ULB

26 septembre



DEFINITION

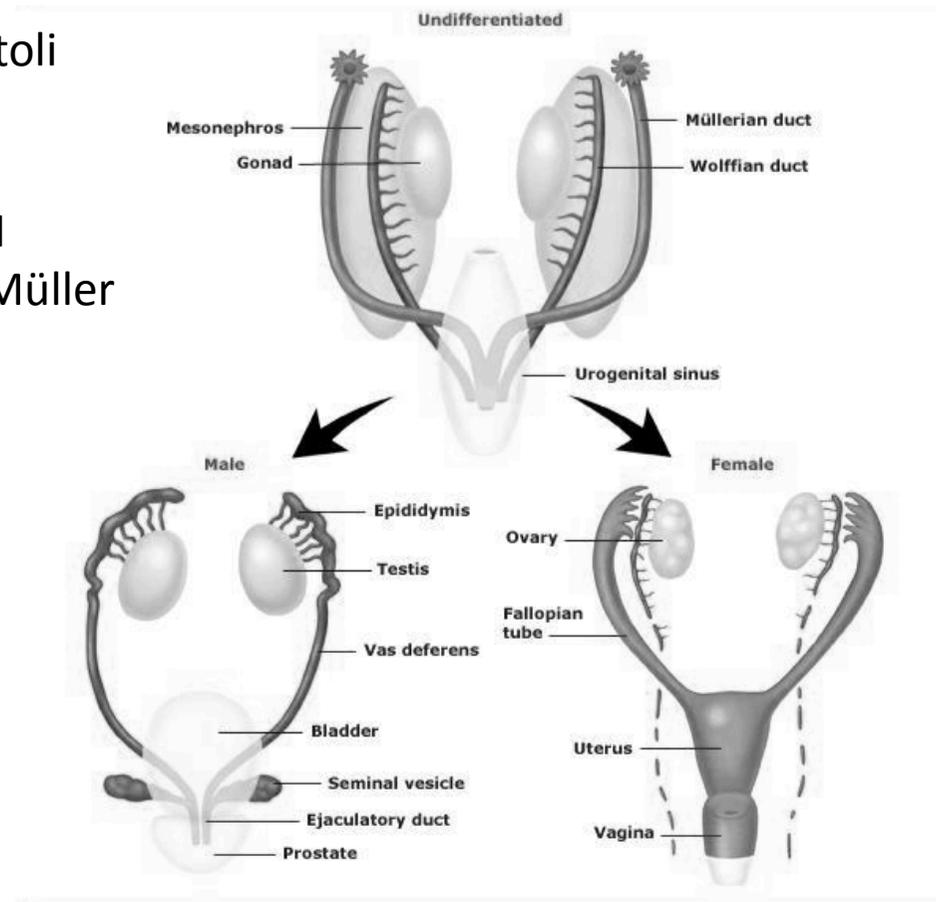
- ◉ L'AMH est une glycoprotéine dimérique 140 k-DA
- ◉ Membre de la famille des TGF- β
- ◉ Action via les récepteur de type II, induisant une cascade de signalisation intracellulaire BMP-like
- ◉ Synthétisés exclusivement par les gonades, à différent moment de la vie



La sécrétion d'AMH par les cellules de Sertoli est induite par le gène SRY.

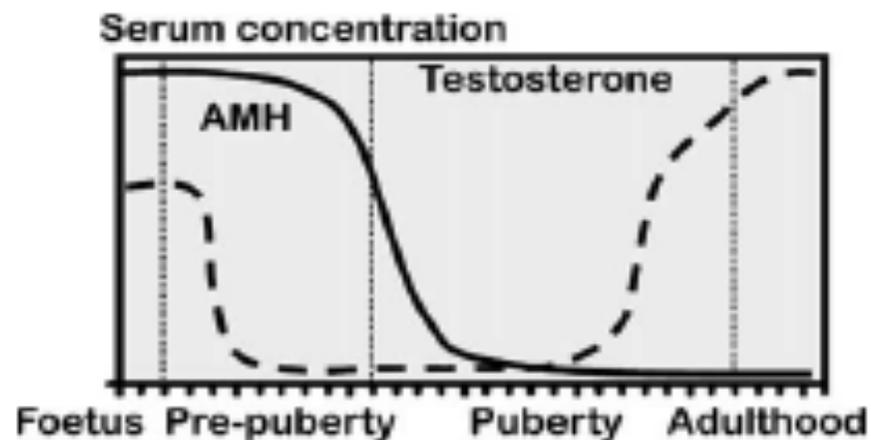
Agit sur récepteur membranaire de type II
Pour induire la régression des canaux de Müller
Vers la 8^{ème} semaine de gestation.

→ Persistant Mullerian Duct Syndrome



In females, the Müllerian ducts give rise to the fallopian tubes, uterus, and upper vagina, and the Wolffian ducts persist in vestigial form. In males, the Wolffian ducts give rise to the epididymides, vasa deferentia, seminal vesicles, and ejaculatory ducts, and the Müllerian ducts regress.

- AMH est sécrétée par les sertoli cells et est down-régulée par la testostérone intratesticulaire.
- Pendant période néonatale et premier mois après la naissance, les deux hormones sont élevées du à l'insensibilité des cellules de Sertoli aux androgènes pendant cette période.
- Taux élevé jusqu'à la puberté (stade II et III), période coïncident avec l'augmentation de la testostérone intratesticulaire.
- Up-reguler par FSH



R. Rey et al. / Molecular and Cellular Endocrinology 211 (2003) 21–31

TABLE 1. Serum AMH in normal males

Age group	n	Serum AMH (pmol/L) ^a	
		Mean ± SEM	Range ^b
<15 days	6	229 ± 59 (32.1 ± 8.3)	76–381 (10.6–53.4)
15 days–1 yr	22	465 ± 93 (65.1 ± 13.0)	251–679 (35.2–95.1)
1.01–4 yr	17	499 ± 66 (69.9 ± 9.2)	360–638 (50.4–89.4)
4.01–7 yr	16	438 ± 61 (61.3 ± 8.4)	309–566 (43.3–79.3)
7.01–9 yr	14	336 ± 47 (47.0 ± 6.6)	234–438 (32.8–61.3)
>9 yr ^c			
I	22	249 ± 26 (34.9 ± 3.7)	194–304 (27.2–42.6)
II	25	159 ± 25 (22.2 ± 3.5)	107–211 (15.0–29.6)
III	8	79 ± 28 (11.0 ± 3.9)	12–145 (1.7–20.3)
IV–V	8	48 ± 14 (6.7 ± 1.9)	14–81 (2.0–11.3)
Adult	21	30 ± 4 (4.2 ± 0.6)	22–38 (3.1–5.3)

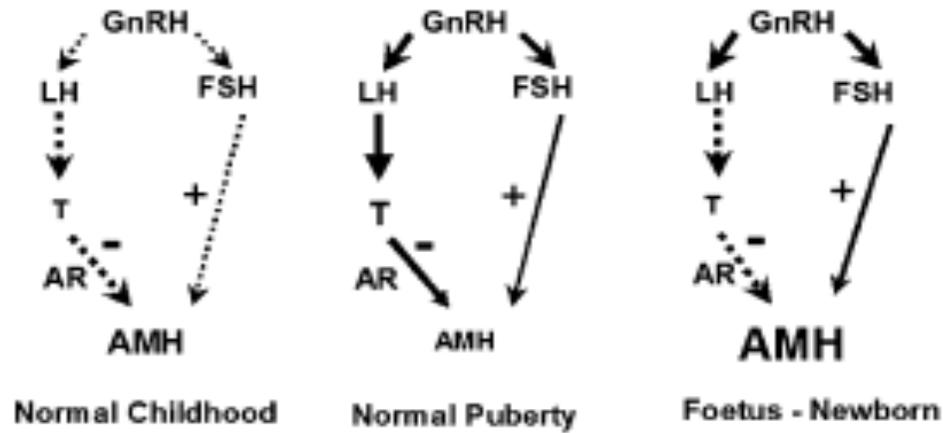
^a Between parentheses, values are also given in ng/mL to allow comparisons with previously reported data. Only pmol/L units will be used throughout this work.

^b 95% confidence interval.

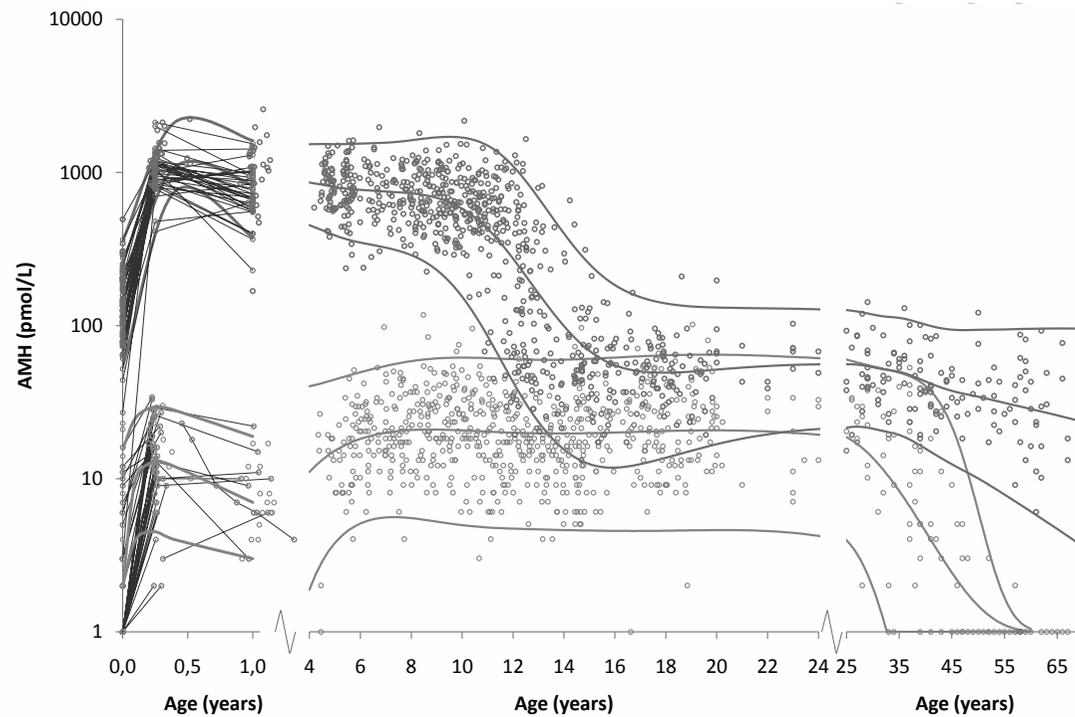
^c Pubertal stages according to Marshall and Tanner (27).

Rey et al.1999

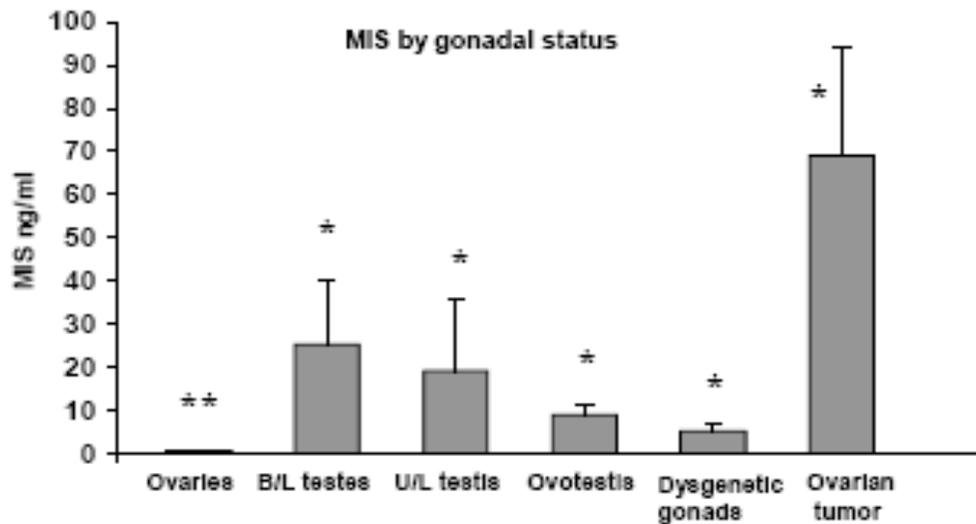
REGULATION DE LA SECRETION AMH



Figure



- Permet de confirmer la présence de testicule fonctionnel chez l'enfant.
- Résidu testiculaire
- Disorders of sex development (DSD): détection de la présence de cellules de Sertoli



Sex chromosome DSD	46,XY DSD	46,XX DSD
(A) 45,X (Turner syndrome and variants)	(A) Disorders of gonadal (testicular) development 1. Complete gonadal dysgenesis (Swyer syndrome)	(A) Disorders of gonadal (ovarian) development 1. Ovotesticular DSD 2. Testicular DSD (eg, SRY+, dup SOX9) 3. Gonadal dysgenesis
(B) 47,XXY (Klinefelter syndrome and variants)	2. Partial gonadal dysgenesis 3. Gonadal regression 4. Ovotesticular DSD	
(C) 45,X/46,XY (mixed gonadal dysgenesis, ovotesticular DSD)	(B) Disorders in androgen synthesis or action 1. Androgen biosynthesis defect (eg, 17-hydroxysteroid dehydrogenase deficiency, 5 α reductase deficiency, StAR mutations) 2. Defect in androgen action (eg, CAIS, PAIS) 3. LH receptor defects (eg, Leydig cell hypoplasia, aplasia) 4. Disorders of AMH and AMH receptor (persistent mullerian duct syndrome)	(B) Androgen excess 1. Fetal (eg, 21-hydroxylase deficiency, 11-hydroxylase deficiency) 2. Fetoplacental (aromatase deficiency, POR) 3. Maternal (luteoma, exogenous, etc)
(D) 46,XX/46,XY (chimeric, ovotesticular DSD)	(C) Other (eg, severe hypospadias, cloacal extrophy)	(C) Other (eg, cloacal extrophy, vaginal atresia, MURCS, other syndromes)

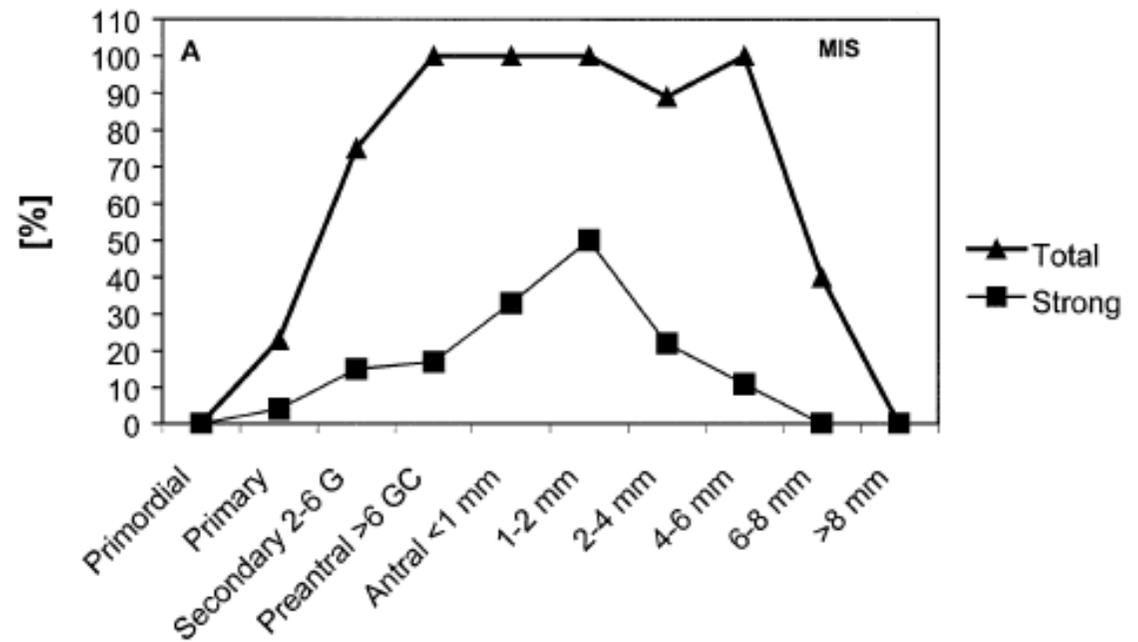
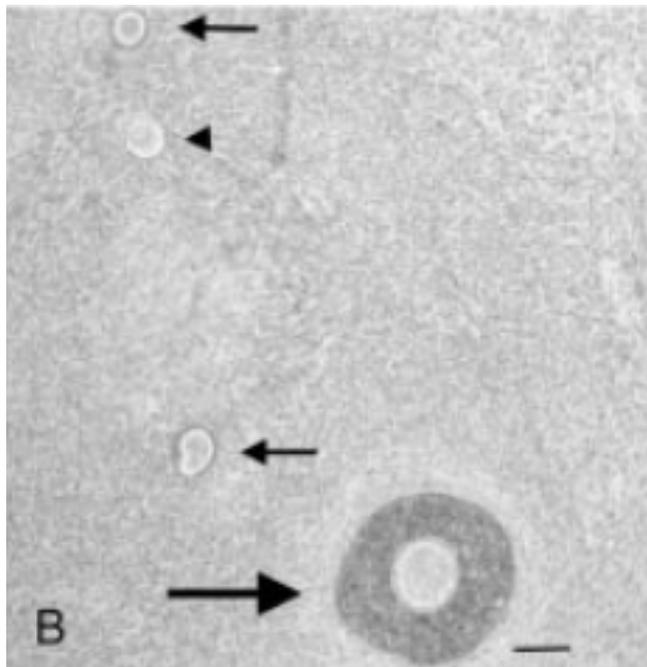
Consensus statement, Arch. Dis Child.2006

➔ Dosage combiné de la testostérone et de l'AMH

IMPORTANCE DE DETECTER LES CELLULES DE SERTOLI

	Germ cells
Grady et al <i>Urology</i> 1998	0/14
Yamazaki <i>Int J Urol</i> 2009	0/3
Merry <i>Eur Urol</i> 1997	4/47
Godbole <i>Br J Surg</i> 1997	0/18
De Luna <i>J Urol</i> 2003	4/71
Rozanski <i>J Urol</i> 1996	5/50
Storm <i>J Urol</i> 2007	8/56
Renzuli <i>J Urol</i> 2005	8/110
Hegarty <i>J Pediat Surg</i> 2007	0/117
Cendron <i>J Urol</i> 1998	0/29
Emir <i>Pediat Surg Int</i> 2007	2/44
Turek <i>J Urol</i> 1994	7/110
TOTAL	38/669 (5,6%)

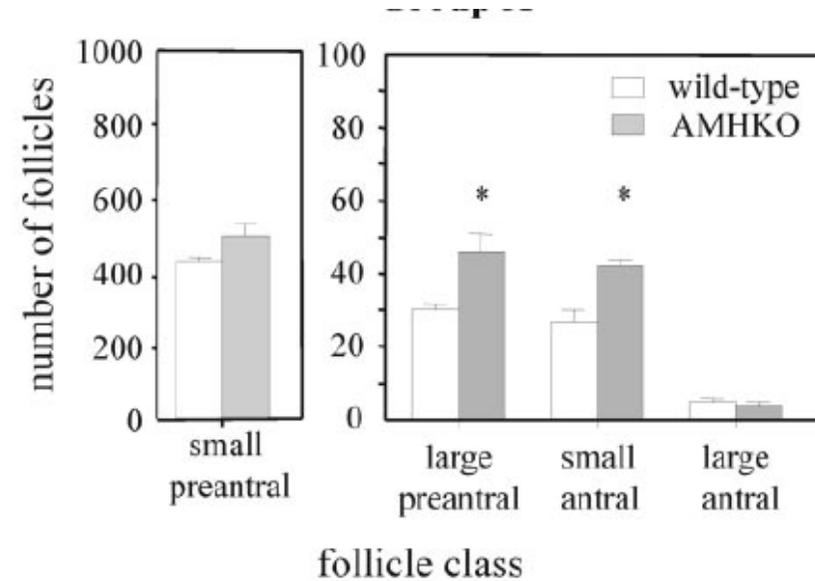
- Sécrétée par les cellules de la granulosa à partir de la 36^{ème} semaine in utero et jusqu'à la ménopause



Weenen et al, 2004



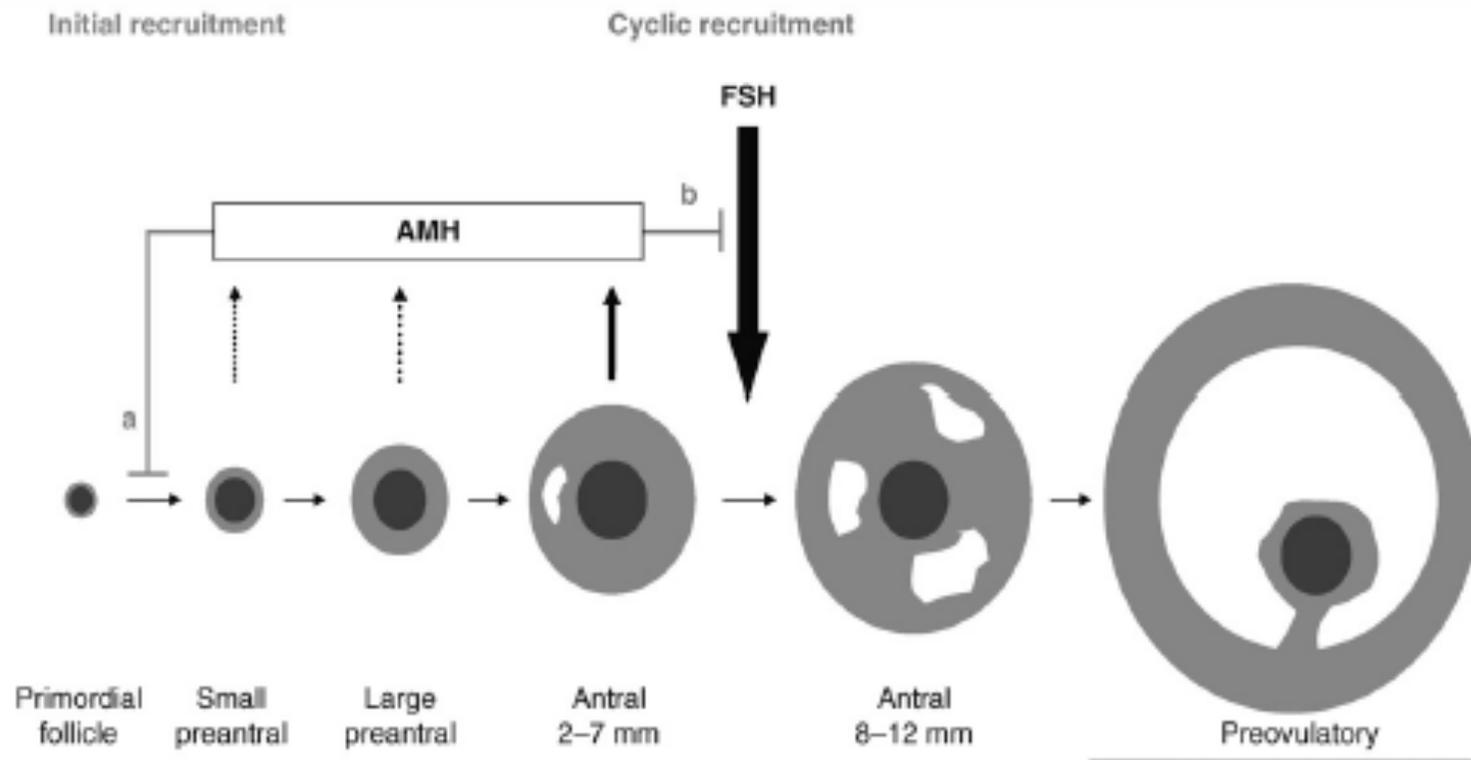
Fertiles mais...



- ↑ follicules en phase de croissance
- ➔ **pool de follicules primordiaux plus rapidement épuisé**
- ↑ développement de follicules qd apport exogène de FSH
- ➔ **Follicules + sensibles à la FSH**

Durlinger et al, 2001

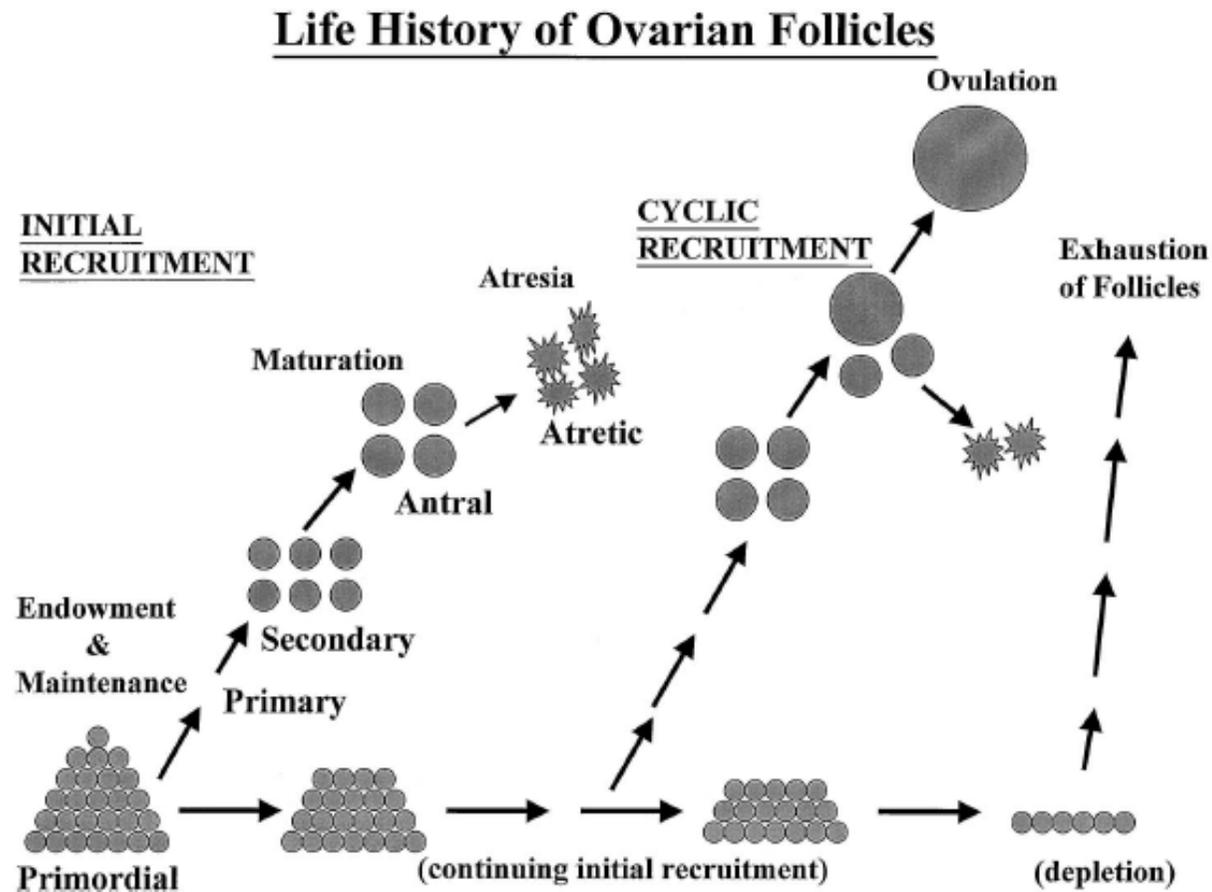
AMH ET FOLLICULOGENESE



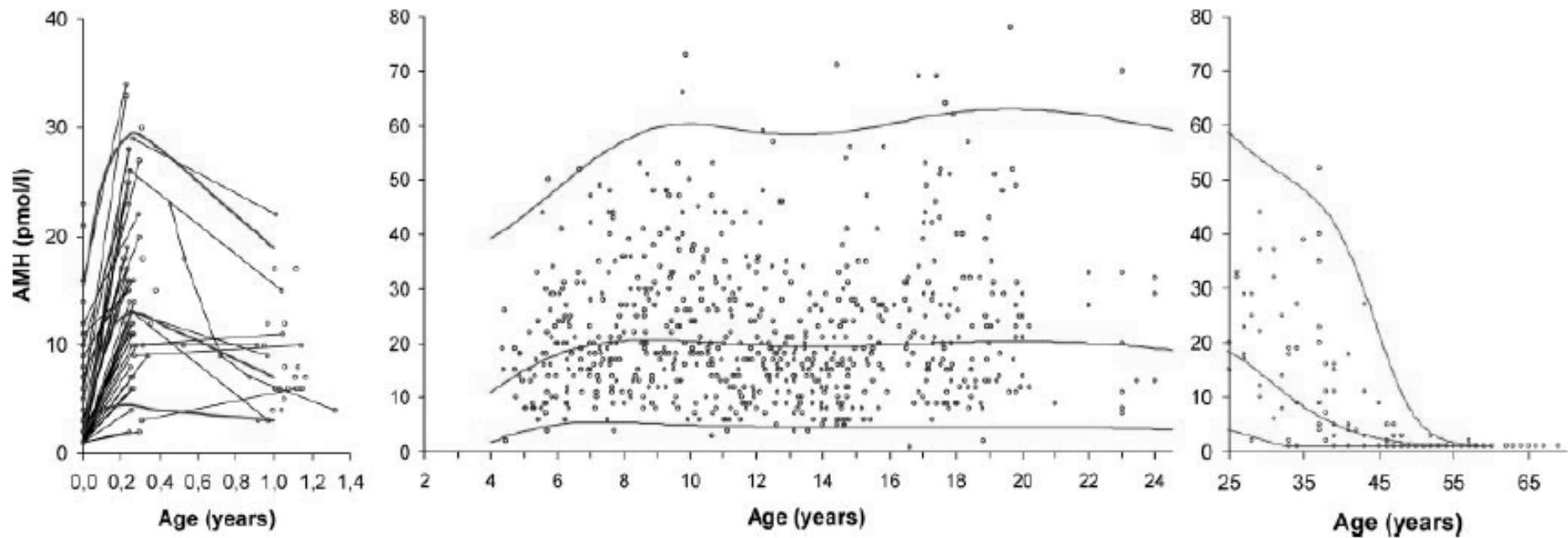
Broeckmans et al. 2008

REFLET DE LA RÉSERVE OVARIENNE

Reflet de la réserve ovarienne et de la durée de vie reproductive
Corréler avec le taux de recrutement folliculaire et détermine la réponse à la FSH exogène



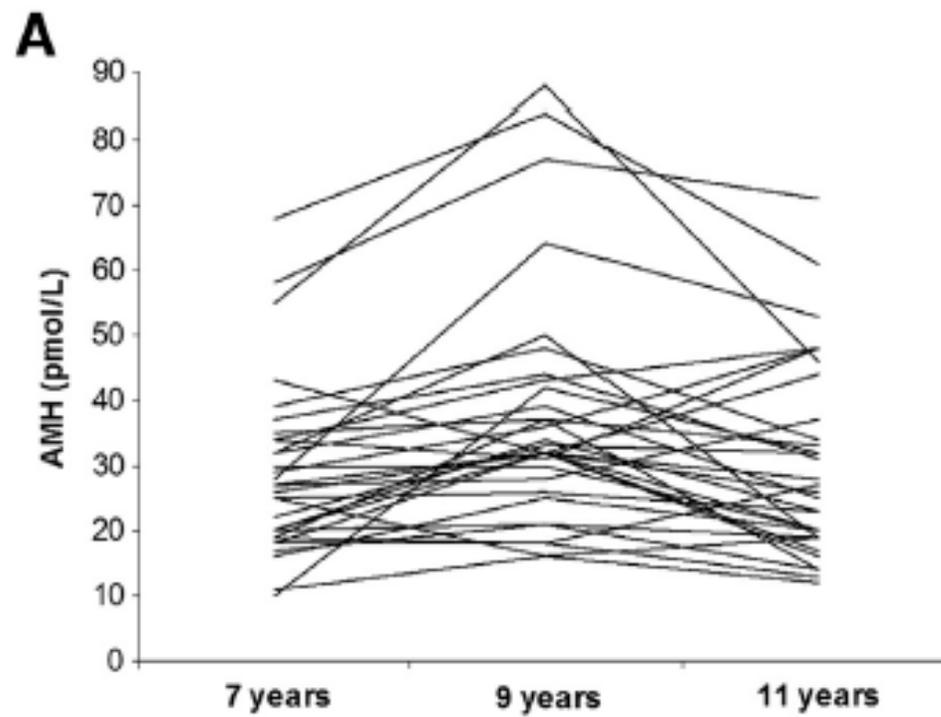
EVOLUTION DES TAUX D'AMH CHEZ LA FEMME



Hagen et al, 2011

AMH CHEZ LES JEUNES FILLES

Diminution d'environ 20% du taux d'AMH entre 9 et 11 ans



Lashen et al. 2013

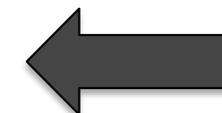
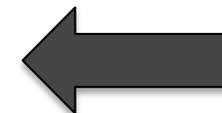
- Poids de naissance: AMH plus élevée chez les enfants de faible/ important poids de naissance?
- Ménarche précoce?
- Grossesse et nbre d'enfants
- Life-style
- PCO- et famille au premier degré (Sir-Petermann et al. 2006)
- Cycle menstruel (Overbeek et al. 2012)
- Pas d'association clairement démontrée avec le risque cardiovasculaire sur une population de 1308 adolescentes (Anderson et al. 2013)

LIFE-STYLE (DOLLEMAN ET AL, 2013)

N=2320

Table 2. Number of Percentiles that AMH Shifts in the Presence of a Specific Reproductive Determinant

Determinants	Linear Regression		P Value
	Percentiles of Change	Standard Error	
Current reproductive status			
Regular cycle	Reference		
Irregular cycle	-11.0	2.2	<.0001
Current OC use	-11.0	1.3	<.0001
Current pregnancy	-16.6	4.1	<.0001
History of OC use			
Never	Reference		
Previous	-0.3	2.3	.88
Current	-9.0	2.3	<.0001
Years of OC use in current or past users (n = 1996)			
<1 y	Reference		
1-5 y	2.6	3.1	.39
5-10 y	-2.2	3.0	.46
10-15 y	-2.6	3.2	.42
15-20 y	-3.9	3.5	.26
>20 y	-8.8	4.2	.04
Age at menarche	0.7	0.4	.09
Parity	2.1	0.9	.02
Age at birth of first child	-0.2	0.2	.39



The association between reproductive determinants and age-specific AMH is quantified as the number of percentiles that AMH shifts in the presence of a specific determinant. Corresponding standard error and P value are shown.

Median (p50) AMH values in subgroups

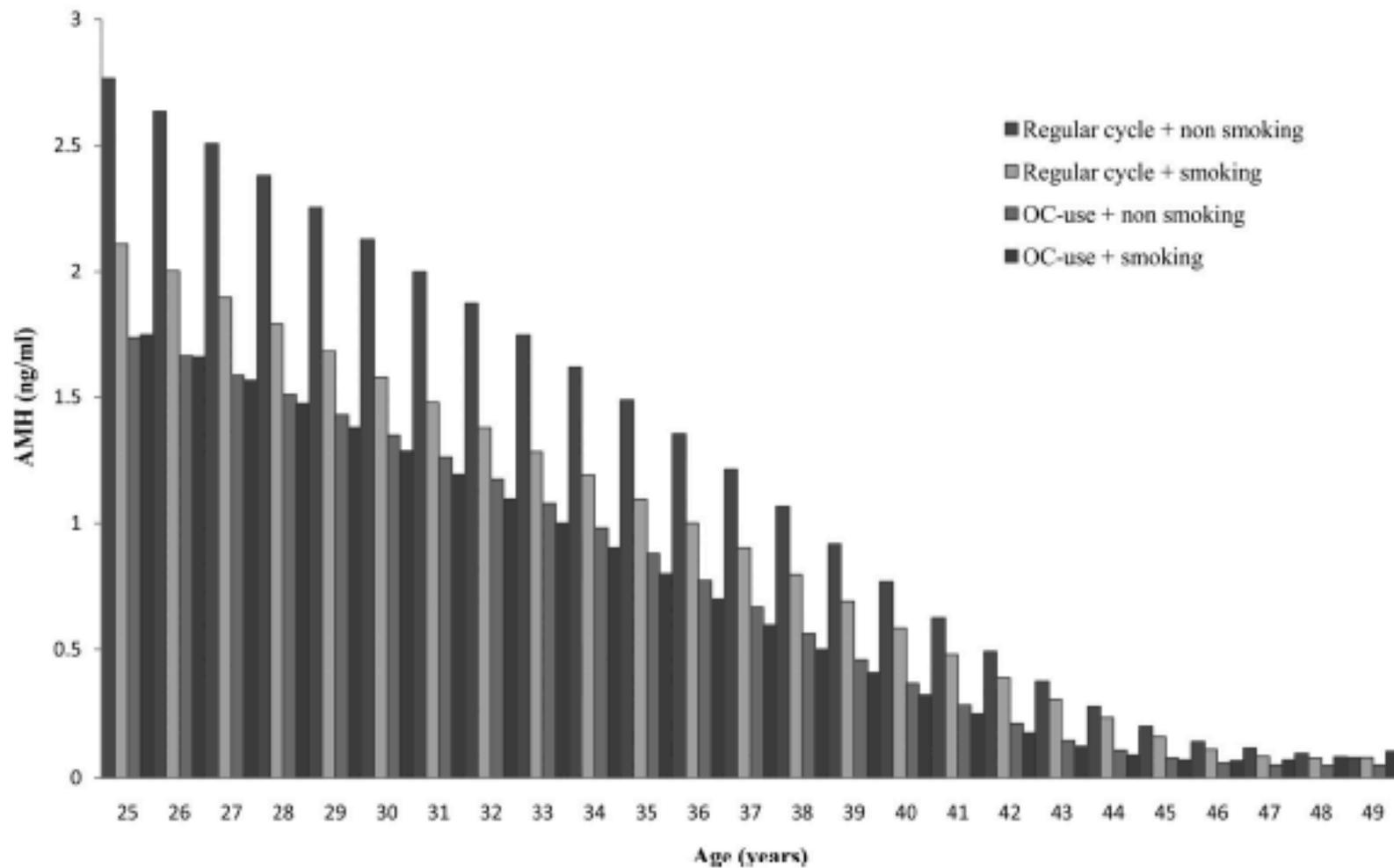
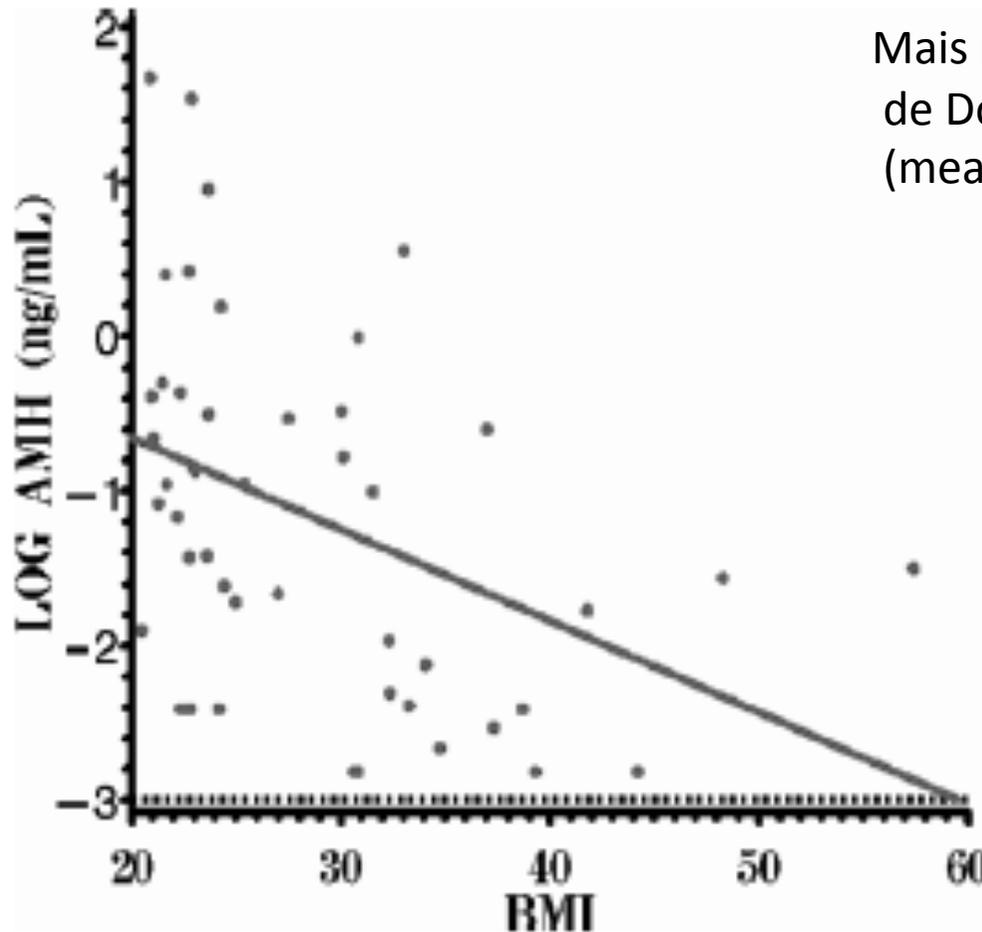


Figure 2. Bar graph showing mean (p50) AMH values with increasing age according to the CG-LMS model for women in 4 subgroups.

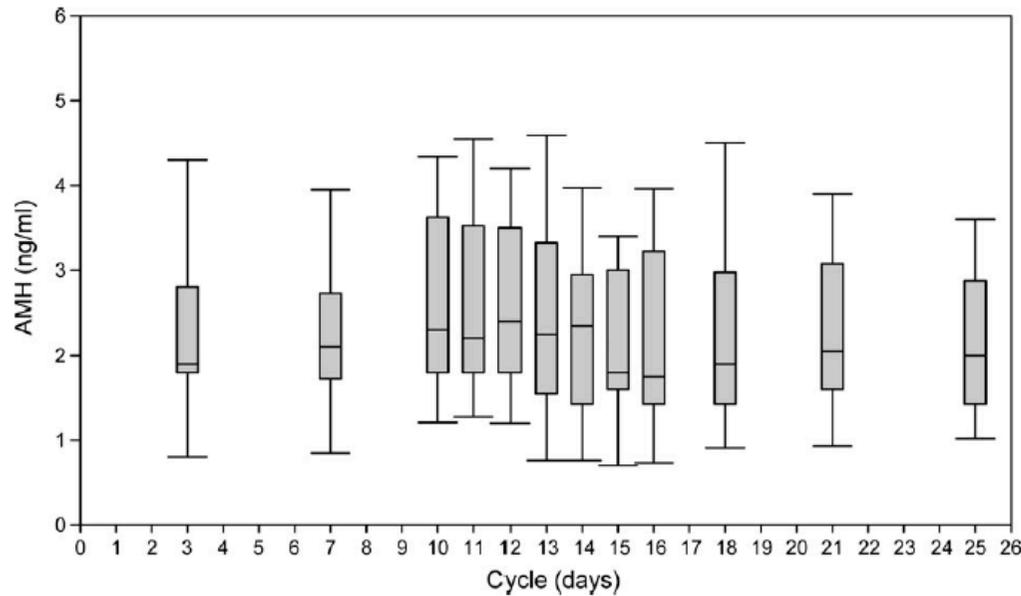
CORRELATION AVEC LE BMI



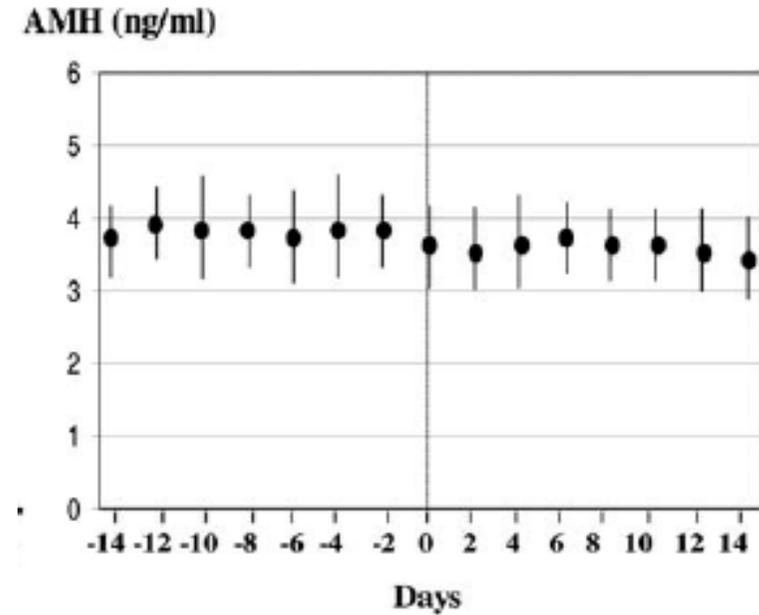
Mais non confirmé par l'étude de Dolleman sur 2320 patients (mean BMI 24.3)...

Freeman et al, 2006

- Stabilité au cours du cycle menstruel



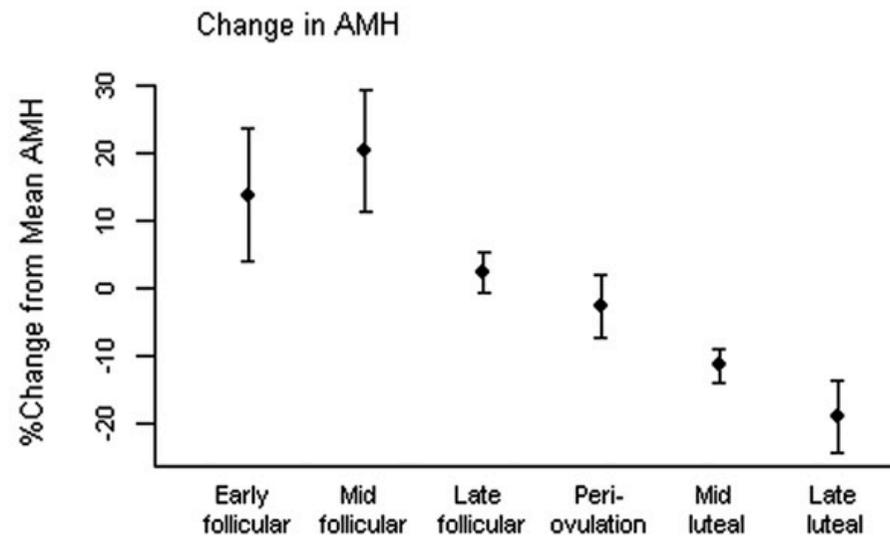
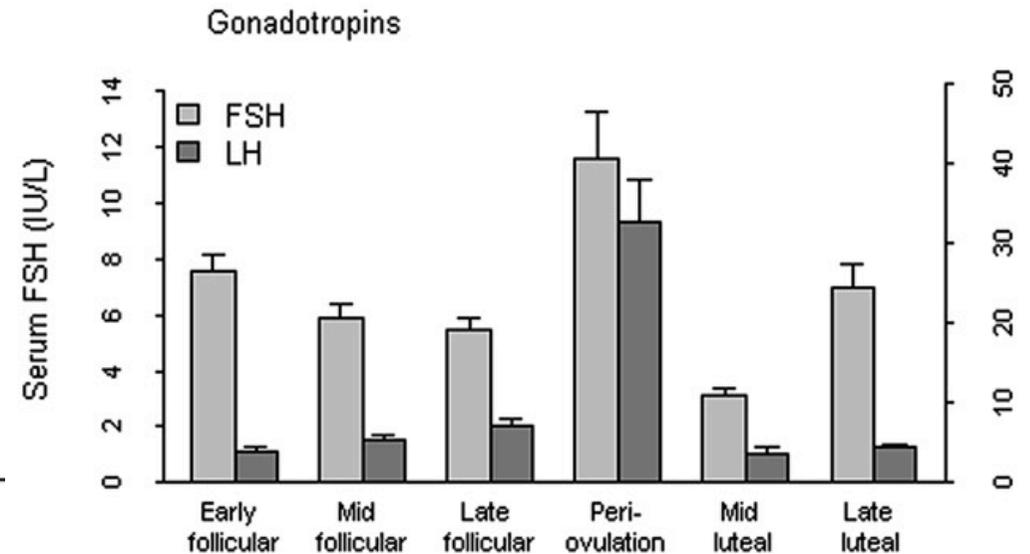
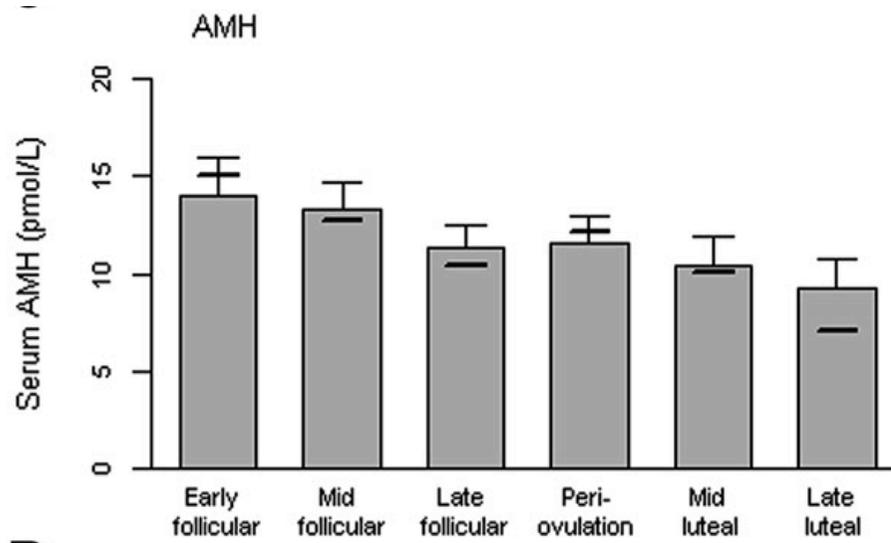
Tsepelidis et al. 2007



La Marca et al. 2006a

VARIATION INTRA-CYCLE

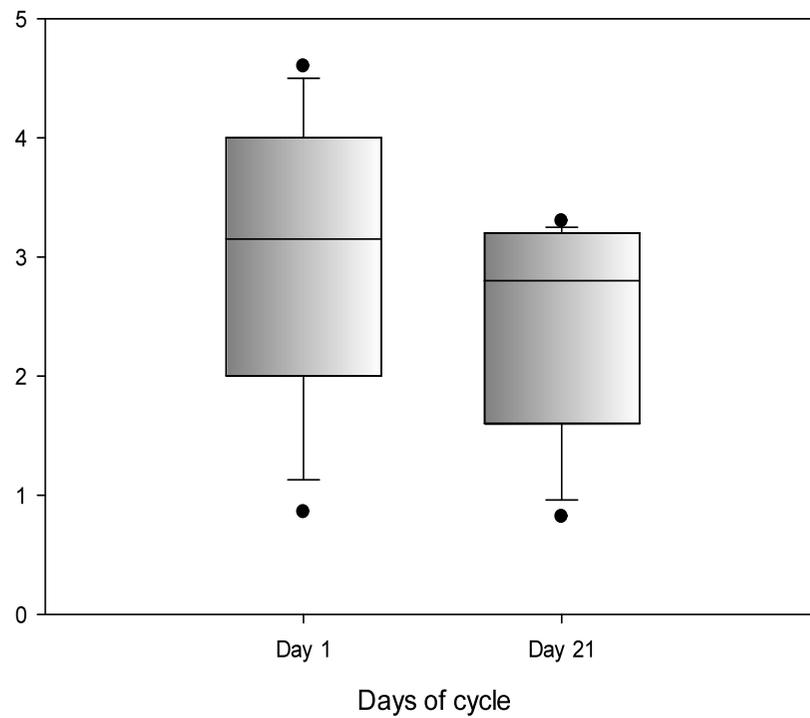
N=12 – AMH>6.4pmol/L



Hadlow et al, 2013

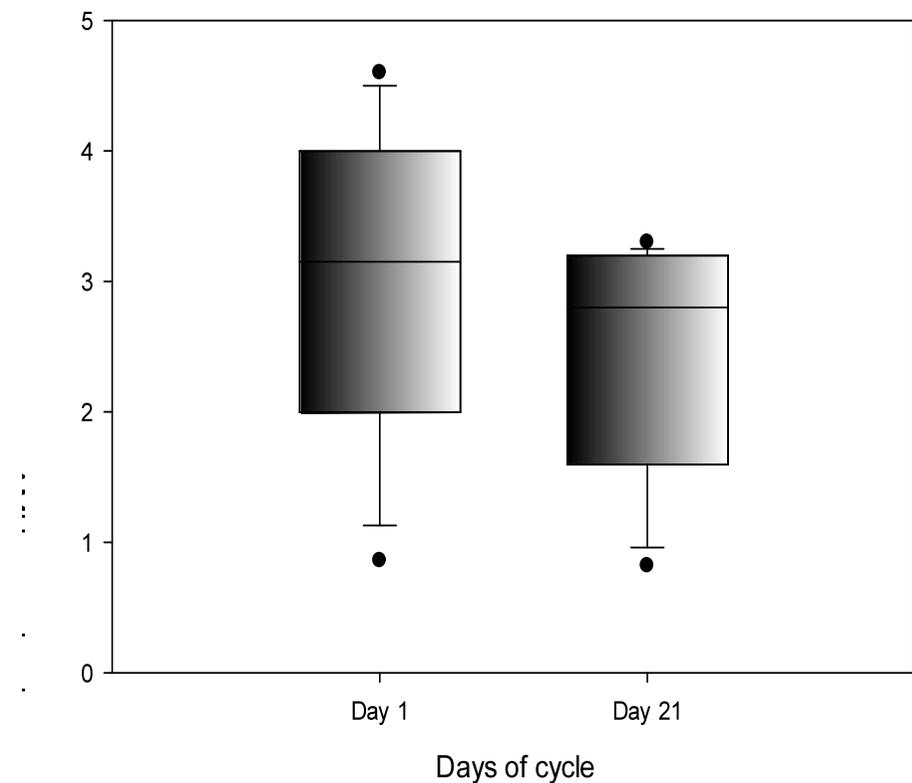
VARIATION INTRA-CYCLE SOUS COC

AMH serum levels during contraception (20gamma)



 Plot 1

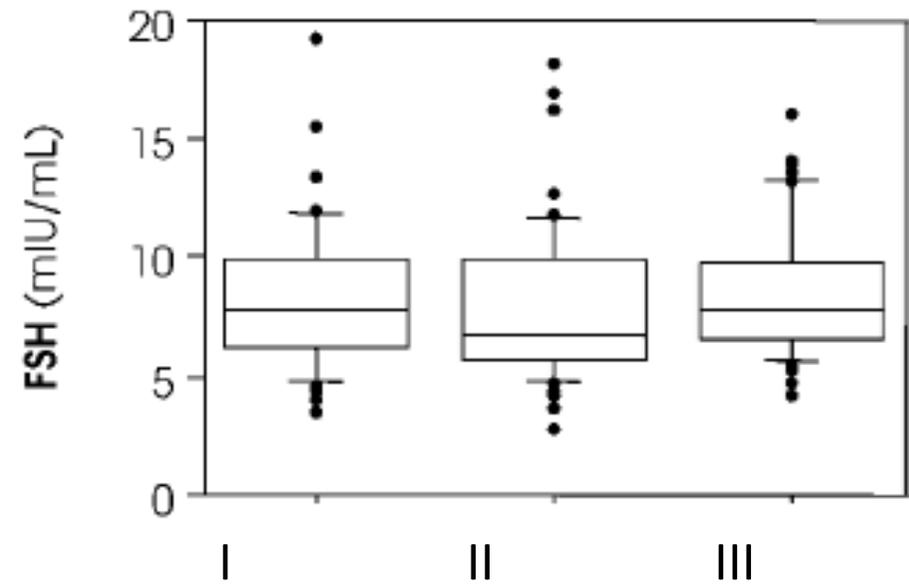
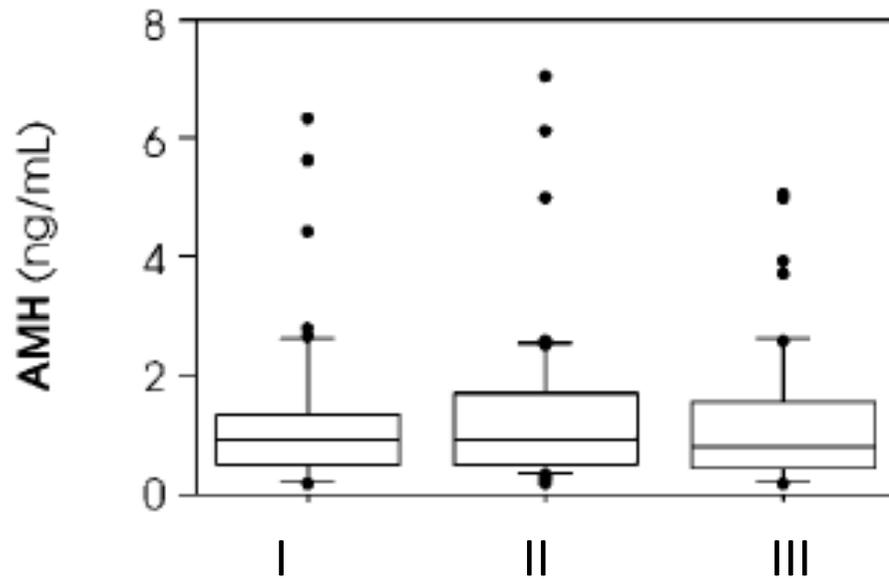
AMH serum levels during contraception (30gamma)



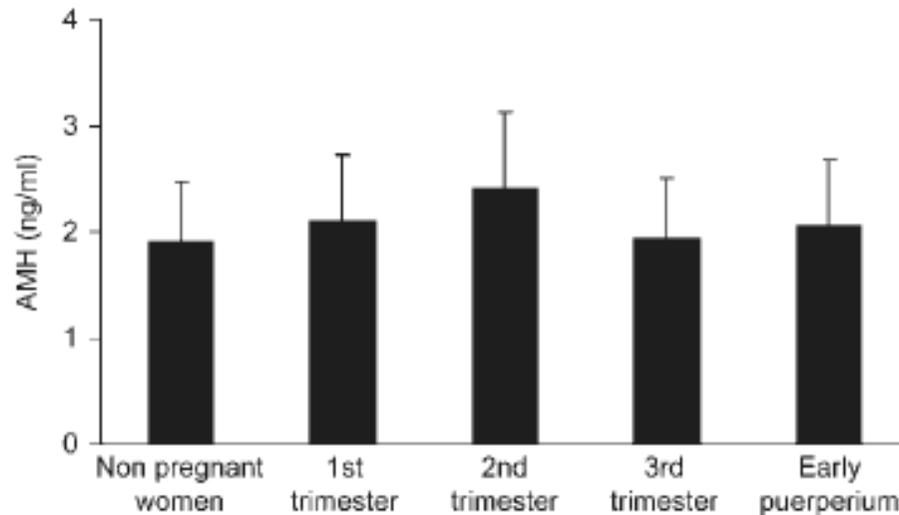
 Plot 1

VARIATION INTER-CYCLE

N=42



Fanchin et al, 2005



La Marca et al. 2005

TABLE 1

Longitudinal profile of metabolic markers.

	T1	T2	T3	Postnatal	<i>P</i> ^a	<i>P</i> ^b
Gestation (wk)	12.4 ± 1.5	26.1 ± 1.3	35.5 ± 1.3	17.3 ± 2.9	—	
AMH (pmol/L)	11.2 (7.6–20)	8.6 (3.6–13.0)	5.5 (2.2–9.2)	14.8 (7.7–26)	<.0001	<.0001

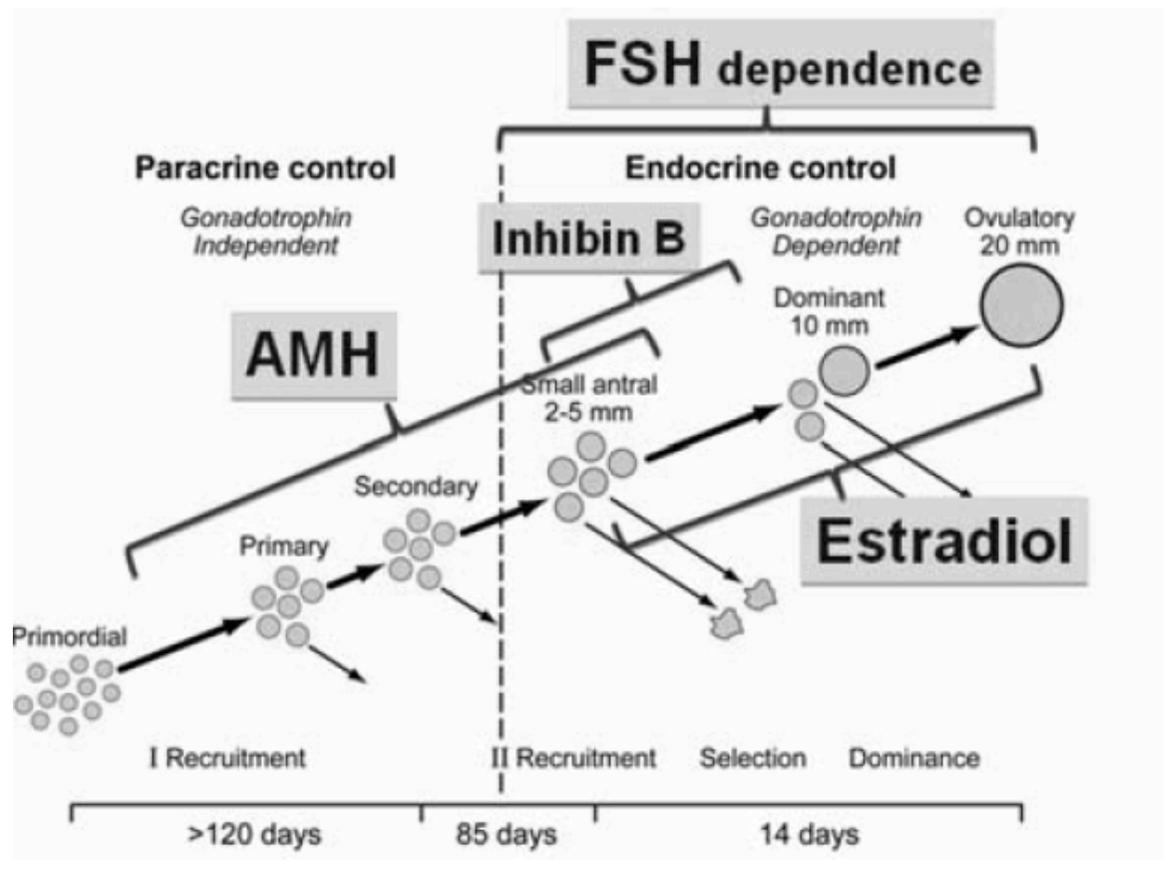
Nelson et al. 2009

- Evaluation de la réserve ovarienne (risque génétique, post-chimiothérapie...)
- Prédiction de la réponse à la stimulation
- Prédiction du risque d'OHSS
- Diagnostic et suivi des tumeurs de la granulosa (76 à 93% des patientes)

PAS PREDICTIF D'UNE GROSSESSE SPONTANÉE



- FSH J3
- Oestradiol
- Inhibine B
- AFC J3
- **AMH**



La Marca 2011

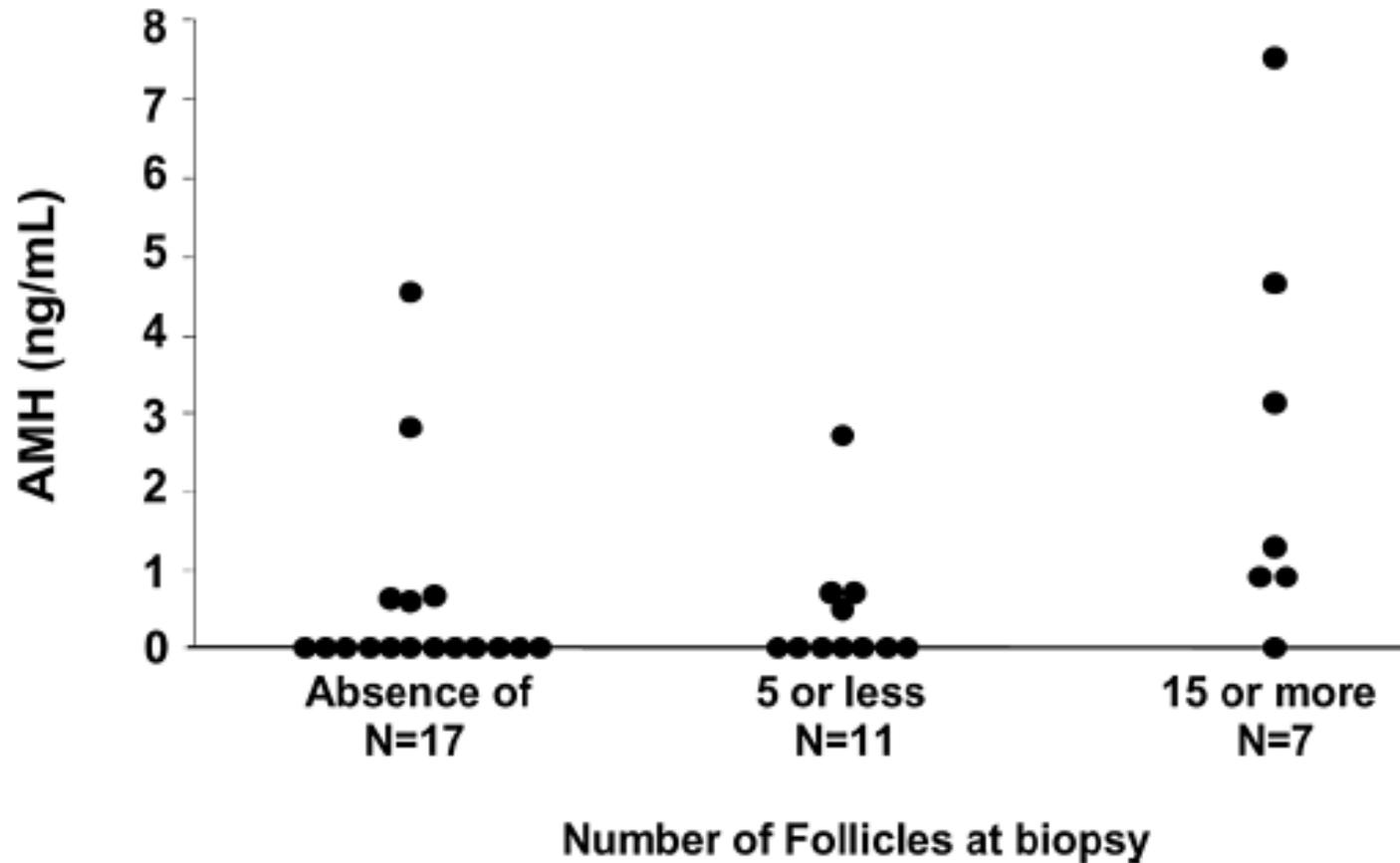
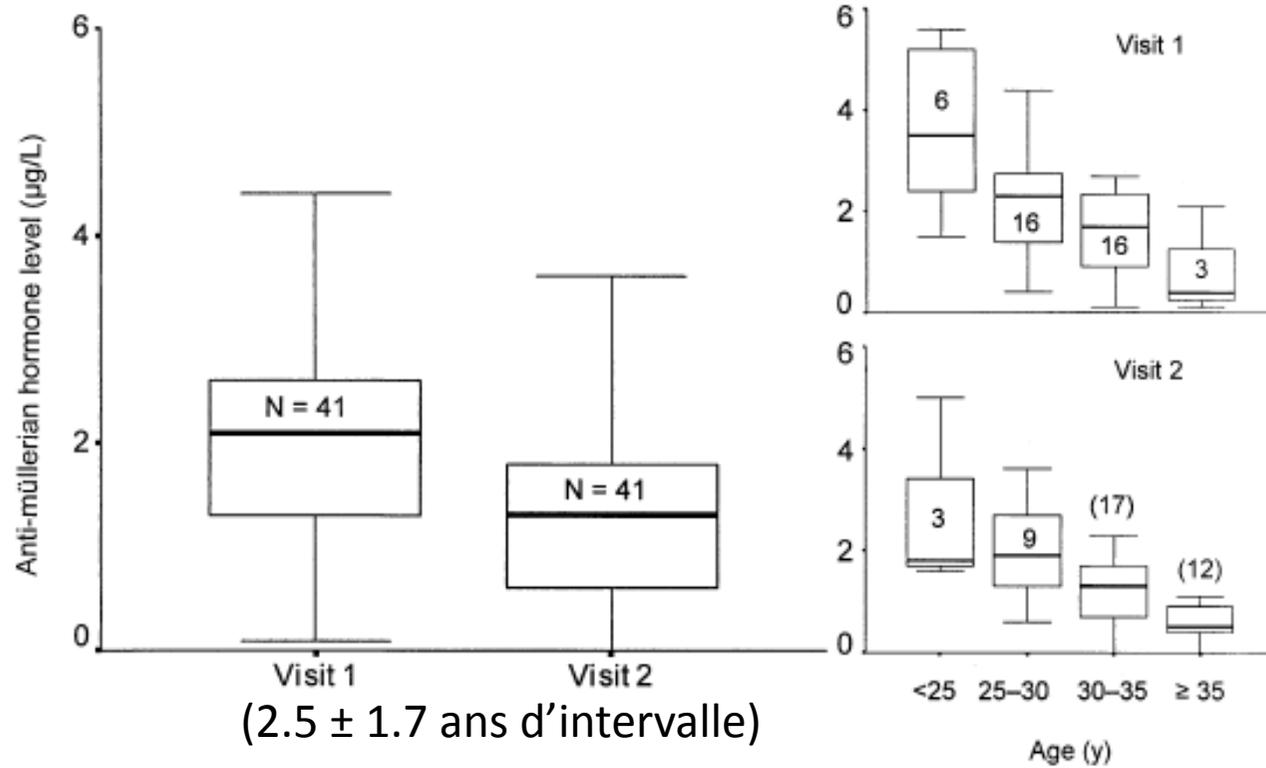


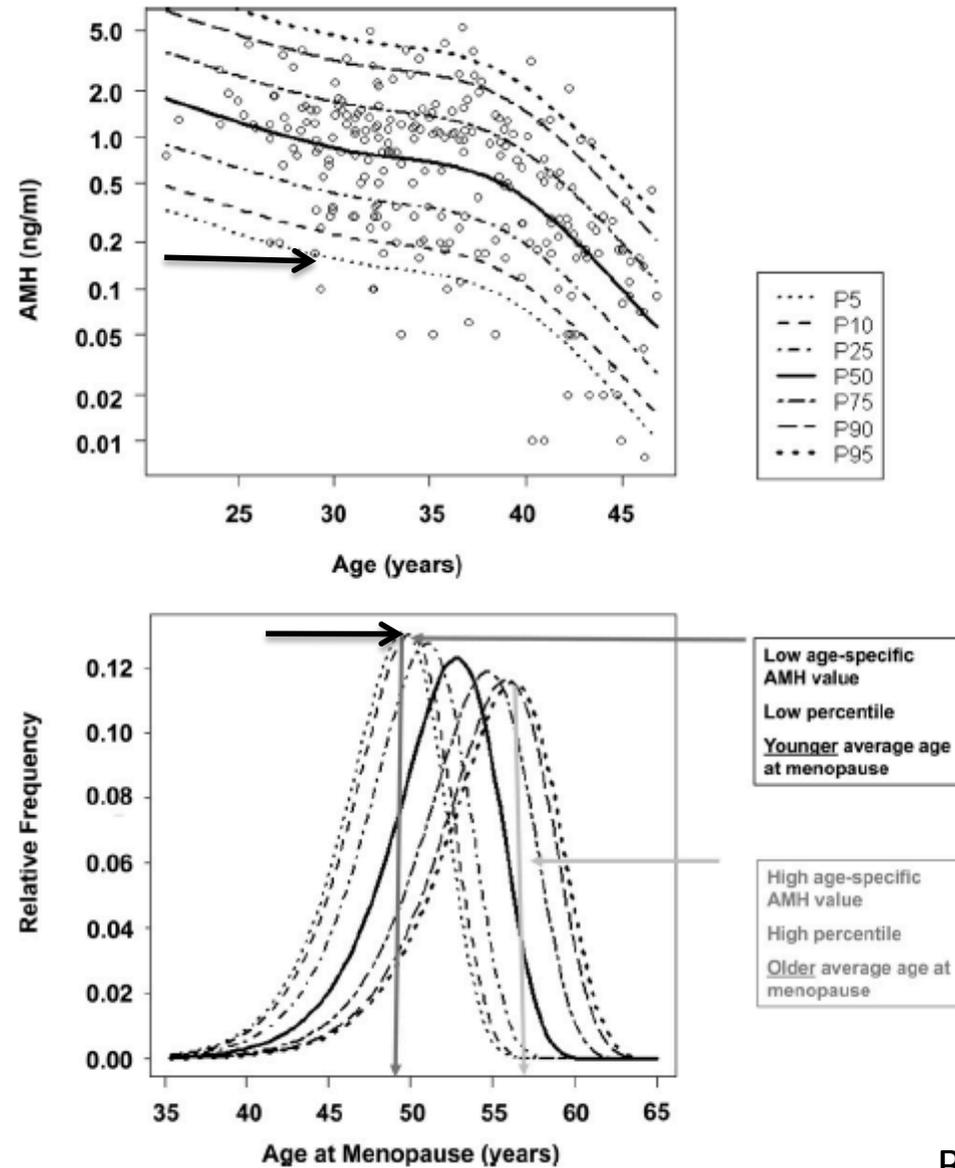
Figure 1. Serum anti-Müllerian hormone (AMH) distribution according to ovarian reserve.

Marqueur plus précis que la FSH: diminue avant modification des valeurs de la FSH

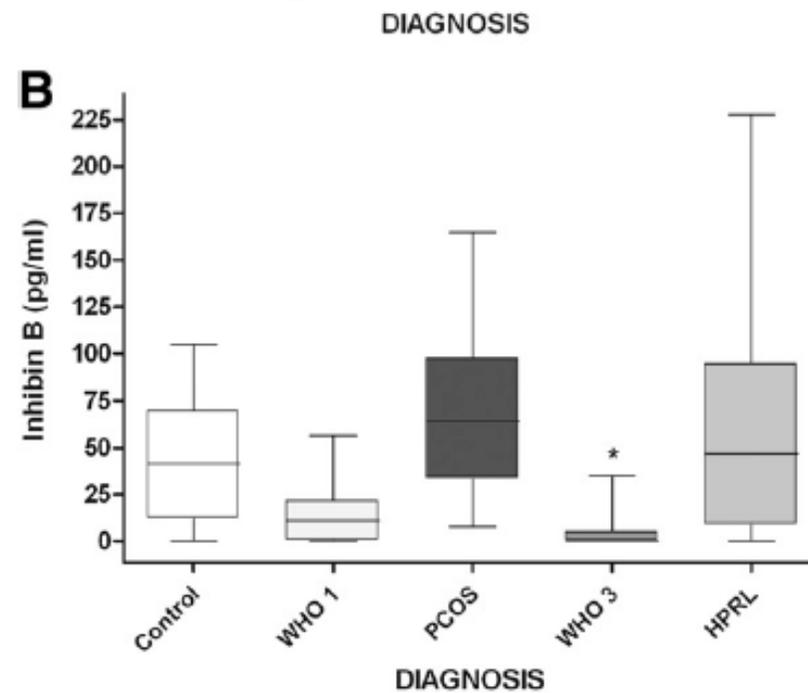
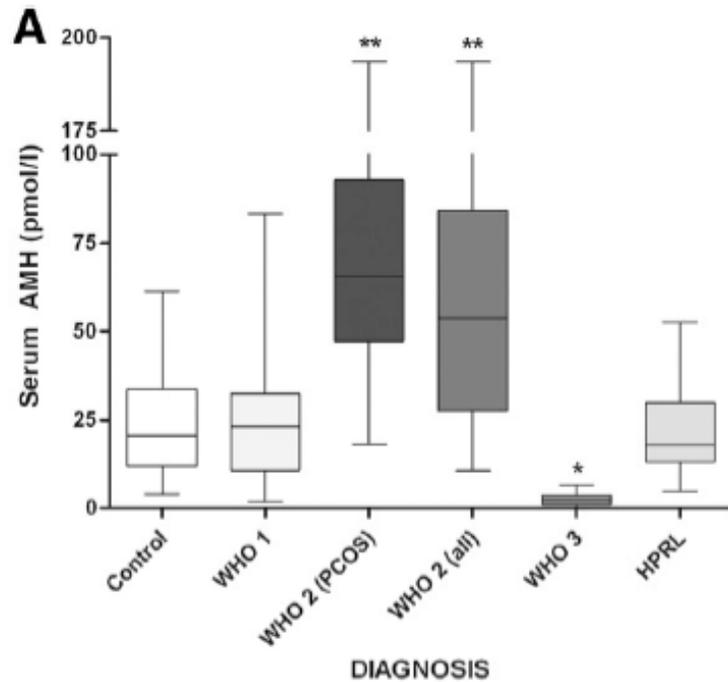


De Vet et al. 2002

PREDICTIF DE L'ÂGE DE LA MENOPAUSE?



DIAGNOSTIC DIFFÉRENTIEL DES OLIGO-AMENORRHÉE



Contrôle: normo-ovulatory
WHO1: HH (low FSH and E2)
WHO2: PCOs (normal FSH and E2)
WHO3: POF
HPRL: Hyperprolactinemia

Li, 2011

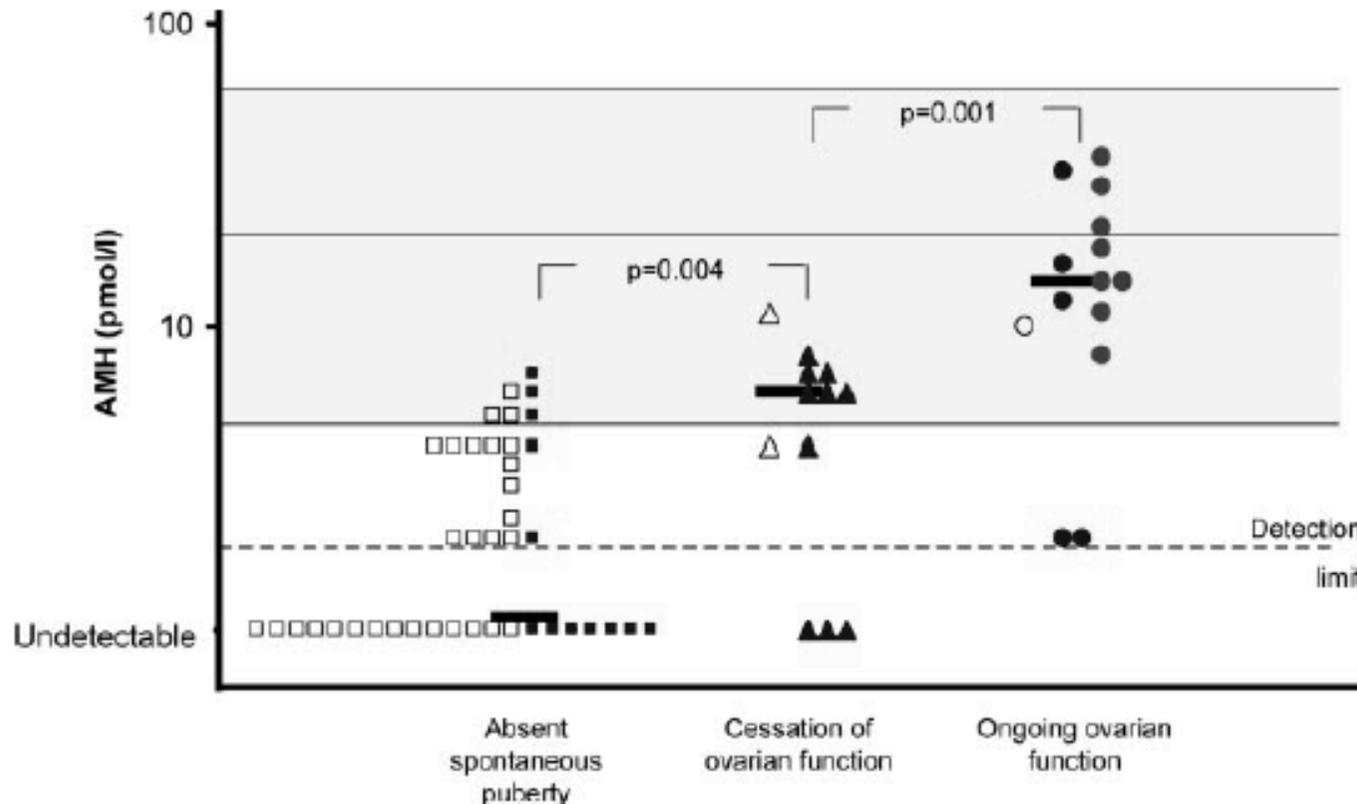
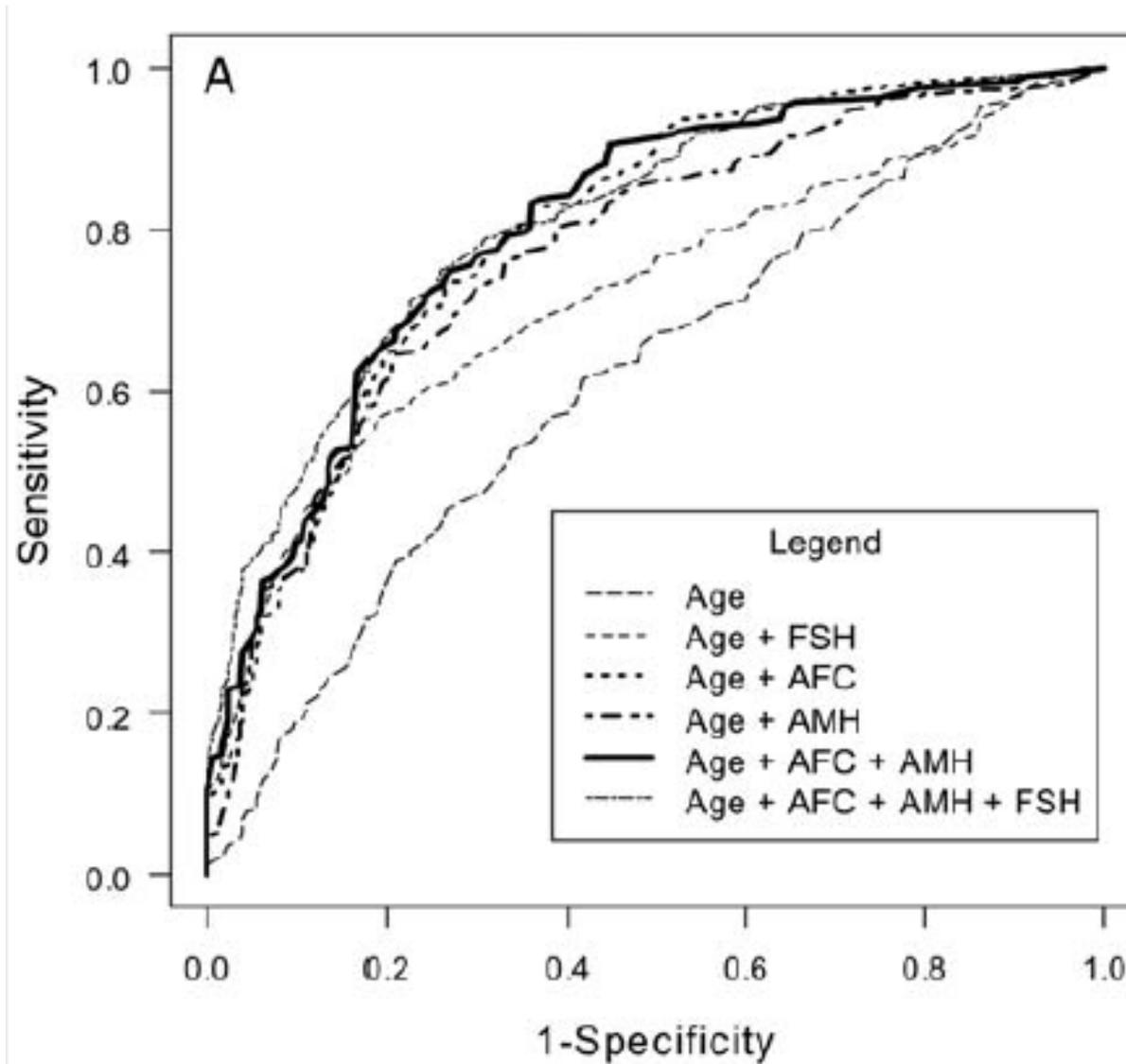


FIG. 3. AMH levels and ovarian function at time of AMH measurement in patients with TS, aged 12–25 yr. The reference range is marked by the *hatched area*, and *lines* represent the median, the 2.5th percentile, and the 97.5th percentile. *Dotted line* represents the detection limit of the assay. *Squares*, Patients with absent puberty; *triangles*, patients with cessation of ovarian function; *circles*, patients with ongoing ovarian function. *Thick black bars*, Median of AMH. *Black*, Patients with 45,X; *blue*, miscellaneous karyotypes; *red*, 45,X/46,XX.

- Corrélation entre taux d'AMH et nombre d'ovocytes récoltés à l'OPU

Parameter	≤6 oocytes (n = 28)	≥11 oocytes (n = 79)	P
Age (y)	35.2 ± 0.7	34.2 ± 0.4	NS
Day 3 FSH (IU/L)	5.9 ± 0.4	4.9 ± 0.2	.01
Day 3 E ₂ (pg/mL)	41.0 ± 3.0	32.0 ± 1.0	.003
MIS (ng/mL)	1.0 ± 0.4	2.5 ± 0.3	.006
E ₂ max (pg/mL)	1,720.0 ± 160.0	2,950.0 ± 140.0	≤.0001
No. of oocytes	4.5 ± 0.2	19.7 ± 0.9	≤.0001
% of mature oocytes	92.0 ± 2.0	90.0 ± 1.4	NS

AMH ET LA RÉPONSE À LA FIV



VALEUR CUT-OFF?

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
Peharrubia 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
Fiçicioglu et al. (2006)	50	Prosp	0.25 pg/ml	90.9	90.9	<5 oocytes	Diagnostic System Laboratories
La Marca et al. (2007)	48	Prosp	0.75 ng/ml	80	93	<4 oocytes or cancellation	Immunotech–Beckman–Coulter
Fréour et al. (2007)	69	Prosp	1.3 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
McIveen 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
Gnoth 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

Corrélation positive entre taux d'AMH et qualité ovocytaire, morpho embryon, taux implantation et naissances vivantes?

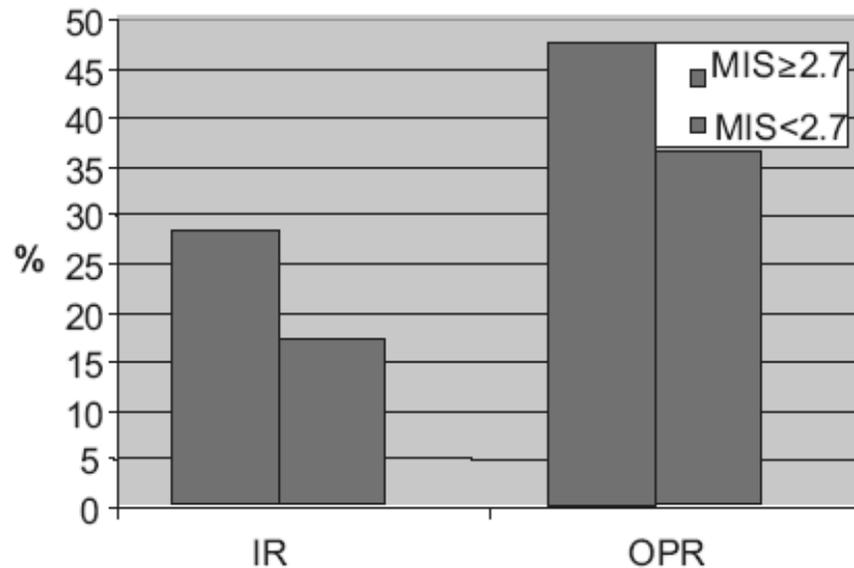
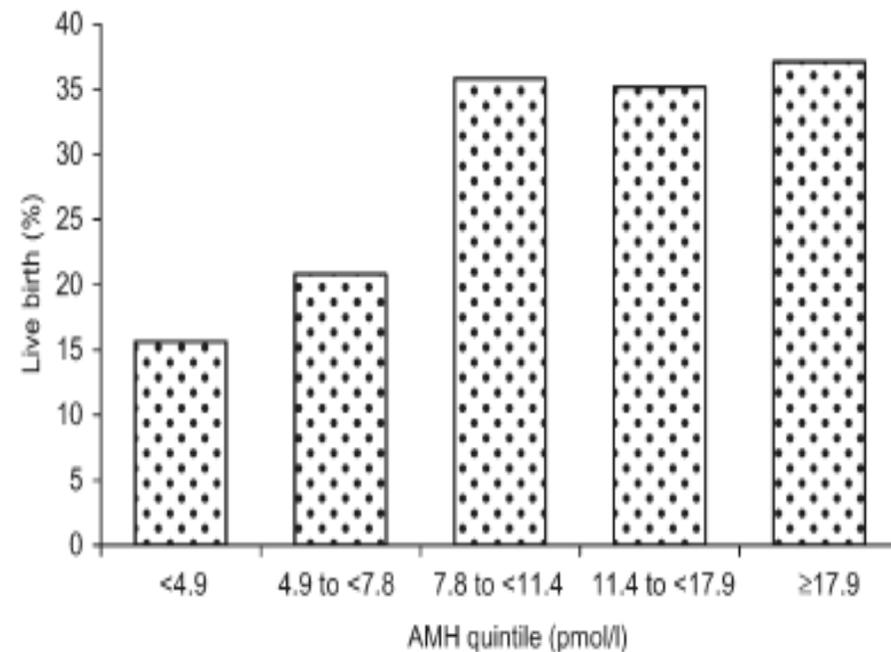


Figure 4. Implantation rate (IR) and ongoing pregnancy rate (OPR) for patients whose Müllerian inhibiting substance (MIS) values were ≥ 2.7 or < 2.7 ng/ml.

Silberstein et al. 2006



Nelson et al. 2007

OMPK ET RISQUE D'HYPERSTIMULATION

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.

^aExcessive response if >20 oocytes retrieved.

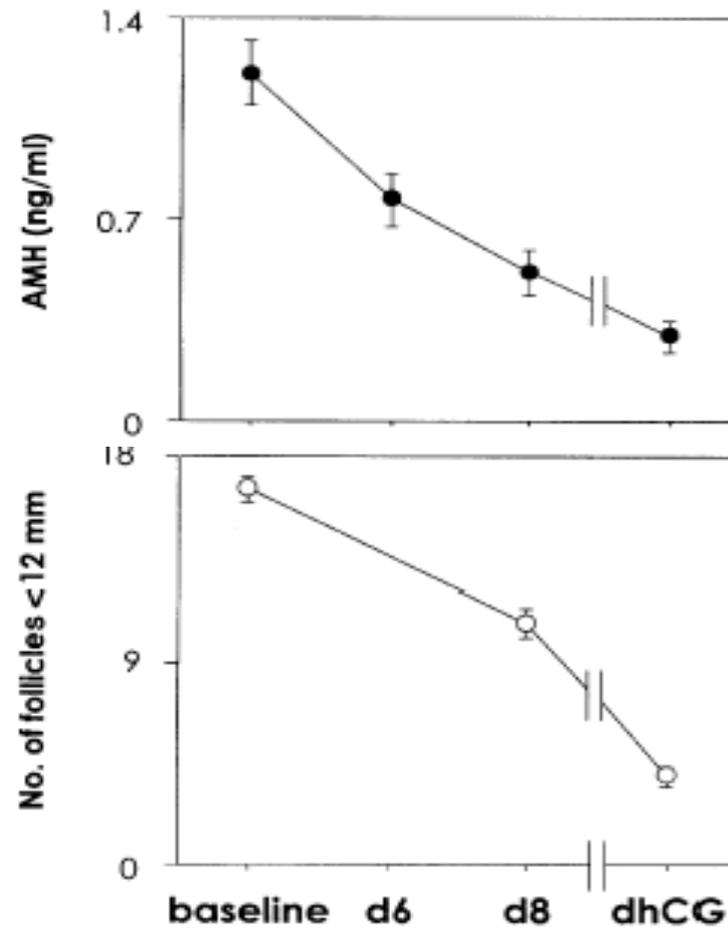
^bExcessive response if ≥ 21 oocytes retrieve.

La Marca et al, 2009

Table III Patient characteristics and controlled ovarian stimulation details relative to anti-Müllerian hormone category for Centre I

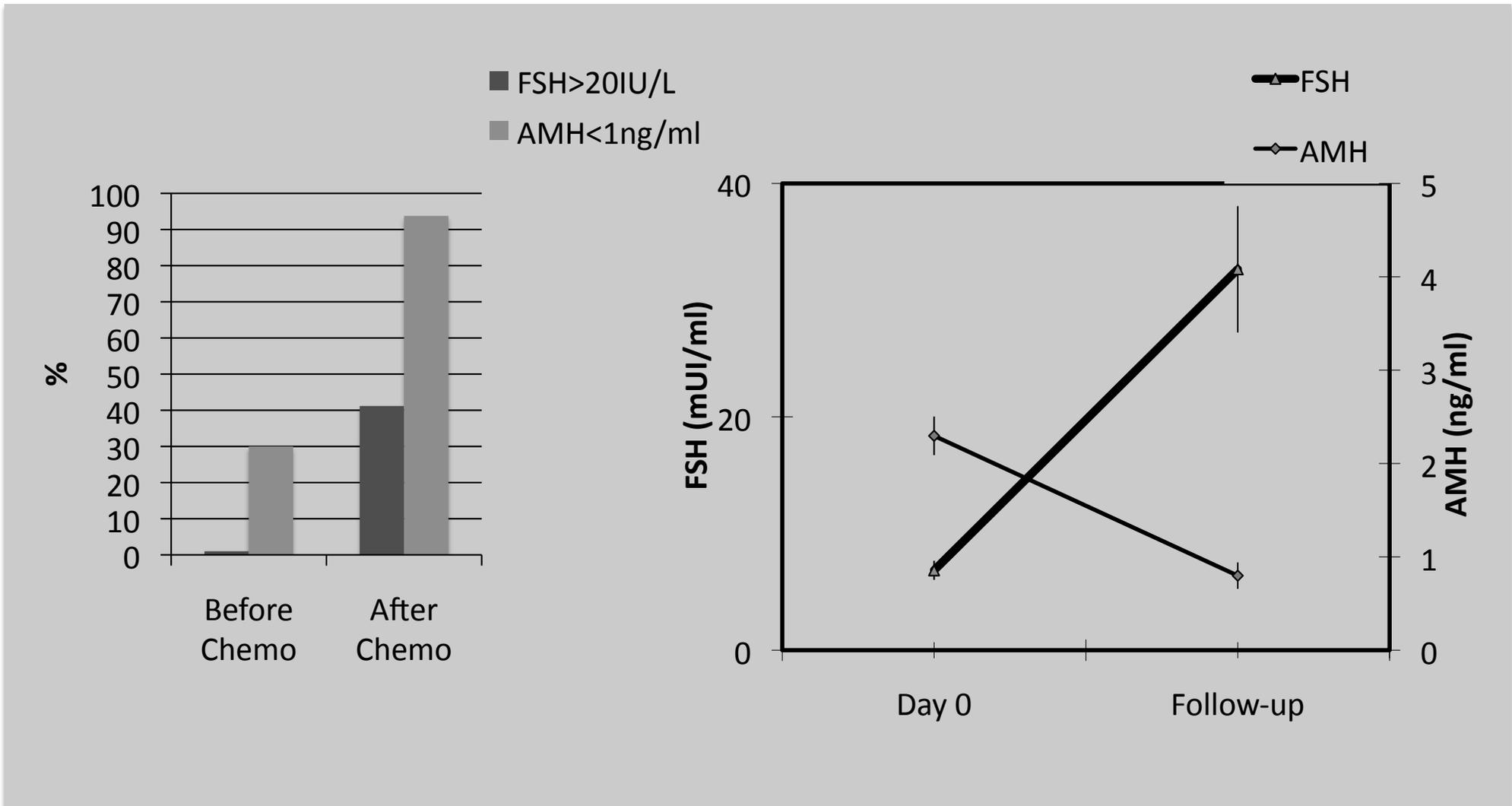
AMH category	1 to <5 pmol/l	5 to <15 pmol/l	≥ 15 pmol/l
Protocol	Agonist + 375 IU	Agonist + 225 IU	Agonist + 150 IU
Patients (n) % of cohort	74 (20%)	128 (34.6%)	148 (40%)
Age (years)	37.3 (34.6–39.3)	35.1 (32.7–37.3)	32.8 (28.8–36.2)
BMI (kg/m ²)	23.9 ± 7.5	23.8 ± 5.6	24.1 ± 5.6
AMH (median (IQR))	2.6 (1.8–3.7)	9.2 (6.8–11.9)	22.4 (18.3–29.9)
Duration of stimulation (days (IQR))	14 (13–15)	14 (13–15)	13 (12–14)
Number of oocytes collected	5 (3–7)	10 (7–15)	14 (10–19)
Number of oocytes fertilized	3 (2–4)	6 (3–9)	7 (5–11)
Low oocyte yield n (%)	7/55 (12.7%)	3 (2.3%)	4 /144 (2.8%)
Freeze all n (%)	1 (1.4%)	13 (10.1%)	27 (18.2%)
Hospitalized for OHSS	0 (0%)	3 (2%)	20 (13.9%)
Cancelled cycle n (%)	19 (25.7%)	3 (2.3%)	4 (2.7%)
Clinical pregnancy per cycle n (%)	6 (8.1%)	29/125 (23.2%)	47 (31.8%)
Clinical pregnancy per OR n (%)	6/55 (10.9%)	29/112 (25.9%)	47/144 (32.6%)
Clinical pregnancy per embryo transfer n (%)	6/54 (11.1%)	128 (34.6%)	47/117 (40.1%)

INFLUENCE DES GONADOTROPHINES



Fanchin et al. 2003

SUIVI DES PATIENTES À RISQUE DE IOP



Etude prospective longitudinale (5 ans) évaluant la fonction ovarienne des patientes traitées pour cancer du sein (N=42).

Valeur prédictive de l'AMH

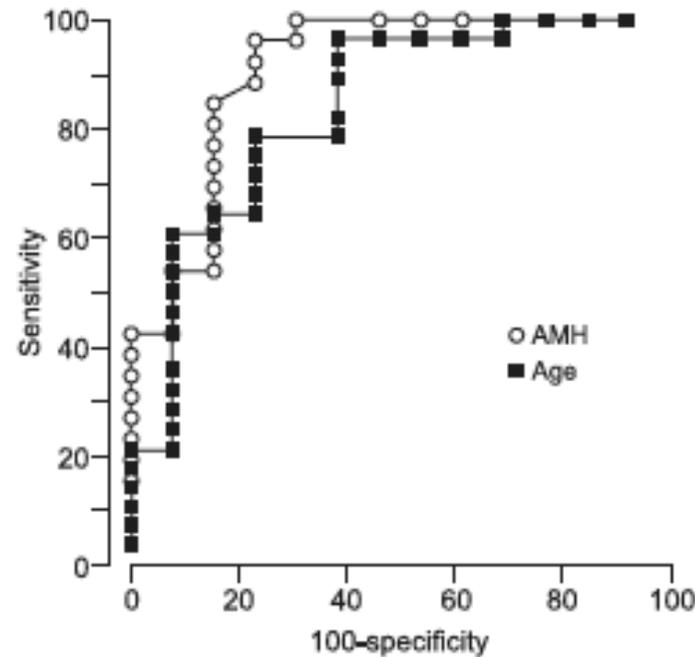


FIG. 3. Receiver operating curve analysis of amenorrhea against pretreatment AMH (○) and age (■). AUC for AMH = 0.91 (95% CI = 0.80–1.01; $P < 0.001$); AUC for age = 0.84 (95% CI = 0.80–0.98; $P = 0.001$).

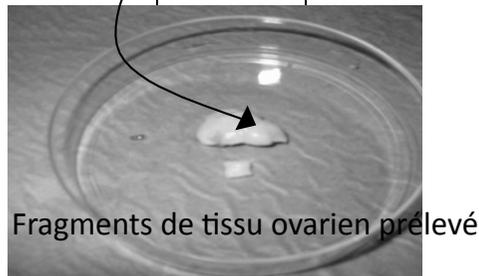
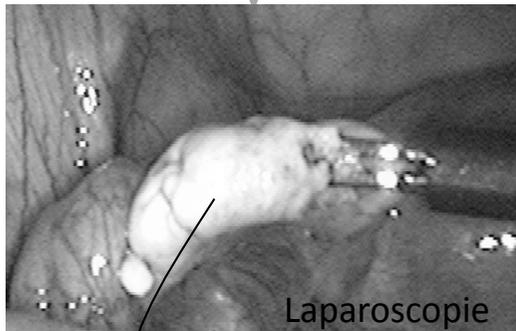
Anderson 2011

SUIVI DE TRANSPLANTATION DE TISSU OVARIEN

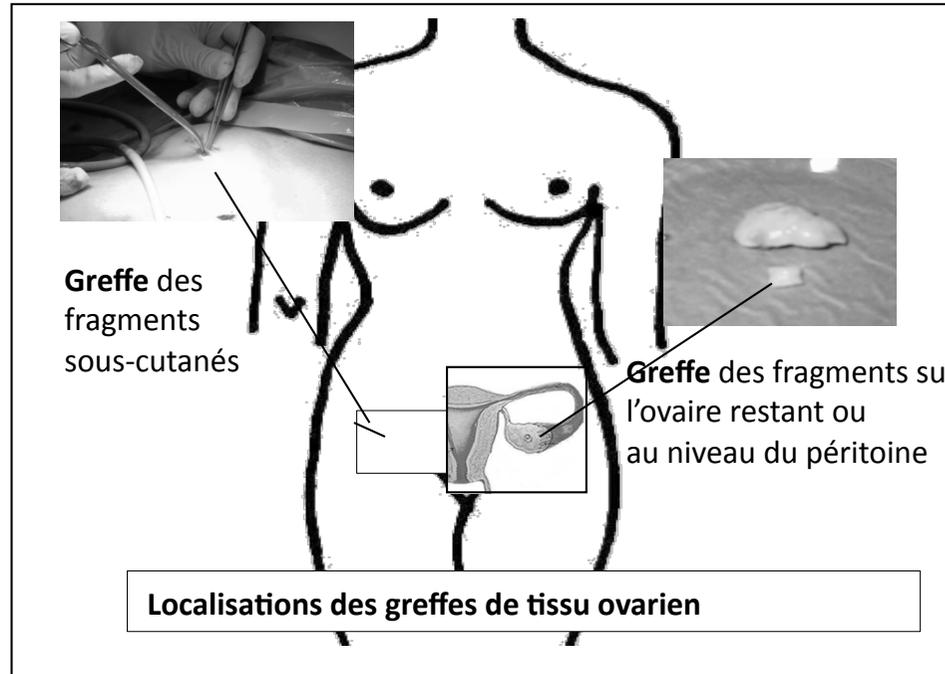
Congélation et greffe des tissus ovarien

1. Diagnostic du cancer

2. Prélèvement de tissu ovarien en vue de préserver la fertilité



26 sept 19



3. Congélation et conservation des fragments de tissu ovarien



4. Traitement du cancer

Rémission complète

5. Décongélation des fragments

6. Greffe des fragments d'ovaire

Récupération des cycles menstruels

7. Grossesse



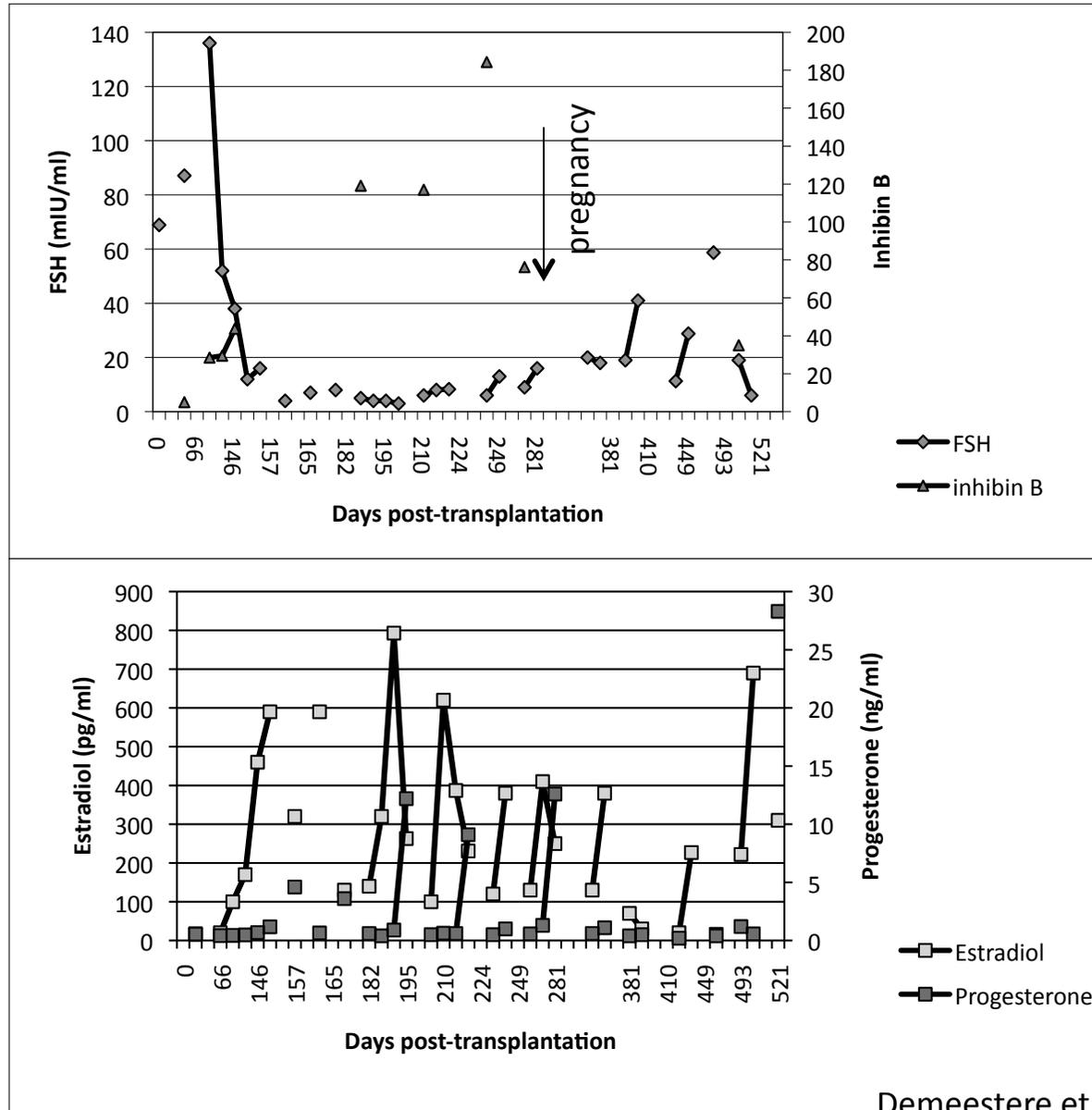
Désir de grossesse

41

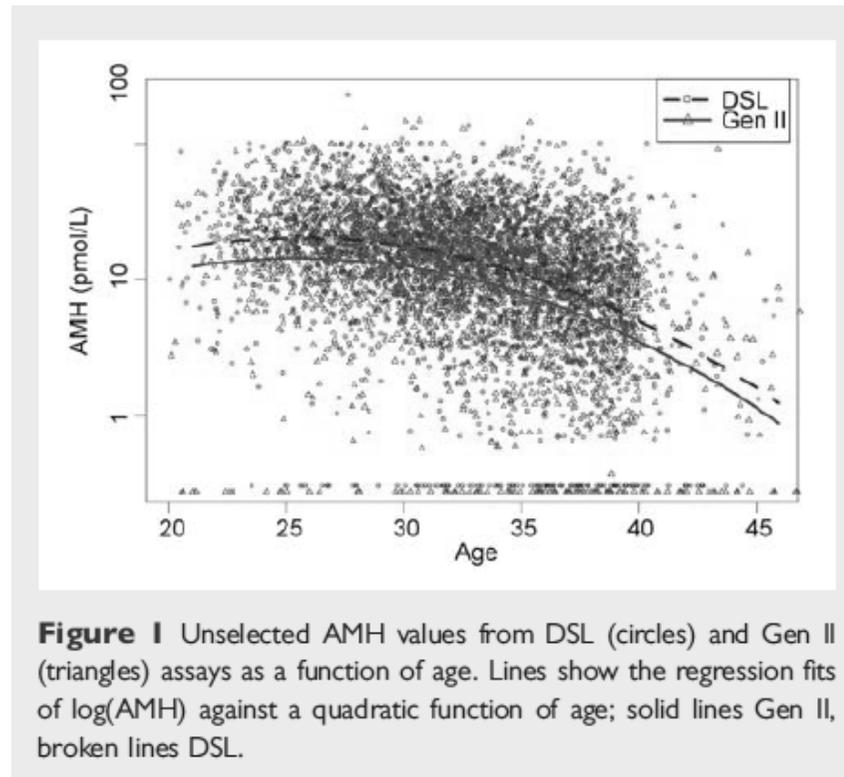
PAS DE VALEUR PREDICTIVE DE L'AMH

Patient	First menses (months since transplantation)	Duration of graft function (months) ^a	Pregnancy	Hormone assays (months since transplantation)	Cycle day	FSH (IU/liter)	E ₂ (pg/ml)	AMH (μg/liter)	Inhibin B (ng/liter)
1	5	>86	Yes ^b	3	6	31	25	<0.025	23
				6	24	21	58	<0.025	30
2	4	43	No	0 ^c	c	c	c	<0.025	c
				7	NA	17	44	<0.025	15
3 (1)	5	30	No	31	32	47	89	0.0–0.01	55
				0 ^c	c	c	c	<0.025	c
3 (2)	5	>32	No	5	NA	42	194	<0.025	27
				14	25	5.5	126	<0.025	12
4	4.5	42	No	5	NA	27	128	<0.025	13
				0 ^c	c	c	c	<0.025	c
5 (1)	6.5	24	No	4	NA	70	13	<0.025	6
				8	6	18	70	<0.025	8
5 (2)	6	18	No	1 ^c	c	c	c	<0.025	c
				4	5	80	26	<0.025	19
6	3.5	>9	Yes ^d	7	96	10	69	<0.025	63
				0 ^c	c	c	c	0.0-0.01	c
7	3.5	>9	Yes ^d	5	16	6.8	186	<0.025	55
				0 ^c	c	c	c	<0.025	c
8	7	28	No	5	43	11	83	<0.025	99
				8	8	6.8	62	<0.025	100
9	3.5	>19	No	2 ^c	c	c	c	<0.025	c
				5	NA	64	12	<0.025	10
10	3.5	>17	No	0 ^c	c	c	c	<0.025	c
				20	120	4	167	<0.025	21
10	3.5	>17	No	1	NA	40	19	<0.025	28
				3	9	6.7	104	0.33	83
10	3.5	>17	No	1 ^c	c	c	c	0.09	c
				5	8	14	196	0.09	97

RESTORATION DE LA FERTILITÉ APRÈS GREFFE



- Immunotech
- AMH Gen II (Elisa sandwich) Beckman Coulter
Seuil detection 0.16 ng/ml
Norme 1-4.6 ng/ml (établie par le laboratoire?)
- Interférence immunologique possible (Cappy et al, 2013)
(maladie autoimmune- présence AC hétérophiles)



METHODOLOGIE!

Serum: Valeurs très stables pour le sérum
 Pas de variation après 5 jours à Temp ambiante puis 4C?

Whole-blood: augmentation des valeurs lorsque l'échantillon est maintenu à 20C

Proportionnalité des dilutions?

Diluants utilisés?

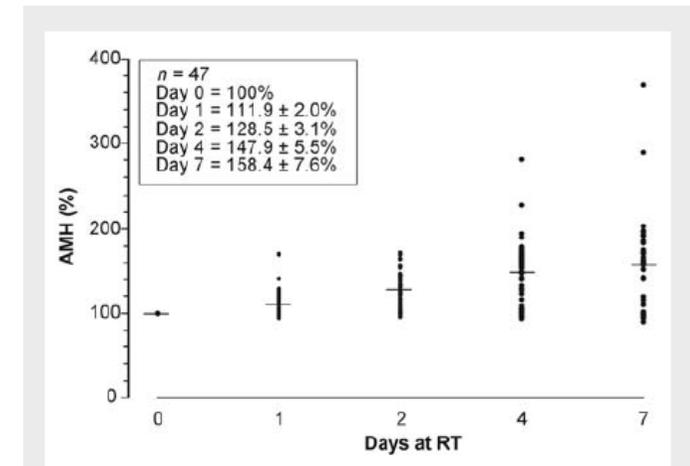


Figure 2 Stability of AMH in serum at RT. Results at each time interval are expressed as a percentage of the patient's AMH concentration at Day 0. Means \pm SEM are indicated.

Table 1 Effect of time and temperature on AMH concentration in whole-blood samples.

	20 h at 20°C (n = 23)	44 h at 20°C (n = 11)	90 h at 20°C (n = 21)	90 h at 4°C (n = 32)
Control AMH (pmol/l) ^a	8.6	12.1	10.5	13.9
Experimental sample AMH (pmol/l)	9.9	13.6	13.8	15.4
Increment (%)	15	12	31	11
P (paired t-test)	<0.001	<0.016	<0.001	0.002

^aControl sample = serum immediately separated from blood cells and refrigerated until assayed within 24 h of venepuncture.

- Intérêt bien démontré chez l'enfant
- Prédicatif des résultats en PMA → dosage systématique
- Avantages multiples par rapport à l'AFC et la FSH pour évaluer la réserve ovarienne
- Suivi des patientes à risque de diminution de la réserve ovarienne

- MAIS...
- Non prédictif de grossesse spontanée
- Non prédictif des reprises de greffe de tissu ovarien
- Variabilité des normes et cut-off en fonction des centres
- Suivi à long-terme des patientes → quid des changements de kit?
- Non remboursé