Prediction of Menopause: the key to subfertility management?

The menopause and its management – a revisit
Edinburgh, October 6&7, 2008

Frank J Broekmans
Reproductive Medicine
UMC Utrecht

Take Home Message

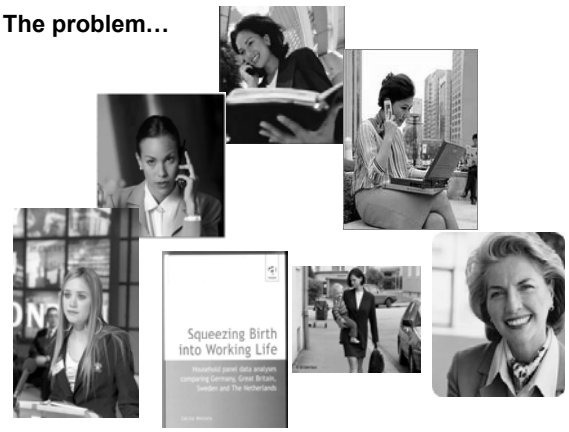
Prediction of Menopause: the key to subfertility management?

Yes and No

Agenda

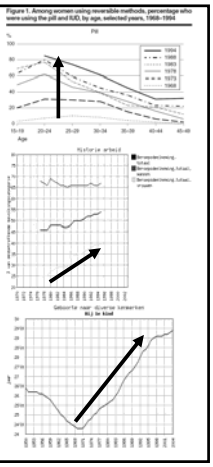
- **The Problem: age related female subfertility**
- **The Key: ovarian ageing**
- **The Solution: prediction menopause??**
- **Discussion**

The problem…

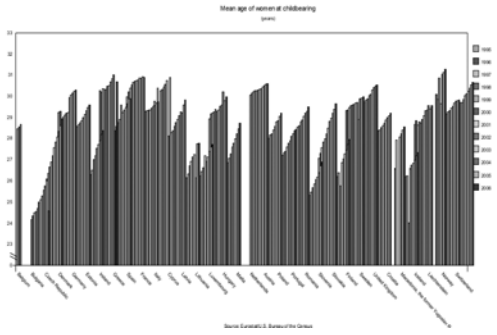


Career Lifestyle

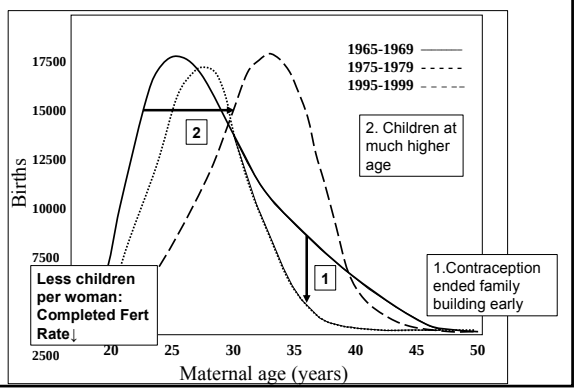
- **Female**
  - **Contraception**
  - **Education**
  - **Labour Participation**
  - **Postponement Childbearing**
- **Male:**
  - **Postponement "Childbearing"**



Mean Female age at childbirth in the EU: although variable across countries…. trend is up and up…..

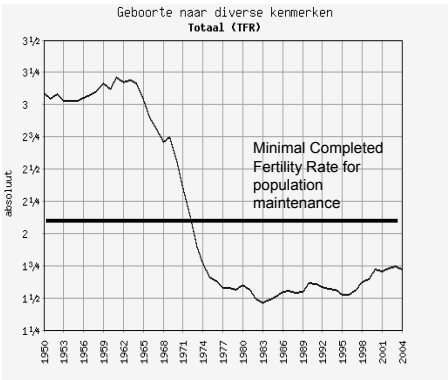


Career Lifestyle - Distribution of childbirths according to maternal age at childbirth for 3 time periods - Netherlands



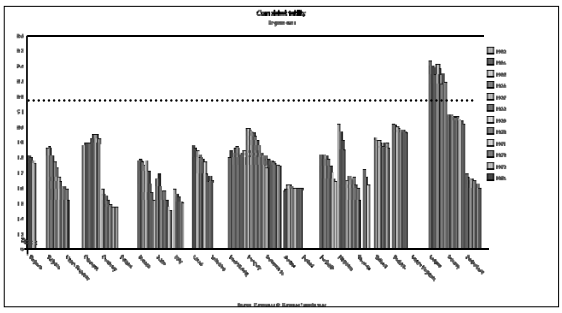
Reduced Fertility Rates..

Replace-ment..?



Source : CBS

Majority of EU countries has a Completed Fertility Rate below the level necessary to maintain the population level. Currently France is doing well (bonus policy)

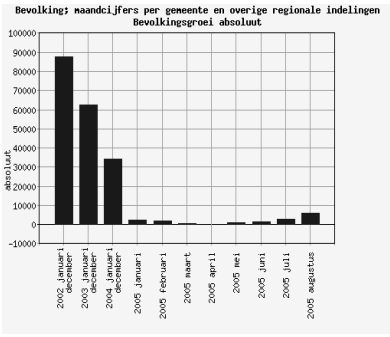


Low Completed Fertility Rate ….

Population effects…

Absent Growth

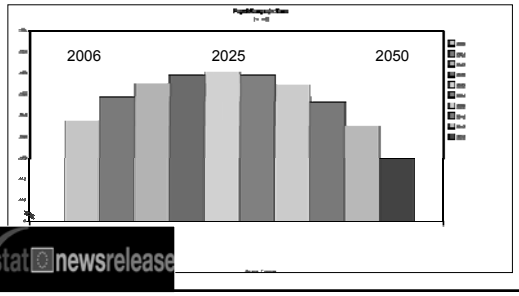
Crimp..



Source: CBS

The annual number of births falls….
The annual number of deaths will continue rising…
2015: deaths will outnumber births….
2035: net migration no longer counterbalances the negative natural change…

Population Projections for EU



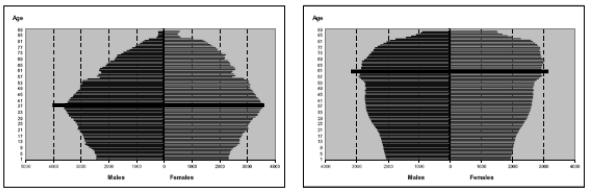
eurostat newsrelease

And there is more: Less young people, more old people

eurostat newsrelease

Almost three times as many people aged 80 or more in 2060

2004 *2050*



Sources: EPC and European Commission (2005)

The Problem

Postponing Childbearing

Reduced Completed Fertility Rates

Population Size Reduction

Economical Impacts….

Age related female fertility decay

Agenda

- **The Problem: age related female subfertility**
- **The Key: ovarian ageing**
- **The Solution: prediction menopause?**
- **Discussion**

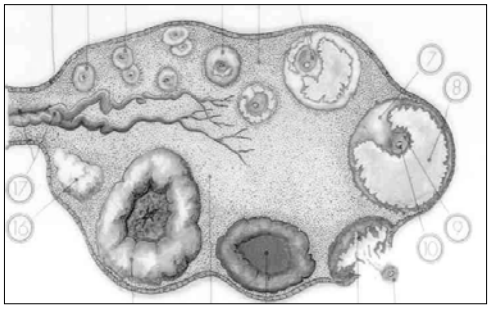
The couple trying to conceive a child without success after one or two years of trying….

No explanation found in the infertility work up…

Which is true for 60% of those couples. What is wrong with them..??

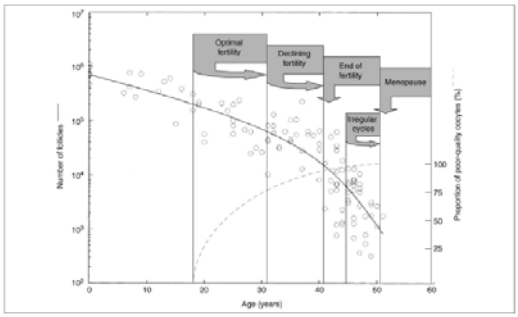
Te Velde, 2002

Reproductive ageing = Ovarian ageing



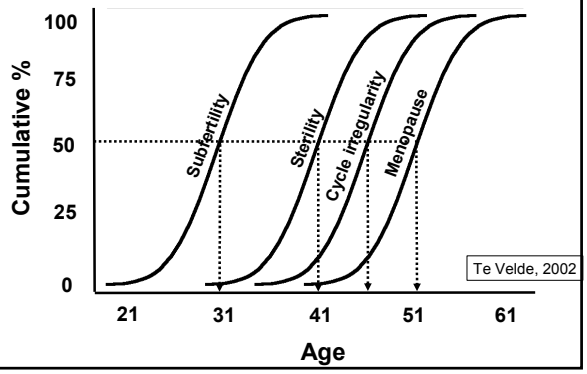
Ovarian reserve

= Quantitative and Qualitative decline of the follicle/oocyte pool

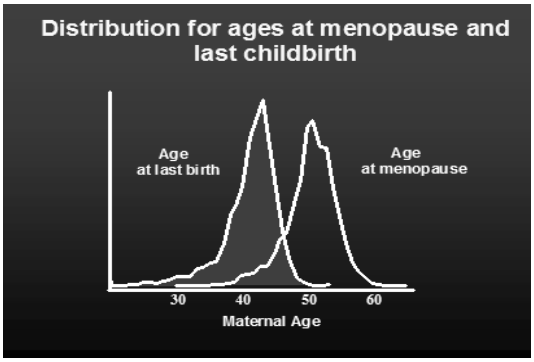


Faddy, 1995, De Bruijn, 2003, O'Connor, 2001

Variation in age at menopause and preceding "reproductive events" Fixed intervals?



Analogy



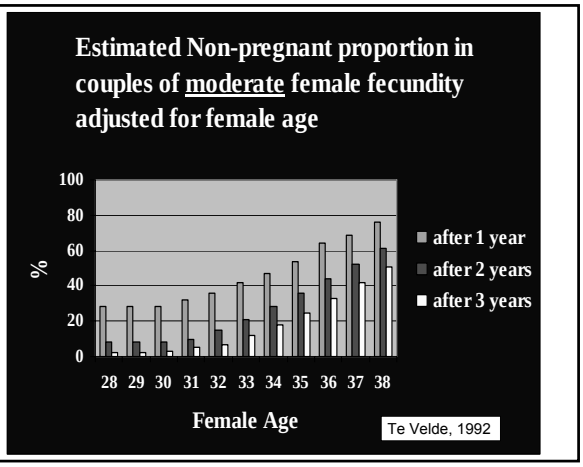
Consistent Poor Responders in IVF (almost sterile) have decreased interval to MP or Transition compared to normal responders (normal fertile)

	Study group				Control group			Adjusted* Odds or Hazard ratio
Study	N	Median Follow Up	MP or Transition	FSH IU/l	N	Median Follow Up	MP or Transition	
Farhi, 1997	12	9 months		23-85				
De Boer, 2003	636	6 years	22%	-	3675	6 years	7%	~3.1 (Odds)
Lawson, 2003	118	5 years	50%	-	265	5 years	16%	~3.1 (Hazard)
Nikolaou, 2002	12	4 years	92%	-	24	4 years	17%	~5.3 (Odds)

* Adjustments were carried our for age and/or smoking behaviour

And…Human Fertility….
is not super….

	MFR	Cumulative pregnancy rate after			
		6 months	12 months	24 months	60 months
Category					
Superfertile	60%	100%	..	..	..
Normally fertile	20%	74%	93%	100%	..
Moderately subfertile	5%	26%	46%	71%	95%
Severely subfertile	1%	6%	11%	21%	45%
Infertile	0%	0%	0%	0%	0%

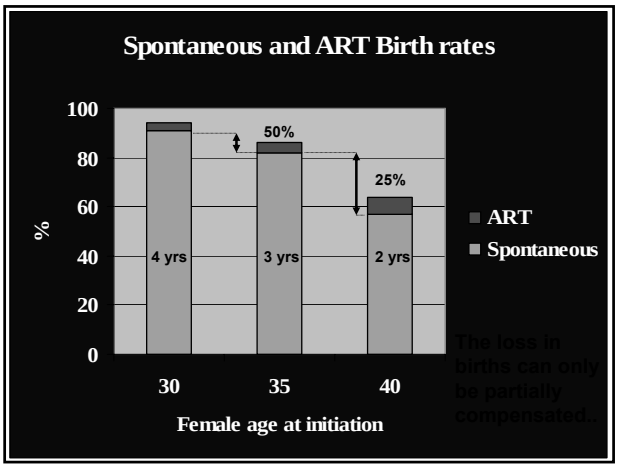


Managing female age related sub/infertility - options

- **More and better infertility treatment ??**
- **Campaign ??**

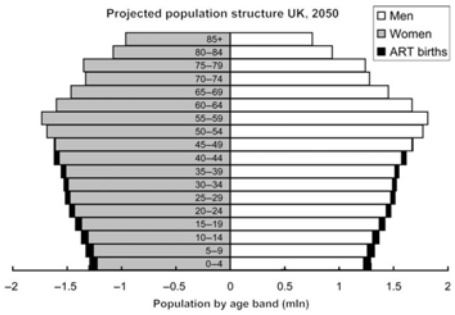
Leridon

- **Monte Carlo simulation model for Live Birth probability**
  - **MFR**
  - **Early pregnancy loss rate**
  - **Rate of becoming naturally sterile**
- **Age corrected**
  - **(starting to conceive at 30, 35 en 40 year)**
- **Effect applying IVF**
  - **(in sub/infertility of 4, 3 or 2 years duration)**



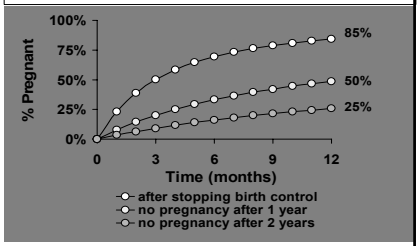
Can ART help to offset population ageing? An assessment of the demographic and economic impact of ART in Denmark and UK
Hoorens, HR 2007 (Rand Cooperation)

Use Danish extent of ART use in UK and Fert Rate will go up by 0.04 or with maximal ART use even by 0.2



However, when using ART early and to the max: many **spontaneous** pregnancies will become "lost"

The proportion of couples having a spontaneous pregnancy leading to a live birth in natural populations (Te Velde *et al.*, Lancet 2000)



Managing female age related sub/infertility - options

- **More and better infertility treatment ??**
  - **Beware of complications: handicapped twin offspring**
- **Campaign ??**

Estimates of Subfertility for the Dutch population based on non-pregnant rates in the Saguenay Population (Eijkemans, 2004)

	Number not pregnant	
Age at Marriage	After 18 months	
20-24	1261	10%
25-29	4660	16%
30-34	6439	20%
35-39	3472	32%
Total Subfertile	15832	18%

Estimates of Subfertility for the Dutch population based on non-pregnant rates in the Saguenay Population (Eijkemans, 2004).

Effect of Preventive campaign

	Number not pregnant		Decrease	%
Age at Marriage	After 18 months			
20-24	1261	10%		
25-29	11547	16%		
30-34	0			
35-39	0			
Total Subfertile	12808	15%	3042	19.1

Campaigning

- **At the population level 20% reduction subfertility: substantial**
- **Measures not necessary for large group of high and normal fertile women who will have no subfertility even if waited until after 30 or 35: overkill**

The Key

Ovarian ageing produces unwanted sub- or in-fertility

Rate of ovarian ageing is variable among women

Events in reproductive ageing have fixed temporal relationship ??

ART and campaigns have limited effect

Agenda

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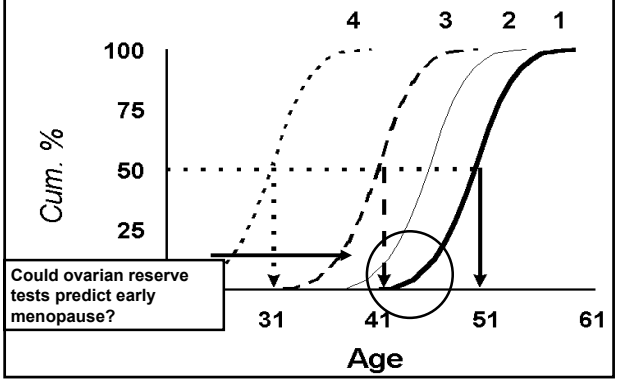
- **The Key: ovarian ageing**
- **The Solution: prediction menopause?**
- **Discussion**

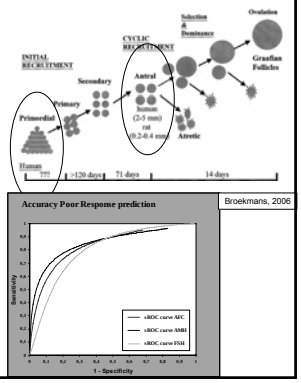
Variation in Reproductive Milestones



Could ovarian reserve tests predict early menopause?

Ovarian Reserve Tests

- Mark the size of the antral follicle cohort
- This cohort is proportionally related the primordial follicle pool
- Identify cases with **damage done**, in ART for instance



Broekmans, 2006

The Solution

Is not in the classical Ovarian Reserve tests

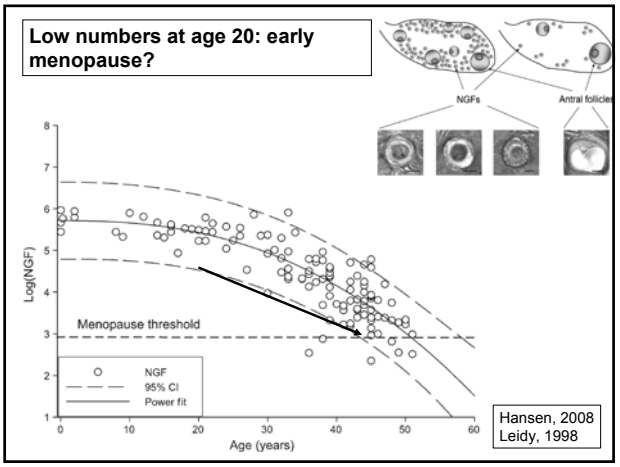
Is not in Campaigning nor in more IVF

Is it in predictors of fertility lifespan, ie menopause?

Counselling Centre

- **All 20-25 year old females**
- **Test predicting menopause**

Low numbers at age 20: early menopause?



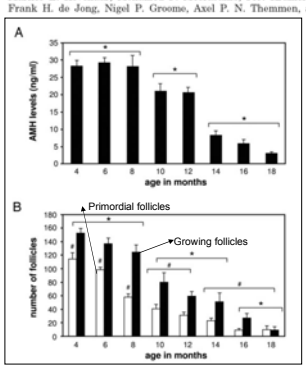
Hansen, 2008
Leidy, 1998

Which marker describes the full period (15-50) of decreasing numbers of follicles??

We don't know really

Serum Anti-Müllerian Hormone Levels Reflect the Size of the Primordial Follicle Pool in Mice

Marlies E. Kevenaar, Mohamed F. Meerasahib, Piet Kramer, Brigitte M. N. van de Lang-Born, Frank H. de Jong, Nigel P. Groome, Axel P. N. Themmen, and Jenny A. Visser

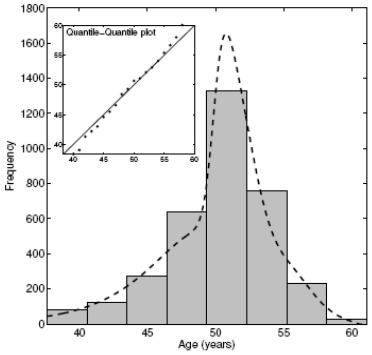


AMH helps us in mice

Is this also true for the human female?

AMH predicted age at menopause conforms with **observed** age at menopause

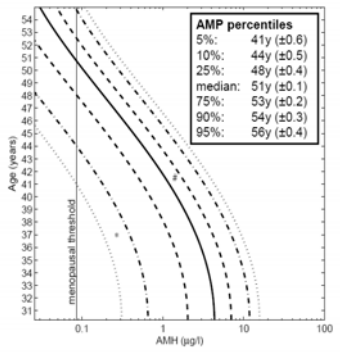
Disseldorp, JCEM 2008



Prediction of Age category at Menopause

from Age/AMH

Disseldorp, JCEM 2008



AMH predicts cycle irregularity, independent from female age, **in 4 year follow up study**, n= 163

Van Rooij, Menopause, 2004

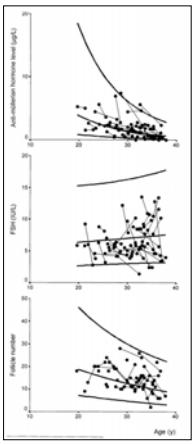
Currently working on follow up 12 years…

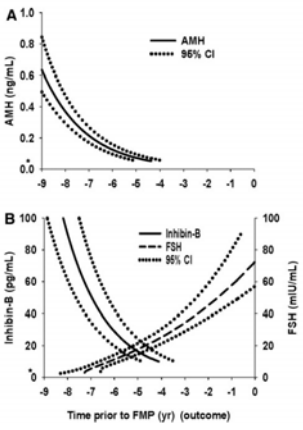
TABLE 3. *Predictive capacity of ovarian reserve markers assessed at T_1 for outcome cycle irregularity within 4 years*

	OR (95% CI)	*P*	ROC_{AUC} (95% CI)
Multivariable analysis, all variables			
AMH (per 0.1 μg/L)	0.81 (0.67–0.99)	0.04	
Inhibin B (per ng/L)	0.98 (0.97–0.997)	0.02	
Age (per y)	1.39 (0.97–1.98)	0.07	
			0.92 (0.86–0.99)[a]

AMH levels decline with female age **in follow up**, as do AF numbers, but not FSH over a mean range of 2.1 years

De Vet, 2002





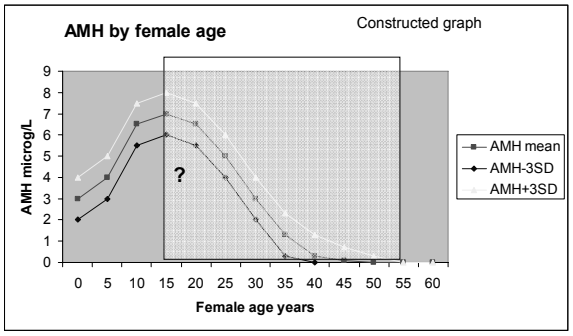
A 9 years follow up showed AMH to drop to undetectable levels some 4-5 years before the actual LMP

Sowers, 2008

Counselling Centre

AMH predicting menopause as proxy for fertility lifespan: we need much longer data….

…or transversal normative data in order to set the limit for "severely at risk for early menopause and short fertility lifespan"

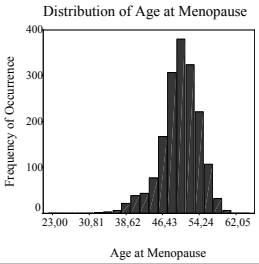


and genes…

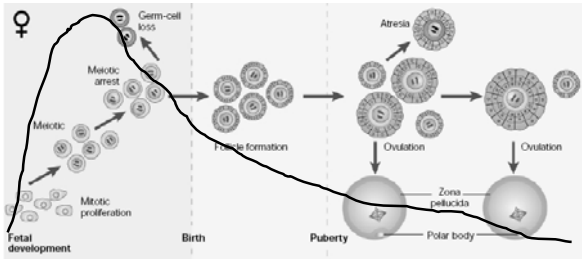
- **Heritability factor for age at MP 0.5-0.8…based on twin studies**
  - **Snieder, 1998**
  - **De Bruin, 2001**
  - **Treloar, 1998**

Menopause Variation

- Genes
  - Not a simple single gene
  - Complex quantitative trait
- Environment, mainly smoking



Genes involved in the built up and wastage of follicles



Hunt, 2001

How to find the genes

Phenotypes: POF vs controls and early/late menopause

Candidate functional genes
Genome Wide screening

Association studies in cohorts with known age at natural menopause

Linkage analysis in familial POF

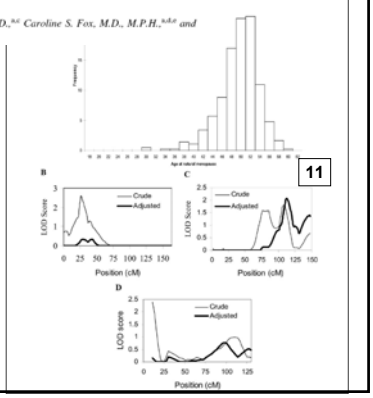
Candidate gene (SNP) Association studies in Natural Menopause cohorts: consistent findings??

Study	Year	Gene	No	Effect on AMP in years	Notes
van Asselt	2003	FV Leiden	373	3.1	S
Kevenaar	2007	AMH II rec	2381	2.6	S, in nullipara
Tempfer	2005	FV Leiden	728	2.4	S
		APOE-2	738	1.5	S
Disseldorp	2008	FV Leiden	742	1.9	NS
		APOE-2	742	2	NS
		FVII	742	0.8	S
Hefler	2006	CYP1B1-4	1345	0.8	S
Dvornyk	2006	ER-α	248	0.2	NS
Hefler	2005	CYP1B1-4	1360	0.8	S
Riener	2003	IL-1RA	90	1	NS
He	2007	ESR2	229	0.3	NS
		CYP-19	229	1.1	S
		CYP-17	229	0.1	NS
Kok	2005	ER-α	385	0.3	NS
Huber	2006	SRD-5A2	323	1.1	NS
Worda	2004	Nos-3	87	0.1	NS
Zhang	2007	HDC	265	1.6	S
Mitchell	2008	CYP-19	152	2.6	S
		HSDB-1	152	1.9	S

Genome-wide linkage analysis to age at natural menopause in a community-based sample: the Framingham Heart Study

Joanne M. Murabito, M.D. Sc.M.,[a,b] Qiong Yang, Ph.D.,[b,c] Caroline S. Fox, M.D., M.P.H.,[a,d,e] and L. Adrienne Cupples, Ph.D.[b,c]

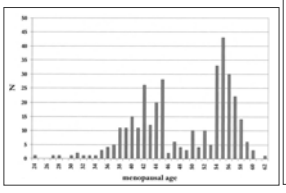
Genome wide (10cM) Linkage in families with known natural MP age: Adjusted LOD 2.1 for locus on chromosome 11

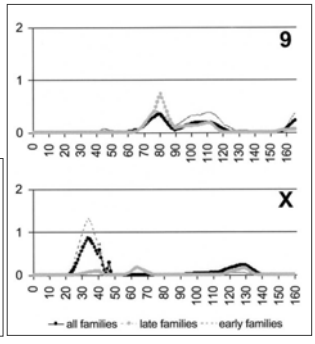


Linkage Analysis of Extremely Discordant and Concordant Sibling Pairs Identifies Quantitative Trait Loci Influencing Variation in Human Menopausal Age

Kristel M. van Asselt,[1,2,3,7] Helen S. Kok,[1,2,3,7] Hein Putter,[4] Cisca Wijmenga,[5] Petra H. M. Peeters,[1] Yvonne T. van der Schouw,[1] Diederick E. Grobbee,[1] Egbert R. te Velde,[2] Sietse Mosselman,[6] and Peter L. Pearson[5]

Genome wide (10cM) Linkage in families with discordant natural MP age





High density GWA

Studies are underway

Candidate functional genes

(identified in POF cases..)

- ZF X (low germ cell number)
- BMP15 (altered folliculogenesis)
- NOBOX (disrupted oogenesis)
- LHX8 (impaired oogenesis)
- GDF9 (disrupted folliculogenesis)
- Fragile X (altered follicle depletion)
- FOXL2 (increased follicle depletion)
- ….

Simpson Ann. N.Y. Acad. Sci., 2008

More Candidate functional genes

Knauff, 2008

Gene*	Gene-name	Locus in human	animal / human		Gene*	Gene-name	Locus in human	animal / human
HSD3B2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	**1p13.1**	human		**KITLG**	KIT ligand	**12q22**	Mouse
DDX20	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20	**1p21.1-p13.2**	Rat		**Foxo1a**	Forkhead box 01	**13q14.1**	Mouse / human
Msh4	MutS homologue 4	**1p31**	Mouse		**Rb1**	Retinoblastoma	**13q14.1-q14.2**	Mouse
LHX8	LIM homeobox 8	**1p31.1**	Mouse / Human		**BCL2L2**	BCL2-like 2	**14q11.2-q12**	Mouse
TGFBR3	transforming growth factor, beta receptor III	**1p33-p32**	human		**Bmp4**	Bone morphogenetic protein 4	**14q22-q23**	Mouse
Wnt4	Wingless-related MMTV integration site 4	**1p35**	Mouse		**ESR2**	Estrogen Receptor β	**14q23.2**	Mouse
GJA4	Gap junction protein, alpha 4	**1p35.1**	Mouse		**POLG**	Polymerase, Dna, Gamma	**15q25**	Human
Bmp8b	Bone morphogenetic protein 8b	**1p35-p32**	Mouse		**cpeb1**	cytoplasmic polyadenylation element binding protein 1	**15q25.2**	Mouse
Gpr3	G protein-coupled receptor 3	**1p36.1-p35**	Mouse / human		**SH2B1**	SH2B adaptor protein 1	**16p11.2**	Mouse
FIGLA	folliculogenesis specific basic helix-loop-helix	**2p13.3**	Mouse / human		**Ybx2**	Y box binding protein 2	**17p11.2-p13.1**	Mouse / human
LHR	Luteinizing Hormone Receptor (LHCGR)	**2p21**	Mouse		**NOG**	Noggin	**17q22**	Human
FSHR	FSH receptor	**2p21-p16**	Mouse / Human		**Bcl2**	B-cell CLL/lymphoma 2	**18q21.3**	Mouse
EIF5B	eukaryotic translation initiation factor 5B	**2q11.2**	Human		**NANOS3**	Nanos homolog 3	**19p13.12**	Mouse / Human
INHA	inhibin, alpha	**2q33-q36**	Human		**AMH**	anti-Mullerian hormone	**19p13.3**	Mouse / Human
Mlh1	MutL homologue 1	**3p21.3**	Mouse		**LHB**	luteinizing hormone beta polypeptide	**19q13.32**	Human

DAZL	deleted in azoospermia-like	**3p24**	Mouse / Human		**NLRP5**	NLR family, pyrin domain containing 5 (Mater)	**19q13.42**	Mouse
FOXL2	forkhead box L2	**3q23**	Mouse / Human		**RFPL4A**	ret finger protein-like 4A	**19q13.42**	Mouse
KIT	Kit receptor, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	**4q11-q12**	Mouse		**Cdc25b**	Cell division cycle 25 homolog B	**20p13**	Mouse
Tert	Telomerase reverse transcriptase	**5p15.33**	Mouse		**BCL2L1**	BCL2-like 1	**20q11.21**	Mouse
Fst	Follistatin	**5q11.2**	Mouse		**BMP7**	bone morphogenetic protein 7	**20q13**	Rat
Adamts19	ADAM metallopeptidase with thrombospondin type 1 motif, 19	**5q31**	Mouse		**SPO11**	SPO11 homolog	**20q13.2-q13.3**	Mouse
GDF9	growth differentiation factor 9	**5q31.1**	Mouse / Human		**AIRE**	autoimmune regulator (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy)	**21q22.3**	Human
MSH5	mutS homolog5	**6p21.3**	Mouse / Human		**DMC1**	Disrupted meiotic cDNA 1 homolog	**22q13.1**	Mouse / Human
POU5F1	POU class 5 homeobox 1	**6p21.31**	Mouse		**USP9X**	Ubiquitin-Specific Protease 9	**Xp11**	Drosophila
Foxa3a	FOXO subfamily 3a	**6q21**	Mouse / Human		**BMP15**	bone morphogenetic protein 15	**Xp11.2**	Mouse / human
ESR1	estrogen receptor α	**6q25.1**	Human		**DDX3X**	Dead box polypeptide 3, X linked	**Xp11.3-p11.23**	Mouse
EGFR	epidermal growth factor receptor	**7p12.3-p12.1**	Zebrafish		**ZFX**	zinc finger protein, X-linked	**Xp22.2-p21.3**	Mouse
Ahr	Aryl-hydrocarbon receptor	**7p15**	Mouse		**AR**	Androgen receptor	**Xq11.2-q12**	Mouse
NOBOX	Newborn ovary homeobox gene	**7q35**	Mouse / Human		**TAF1**	TATA box binding protein (TBP)-associated factor	**Xq13.1**	Drosophila
NR6A1	nuclear receptor subfamily 6, group A, member 1	**9q33-q34.1**	Mouse		**DACH2**	dachshund homolog 2 (Drosophila)	**Xq21**	Human
SOHLH1	spermatogenesis and oogenesis specific basic helix-loop-helix 1	**9q34.3**	Mouse		**POF1B**	premature ovarian failure, 1B	**Xq21.1-q21.2**	Human
CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	**10q11.1**	Mouse		**DIAPH2**	diaphanous homolog 2 (Drosophila)	**Xq21.33**	Drosophila / Human
PTEN	phosphatase and tensin homolog	**10q23.3**	Mouse		**CENPI**	Centromere protein I	**Xq22.1**	Rat

BDNF	brain-derived neurotrophic factor	**11p13**	Mouse		**BHLHB9**	basic helix-loop-helix domain containing, class B, 9	**Xq23**	Human
FSHB	FSH hormone β-subunit	**11p13**	Mouse / Human		**XPNPEP 2**	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound	**Xq25**	Human
ATM	Ataxia telangiectasia	**11q22.3**	Mouse		**POF1**	premature ovarian failure 1	**Xq26 -q28**	Human
XIST	X (inactive)-specific transcript (non-protein coding)	**inactiveXq13.2**	Human		**SOX3**	SRY (sex determining region Y)-box 3	**Xq27.1**	Mouse
FMR2	fragile X mental retardation 2	**Xq28**	Human		**FMR1**	fragile X mental retardation 1	**Xq27.3**	Human

Genetics of menopause variation

Problems

- Power, large cohorts needed
- Confirmation, on other large cohorts
- Effect size small: 1-3 years
- Multigene predictions: will they ever do?

Counselling Centre

Genes predicting menopause as proxy for fertility lifespan: they are there, but in a complex fashion….

Agenda

- **The Problem: age related female subfertility**
- **The Key: ovarian ageing**
- **The Solution: prediction menopause?**
- **Discussion**

Counselling Centre

Test to be developed
Counsel on fertility lifespan and associated issues, like CV disease
Counsel on lifestyle, like smoking

Would young women be interested?

Prediction of Fertility Lifespan

- Endocrine markers: AMH?
  - Follow up studies, many underway
  - Cross-sectional data for age specific reference values
- Genetic markers: to be assessed!?
  - Large studies needed and confirmation!
  - Follow up studies
- Lifestyle factors: smoking

Take Home Message

Prediction of Menopause: the key to subfertility management?

Yes and No

Thank you

Or will the credi(bili)t(y) crisis do the job?