eshre

SCIENCE MOVING PEOPLE MOVING SCIENCE

Ovarian aging

Special Interest Group Reproductive Endocrinology

5

3 July 2011
Stockholm, Sweden

Ovarian aging

Stockholm, Sweden
3 July 2011

Organised by
Special Interest Group Reproductive Endocrinology

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Course coordinators

Adam Balen (United Kingdom)

Course description

The aims of this course are to provide an understanding of how oocytes are formed in the human ovary and then how they are lost. The attendee will leave with knowledge relating to the differences between embryo number and quality and how these can be determined. This is of key importance for advising women of their potential fertility and in the management of infertility.
This course will cover how oocytes are formed in the ovary and determinants of their rate of loss. Detailed descriptions will be given of factors that influence oocyte quality and thereby potential fertility and how these may be quantified. The causes and management of premature ovarian failure will be described as will ways to preserve fertility by either oocyte or ovarian tissue cryopreservation. We will conclude with a socio-ethical talk on the effect of postponing pregnancy on society as a whole with respect to population impact, demand for or access to infertility treatment and its financial implications.

Target audience

Reproductive physicians, paramedicals, basic scientists and embryologists.

Scientific programme

Chair: Adam Balen (United Kingdom)

09.00 - 09.10	Introduction
09.10 - 09.40	The genesis of the oocyte store: does it really stop in utero? - **Claus-Yding Andersen (Denmark)**
09.40 - 09.50	Discussion
09.50 - 10.20	Determinants of ovarian aging and premature ovarian failure - **Richard Anderson (United Kingdom)**
10.20 - 10.30	Discussion
10.30 - 11.00	Coffee Break

Chair: Georg Griesinger (Germany)

11.00 - 11.30	Oocyte quality, genetics and metabolism - **Helen Picton (United Kingdom)**
11.30 - 11.40	Discussion
11.40 - 12.10	Is the oocyte the main determinant of embryo quality? - **Ursula Eichenlaub-Ritter (Germany)**
12.10 - 12.30	Discussion
12.30 - 13.30	Lunch

Chair: Richard Anderson (United Kingdom)

13.30 - 14.00	Do ovarian reserve tests correlate with oocyte quality and natural fertility or simply numbers of oocytes available during ART? **- Scott Nelson (United Kingdom)**
14.00 - 14.15	Discussion
14.15 - 14.45	Preservation of fertility: oocyte or ovarian tissue freezing? - **Dror Meirow (Israel)**
14.45 - 15.00	Discussion
15.00 - 15.30	Tea break

Chair: Frank Broekmans (The Netherlands)

15.30 - 16.00	Hormone replacement therapy for Premature Ovarian Failure and the menopause – **Melanie Davies (United Kingdom)**
16.00 - 16.15	Discussion
16.15 - 16.45	Effect of postponing pregnancy on society as a whole: population impact, demand for/access to infertility treatment, financial implications – **Siladitya Bhattacharya (United Kingdom)**
16.45 - 17.00	Discussion
17.00	Close

ESHRE – European Society of Human Reproduction and Embryology

What is ESHRE?

ESHRE was founded in 1985 and its **Mission Statement** is to:

- promote interest in, and understanding of, reproductive science
- facilitate research and dissemination of research findings in human reproduction and embryology to the general public, scientists, clinicians and patient associations.
- inform policy makers in Europe
- promote improvements in clinical practice through educational activities
- develop and maintain data registries
- implement methods to improve safety and quality assurance

Executive Committee 2009/2011

Chairman	Luca Gianaroli	Italy
Chairman Elect	Anna Veiga	Spain
Past Chairman	Joep Geraedts	Netherlands
	Jean François Guérin	France
	Timur Gürgan	Turkey
	Ursula Eichenlaub-Ritter	Germany
	Antonis Makrigiannakis	Greece
	Miodrag Stojkovic	Serbia
	Anne-Maria Suikkari	Finland
	Carlos Plancha	Portugal
	Françoise Shenfield	United Kingdom
	Etienne Van den Abbeel	Belgium
	Jolieneke Schoonenberg-Pomper	Netherlands
	Veljko Vlaisavljevic	Slovenia
	Søren Ziebe	Denmark

ESHRE Organisation

- General Assembly of Members
  - Executive Committee
  - Committee of Nat. Representatives
  - Central Office
  - ESHRE Consortia
    - EIM Consortium
    - PGD Consortium
  - Sub-Committees
    - Finance Sub-Committee
    - Comm. Sub-Committee
    - Publ. Sub-Committee
      - Editorial Office
      - Publisher
      - Editors-in-Chief
    - Int'l Scientific Committee
    - SIG Sub-Committee
      - SIG Coordinators
  - Task Forces

ESHRE Journals

Human Reproduction with impact factor 3.859

Human Reproduction Update with impact factor 7.042

Molecular Human Reproduction with impact factor 3.005

Campus Activities and Data Collection

Campus / Workshops

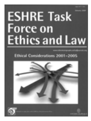
- Meetings are organised across Europe by Special Interest Groups and Task Forces
- Visit www.eshre.eu under CALENDAR

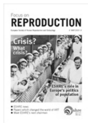
Data collection and monitoring

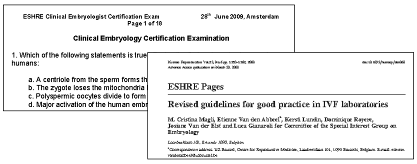
- European IVF Monitoring Group data collection
- PGD Consortium data collection

ESHRE Activities

- Embryology Certification
- Guidelines
- Position papers
- News magazine "Focus on Reproduction"







ESHRE Clinical Embryologist Certification Exam 29th June 2009, Amsterdam
Page 1 of 10

Clinical Embryology Certification Examination

1. Which of the following statements is true … humans:

a. A centriole from the sperm forms th…
b. The zygote loses the mitochondria …
c. Polyspermic oocytes divide to form …
d. Major activation of the human embr…

ESHRE Pages

Revised guidelines for good practice in IVF laboratories

M. Cristina Magli, Etienne Van den Abbeel, Kersti Lundin, Dominique Royere, Josiane Van der Elst and Luca Gianaroli for Committee of the Special Interest Group on Embryology

ESHRE COMMUNITY

RSS feeds for news in reproductive medicine

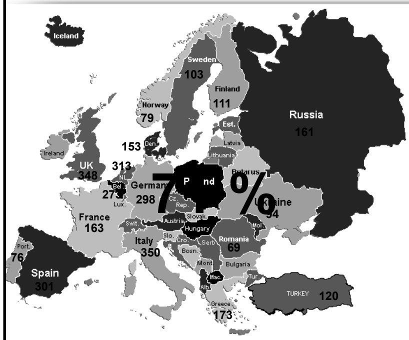
facebook — Since launch 12/2009: **1,360 Fans**

twitter — Since launch 12/2009: **190 followers** (journalists, scientific organisations, patient societies, governmental bodies)

MHR — Retweets to MHR

YouTube Broadcast Yourself — IVFPODCASTS — **Find a member**

ESHRE Membership (1/3)



4,017	Europe
476	Asia
372	North America
332	Middle East
221	Africa
142	Oceania
99	South America

TOTAL MEMBERSHIP*: 5 659 members

* as of July 2010

ESHRE Membership (2/3)

	1 yr	3 yrs
Ordinary Member	€ 60	€ 180
Paramedical Member*	€ 30	€ 90
Student Member**	€ 30	N.A.

*Paramedical membership applies to support personnel working in a routine environment such as nurses and lab technicians.
**Student membership applies to undergraduate, graduate and medical students, residents and post-doctoral research trainees.

ESHRE Membership – Benefits (3/3)

1) Reduced registration fees for all ESHRE activities:

Annual Meeting	Ordinary	€ 480	(€ 720)
	Students/Paramedicals	€ 240	(€ 360)
Workshops*	All members	€150	(€ 250)

2) Reduced subscription fees to all ESHRE journals – e.g. for Human Reproduction €191 (€ 573!)

3) ESHRE monthly e-newsletter

4) News Magazine “Focus on Reproduction” (3 issues p.a.)

5) Active participation in the Society’s policy-making

*workshop fees may vary

Special Interest Groups (SIGs)

The SIGs reflect the scientific interests of the Society’s membership and bring together members of the Society in sub-fields of common interest

- Andrology
- Early Pregnancy
- Embryology
- Endometriosis / Endometrium
- Ethics & Law
- Safety & Quality in ART
- Psychology & Counselling
- Reproductive Genetics
- Reproductive Surgery
- Stem Cells
- Reproductive Endocrinology

Task Forces

A task force is a unit established to work on a single defined task / activity

- Fertility Preservation in Severe Diseases
- Developing Countries and Infertility
- Cross Border Reproductive Care
- Reproduction and Society
- Basic Reproductive Science
- Fertility and Viral Diseases
- Management of Infertility Units
- PGS
- EU Tissues and Cells Directive

ESHRE – Annual Meeting

- One of the most important events in reproductive science
- Steady increase in terms of attendance and of scientific recognition

Track record:
ESHRE 2010 – Rome: 9,204 participants
ESHRE 2009 – Amsterdam: 8,055 participants
ESHRE 2008 – Barcelona: 7,559 participants

Future meetings:
ESHRE 2011 – Stockholm, 3-6 July 2011
ESHRE 2012 – Istanbul, 1-4 July 2012

ESHRE 2011, Stockholm, Sweden

When: **3 - 6 July 2011**
Where: **Stockholmsmässan,**
Mässvägen 1, Älvsjö, Sweden
www.stockholmsmassan.se

Chair of conference: **Kersti Lundin**

Hotel and Travel:
MCI - Stockholm Office
Phone: +46 (0)8 54651500
E-mail: eshre@mci-group.com

For updates visit www.eshre.eu

ESHRE 2011, Stockholm

Keynote Lectures

Aneuploidy in humans: what we know and we wish we knew – Terry Hassold (USA)

Historical Lecture

A brave new world with a brave old humankind; quo vadimus – E. Diczfalusy (SE)

MHR Symposium – The paternal genome

Sperm chromatin packaging – B. Robaire (CDN)

The human sperm epigenome – B. Cairns (USA)

ESHRE 2011, Stockholm: Debates

This house believes that obese women should not receive treatment until they have lost weight

- **Yes: Mark Hamilton *(UK)***
- **No: Guido de Wert *(NL) - TBC***

Paramedical invited session: Should we pay donors?

- **Yes: Herman Tournaye (BE)**
- **No: Laura Witjens (UK)**

Annual Meeting – Pre-Congress Courses

- PCC 1: The challenges of embryo transfer (Paramedical Group)
- PCC 2: The blastocyst: perpetuating life (SIG Embryology and SIG Stem Cells)
- PCC 3: From genes to gestation (SIG Early Pregnancy and SIG Reproductive Genetics)
- PCC 4: Lifestyle and male reproduction (SIG Andrology)
- PCC 5: Ovarian ageing (SIG Reproductive Endocrinology)
- PCC 6: The impact of the reproductive tract environment on implantation success (SIG Endometriosis/Endometrium)
- PCC 7: Adhesion prevention in reproductive surgery (SIG Reproductive Surgery)

Annual Meeting – Pre-congress Courses

- PCC 8: Theory and practice update in third party reproduction
  (SIG Psychology and Counselling)
- PCC 9: Ethical aspects of non-invasive prenatal diagnosis
  (SIG Ethics & Law)
- PCC 10: Patient-centered fertility services
  (SIG SQUART)
- PCC 11: Clinical management planning for fertility preservation in female cancer patients
  (TF Basic Science and TF Preservation in Severe Disease in collaboration with the US OncoFertility Consortium)
- PCC 12: Opportunities for research in female germ cell biology
  (TF Basic Science)

Annual Meeting – Pre-congress courses

- PCC 13: Assisted reproduction in couples with HIV
  (TF Fertility and Viral Diseases)
- PCC 14: Prevention of infertility – from preconception to post-menopause
  (TF Reproduction and Society)
- PCC 15: Hot topics in male and female reproduction
  (ASRM exchange course)
- PCC 16: Academic Authorship programme
  (Associate Editors ESHRE journals)
- PCC 17: Science and the media, an introduction to effective communication with the media
  (Communications SubCommittee ESHRE)

Certificate of attendance

1/ Please fill out the evaluation form during the campus

2/ After the campus you can retrieve your certificate of attendance at www.eshre.eu

3/ You need to enter the results of the evaluation form online

4/ Once the results are entered, you can print the certificate of attendance from the ESHRE website

5/ After the campus you will receive an email from ESHRE with the instructions

6/ You will have TWO WEEKS to print your certificate of attendance

Contact

ESHRE Central Office
Tel: +32 (0)2 269 09 69
info@eshre.eu / www.eshre.eu

The genesis of the oocyte store: does it really stop in utero?

Claus Yding Andersen

Contribution not submitted by speaker

MRC Centre for Reproductive Health

Determinants of ovarian aging and premature ovarian failure

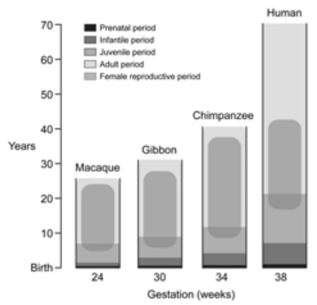
Richard A Anderson

ESHRE Rep Endo SIG PCC, Stockholm June 2011

Ovarian ageing: learning objectives

- Background to ovarian ageing
- Environmental and genetic determinants
- Single-gene models
- Granulosa cell and oocyte contributions
- Is there potential for extending ovarian life?

Humans have a limited reproductive lifespan

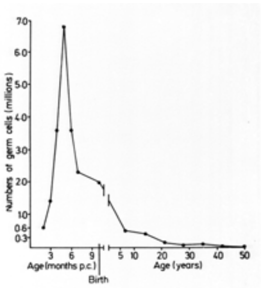


Modified from A. H. Schultz (1969) The Life of Primates (20), 149

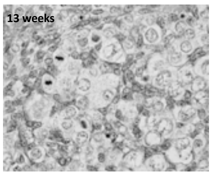
Age at menopause: health impact

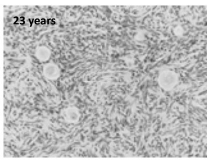
- Early
  - Osteoporosis
  - Cardiovascular risk
- Late
  - Breast cancer

Human oocyte dynamics

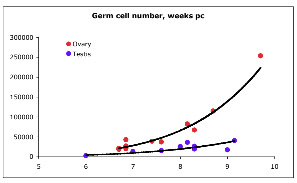


Data from Block 1952; Baker 1963



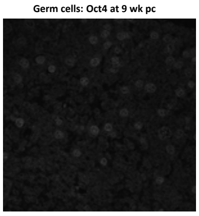


Germ cell proliferation in ovary and testis

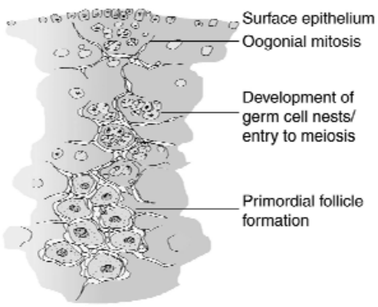


Data from Bendsen et al 2003, 2006

Germ cells: Oct4 at 9 wk pc



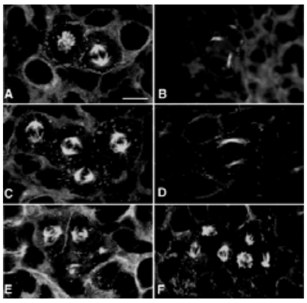
MRC Centre for Reproductive Health



Drawing by Paul Hartley

MRC Centre for Reproductive Health

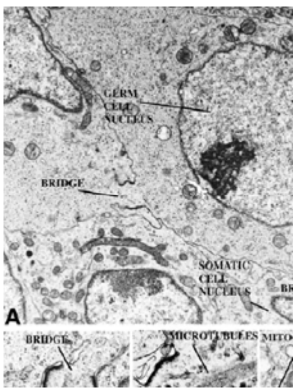
Synchronous division in germ cell cysts



d13.5 p.c.
EMA: red
Anti-tubulin: green

Pepling and Spradling 1998

MRC Centre for Reproductive Health

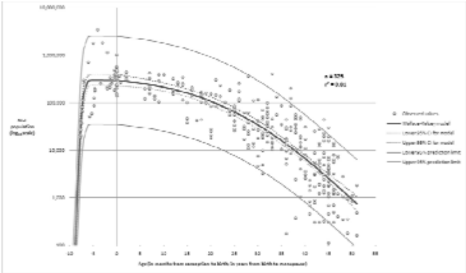


Germ cells are connected by ring canals

d13.5 p.c.

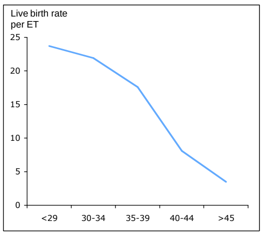
Pepling and Spradling 1998

Current model of follicular depletion

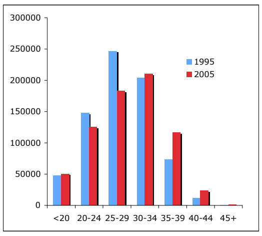


Wallace and Kelsey 2010 PLoS One 5; e8772

Age and reproductive success

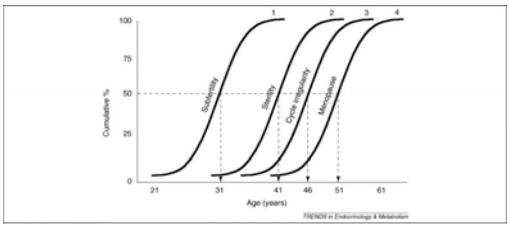


Templeton et al 1996 Lancet 384; 1402



OHE Compendium of Health Statistics

Progressive loss of reproductive function



From Broekmans et al 2007 TEM 18, 58

Environmental determinants of age at menopause

- Age at first childbirth
- Age at last childbirth
- Age at menarche
- Alcohol use
- Birth weight
- Body mass index
- Coffee consumption
- Cognition
- Depression
- Diet
- Educational level
- Ethnicity
- Employment
- Height
- Income
- Left-handedness
- Marital status
- Meat consumption
- Menstrual cycle irregularity
- Menstrual cycle length
- Miscarriages
- Oral contraceptive use
- Parity
- Physical acitivity
- Psychosocial stress
- Rank in birth order
- Religion
- Siblings
- Smoking
- Type 2 diabetes
- Unilateral Oophrectomy
- Year of birth
- Weight
- Weight gain
- Weight reduction diet

Total impact: 3%

From Kok et al 2005, Hum Reprod Update 11, 483

Factors determining the age of menopause

Environmental factors

Women who smoke reach menopause 2 years earlier than non smokers

Midgette 1990 Epidemiology 1; 1479-480
Bromberger 1997 Am J Epidemiol 145; 124-133
Gold 2001 Am J Epidemiol 15; 634-639

Premature ovarian failure

Etiology unknown in more than 90%

-other than surgery chemotherapy, radiotherapy and Turner syndrome

Genes and POF

- Familial cases
- 15-20% of cases

Approaches

- ☐ Genome scanning of familial cases
- ☐ Genome scanning of sporadic cases
- ☐ Candidate genes from animal models?

Premature Ovarian Failure (insufficiency)

- Autosomes
  - *FSHR*
  - *FOXL2*
  - *GDF9*
  - *ATM*
  - *AIRE*
  - *NOBOX*
  - *GALT*
  - *EIF2B*
  - *NSB1*
  - *DMC1*
  - Parathyroid responsive B1 gene
  - *FIGLA*
  - *Progesterone receptor membrane component-1 (PGRMC1)*
- X linked
  - X Monosomy
  - X,XX mosaicism
  - X ring
  - Triple X
  - X Deletions
  - X, autosome translocations
  - *FMR1*
  - *BMP15* ……..

Genome-wide scanning

- 165 Dutch families
- 9q21.3 and Xp21.3

Chromosome 9 locus: Bcl2

van Asselt et al. 2004 Am J Hum Genet 74, 444

GWAS in 2979 women
'Loci at chromosomes 13, 19 and 20 influence age at natural menopause'

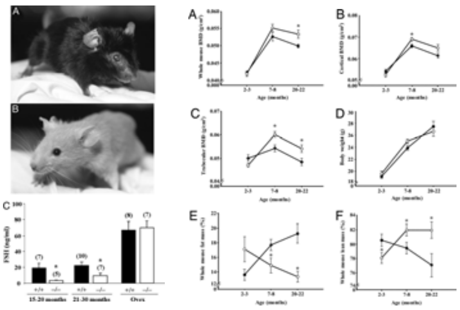
Stolk et al Nature Genetics 2009, 41, 645

Human studies on genetics of the age at natural menopause: a systematic review

Voorhuis et al Hum Reprod Update Jan 2010

'…very few consistent associations were found'

Non-reproductive function in Bax-deficient mice

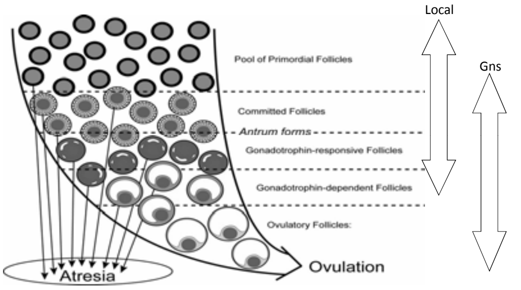


Perez et al. 2007 Proc. Natl. Acad. Sci. USA 104, 5229

Follicle recruitment rate by age

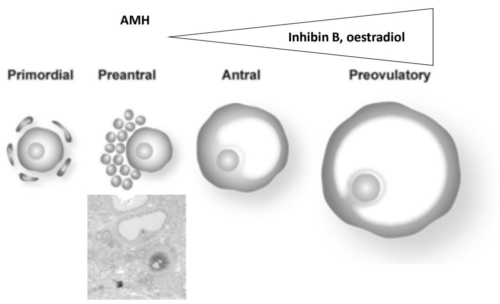
Wallace and Kelsey 2010 PLoS One 5; e8772

Follicle interactions

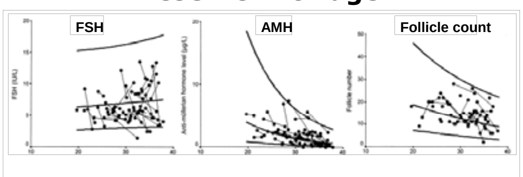


Scaramuzzi R et al 1993 Reprod Fertil Develop 5: 459-478

The growing follicle produces changing hormones



Changes in markers of the ovarian reserve with age



de Vet et al, 2002

In COS, AMH predicts no of oocytes (better than inhibin B)

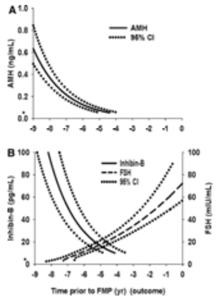
Conveniently, AMH does not vary across the menstrual cycle

Seifer et al., 2002: Fanchin et al., 2003

Prediction of menopause

50 women followed prospectively
(Michigan Bone Health and Metabolism Study)
6 annual assessments

Mean initial age 42 yr



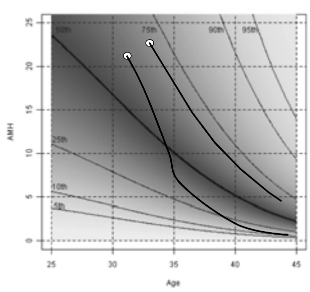
Sowers MR et al. J Clin Endocrinol Metab 2008;93:3478-3483

The association of age at FMP with AMH and inhibin B profile

	β ± SE	P value
Log AMH intercept	0.83 ± 0.38	0.035
Log AMH slope	0.75 ± 3.52	0.83
Log Inhibin B intercept	1.83 ± 1.77	0.31
Log Inhibin B slope	–0.07 ± 3.52	0.98

Sowers MR et al. J Clin Endocrinol Metab 2008;93:3478-3483

AMH nomogram with age



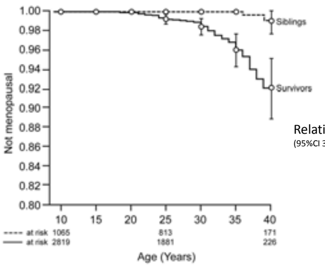
AMH in 9600 infertile women: Nelson et al Fertil Steril 2010

Poor responders=earlier menopause

	IVF poor responders			IVF normal responders			OR or HR
	n	Median follow-up	% menopausal	n	Median follow-up	% menopausal	
Retrospective cohort	636	6 years	22	3675	5 years	7	3.1
Retrospective cohort	118	5 years	50	265	5 years	16	3.1
Case control	12	7 years	92	24	7 years	17	5.3

Data from De Boer et al 2002, 2003; Nikolaou et al 2002; Lawson et al., 2003

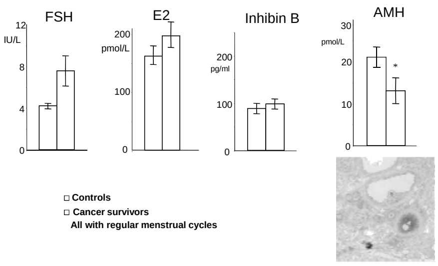
Premature menopause in childhood cancer survivors



Relative risk 13.2
(95%CI 3.3-53.5)

Sklar et al. J. Natl. Cancer Inst. 2006 98:890

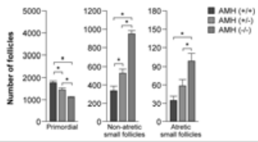
Reduced ovarian reserve in childhood cancer survivors



Bath et al 2003 Hum Reprod 18, 2368

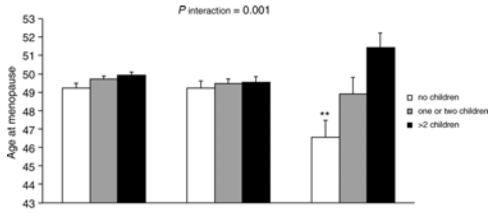
Follicle activation and depletion in AMH ko

4 months



Durlinger, A. L. L. et al. Endocrinology 1999;140:5789-5796

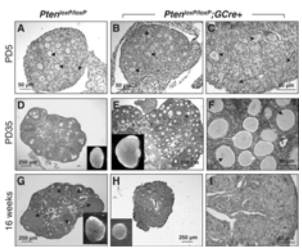
AMHR2−482 A>G genotypes and parity

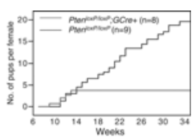


AMHR2	A/A			A/G			G/G		
n	351	670	541	135	330	275	25	23	31
Age at Menopause	49.2	49.7	49.9	49.2	49.5	49.6	46.6	48.9	51.4

Kevenaar M E et al. Hum. Reprod. 2007;22:2382-2388

Oocyte-dependent follicle activation





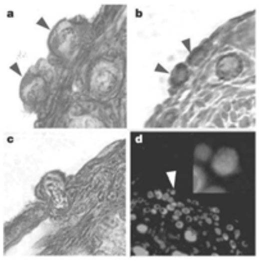
Oocyte-specific expression (GDF-9 promoter)

Pi3K inhibitor

Normal at PD5
Widespread activation from PD5, few primordials by PD23
Excess follicles at transient stage ( ) ↗

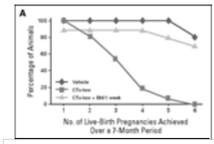
Reddy P et al 2008 Science 319, 611

Germ stem cells in the ovary?



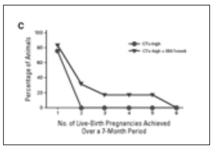
Johnson J, Canning J, Kaneko T, Pru JK and Tilly JL (2004) Nature 428, 145-150

Ovarian regeneration?



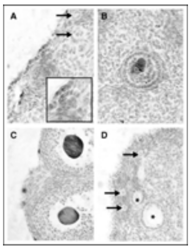
Restoration of fertility in BMT recipients after busulphan/cyclophosphamide (CTx)

All offspring from recipient germline



Lee et al 2007 J Clin Oncol 3198

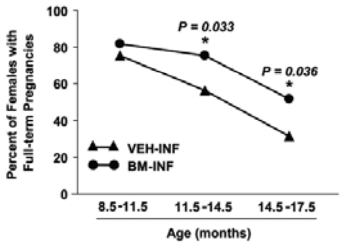
Bone marrow-derived oocytes in recipients



A,B: GFP (brown) oocytes in recipients
C +ve control
D -ve control

Lee et al 2007 J Clin Oncol 3198

Bone-marrow infusion prolongs reproductive life



Also improvement in pup survival in older dams

Selesmiemi et al 2008 Aging 14, 49

Conclusions

- The ovary has a finite lifespan, shorter than any other major organ
- Evolutionary benefits vs individual detriment
- Major genetic component: irreversible?
- Prediction in the individual
- Stem oocytes: a real contributor?

Oocyte Quality:
Genetics and Metabolism

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Leeds Institute Of Genetics, Health and Therapeutics
University of Leeds
UK

LIGHT Leeds Institute of Genetics, Health and Therapeutics

UNIVERSITY OF LEEDS

Oocyte Quality: Genetics and Metabolism

Learning Objectives

1. Define oocyte quality in health and infertility
2. Discuss the dynamics of follicular fluid and granulosa markers of oocyte quality
3. Evaluate molecular markers as indices of oocyte quality
4. Assess cytogenetic markers of oocyte quality
5. Explore the links between energy and protein metabolism and oocyte developmental competence

Genetic and Metabolic Markers Of Oocyte Quality

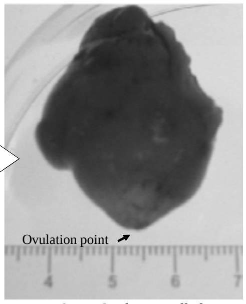
Oocyte quality is defined as the ability of an egg to complete meiosis and undergo fertilization to produce a healthy embryo which has the potential to progress to the blastocyst stage *in vivo* or *in vitro* and/or implant to produce healthy offspring

Follicular Fluid And Granulosa Cell Markers Of Follicle And Oocyte Development

Nutrients
Growth factors
Hormones
Oxygen tension
Granulosa cells

1.5 mm Antral Follicle

Metabolic requirements change as:
i) Follicle size and vascularity ⇧
ii) Follicle differentiation ⇧
iii) Follicle exposure to LH/ hCG



18 mm Ovulatory Follicle

Genetic And Metabolic Markers Of Oocyte Development & Quality

1. Molecular Markers (Genomics)
2. Cytogenetic Markers
3. Metabolic Markers (Metabolomics)

Species: Mouse, Cow, Human

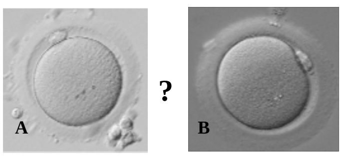
In vivo animal and human studies

In vitro growth & maturation of oocytes

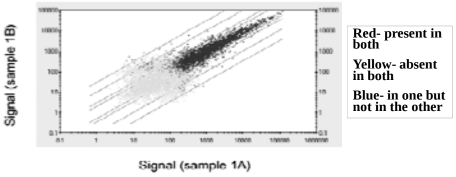
In vitro production of embryos

Strategies To Study Molecular Aspects Of Egg Quality

- Targeted molecular studies of known genes
- Expression analysis across all stages of egg development
  - Global screening- e.g. microarray analysis
  - Characterise known and novel gene function



Microarray Analysis Of 8793 Gene Products In Single Vs Pooled (5) Human Oocytes

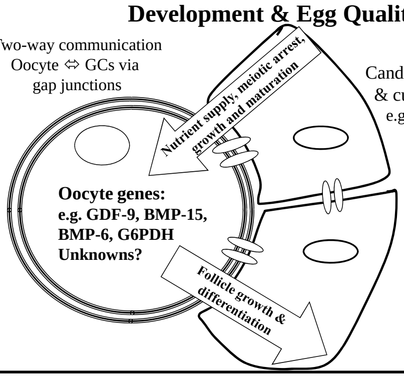


Sample type	*Total genes expressed*	*Exclusively expressed genes*	*Genes in common*
Single oocytes	1467	106	1361
Multiple oocytes	1823	462	

Bermúdez et al 2004 *RBM On Line* 8, 323-335

Summary Of Molecular Regulation Of Follicle Development & Egg Quality

Two-way communication
Oocyte ⇔ GCs via
gap junctions



Candidate granulosa & cumulus genes:
e.g. Gremlin
BMPR1A,1B, 2
SERPINE2
3βHSD
Aromatase
HAS2
COX2
PTX3
EGF-R
TBC1D1
STX7
Ferredoxin 1
Unknowns?

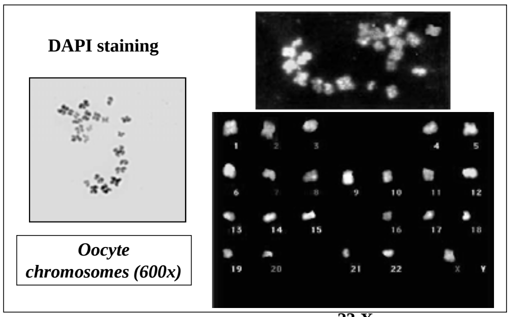
Genetic And Metabolic Markers Of Oocyte Quality

1. Molecular Markers
   - Valuable & generates a lot of data but:
   - Destructive of cells of interest
   - Analyses often conducted on pooled eggs of “different quality”
   - Must be followed up by functional studies
   - No insight into the chromosomal health of the gamete
2. Cytogenetic Markers
3. Metabolic Markers

Cytogenetic studies are highly relevant as oocyte quality is known to decline with advancing maternal age.
This decline is due to:

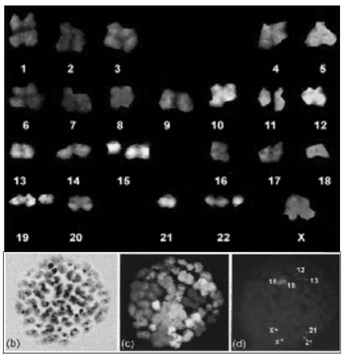
1. Increased chromosomal error/aneuploidy in oocytes and embryos
2. Accumulation of mitochondrial deletions and reduced mitochondrial activity in oocytes

24-colour M-FISH On Human Oocytes



Clyde and Picton

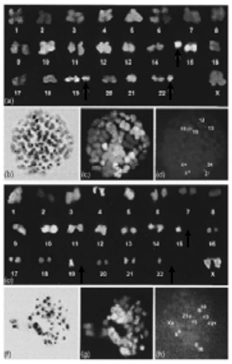
Oocyte Karyotype: 23,X +15cht,+19cht,+22cht



(b) = DAPI
(c) = M-FISH
(d) = Re-probing
- 13
- 18
- 21
- X

Clyde et al., 2001

1st Polar Body Karyotype: 23,X -15cht,-19cht,-22cht



(b) & (f) = DAPI

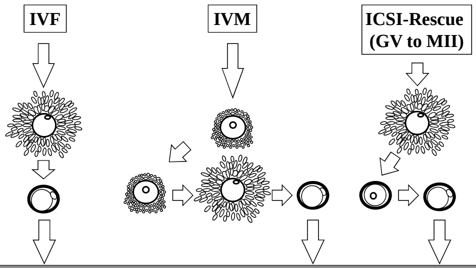
(a,c) & (e,g) = M-FISH

(c = Re-probing

- 13
- 18
- 21
- X

Clyde et al., 2001

Analysis of Human Oocytes



Molecular and metabolism assays and cytogenetic evaluation
after 30-36hrs of "true" IVM of GV oocytes to MII
after 12-16hrs of culture of ICSI GV/MI oocytes to MII

Genetic Analysis of <u>Human</u> Oocyte Karyotypes By M-FISH or SKY

	IVF (n=50)	IVM (n=79)	ICSI-Rescue (n=38)
Normal	**45 (90%)**	**57 (72%)**	**24 (63%)**
Abnormal	**4 (8%)**	**18 (22%) 30hrs:7-8% 36hrs:16-21%**	**14** (37%)**

** 13% n<23, 21% n>23,
True hypoploidy recorded for Chr 3, 8, 20, 22, X
1 oocyte with a balanced predivision of Chr 16, 1 diploid
Non-disjunction (+/- univalent) most frequent followed by predivision

Results of <u>Human</u> Oocyte Karyotyping Studies

Study	Patient No.	Age (yrs)	K-type	Tissue Type	Abnormal
1. *Pellestor et al 2003*	792	19-46	1397	IVF-FF	22.1%
2. *Sandalinas et al 2002*	13	20-45	47	Donor	42.5%
		20-34	31		35%
		35-45	16		75%

Aneuploidy rates increase with advancing maternal age and increasing FSH dose.
Chromosomal errors induced by ART may compromised egg quality.

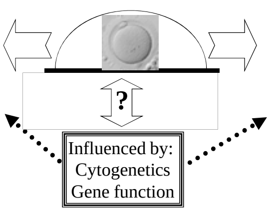
Genetic And Metabolic Markers Of Oocyte Quality

1. Molecular Markers
   Targeted molecular studies are valuable but invasive & destructive of the tissue under study.
2. Cytogenetic Markers
   Information on oocyte genetic health: – FISH studies are informative but time consuming; 1st polar body analysis by CGH array is likely to be a valuable tool to study impact of ovarian ageing on oocytes
3. Metabolic Markers
   Non-invasive & sensitive at single oocyte level

Measurement Of Metabolism During Egg Development *In Vitro*

Uptake From media

Pyruvate
Glucose
Lactate
Lipid
Amino acids
Oxygen



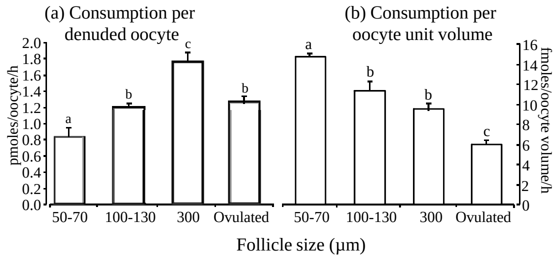
Production Into media

H_20
CO_2
Lactate
NH_4^+
Amino acids
Enzymes
Growth factors /peptides

Evidence Of The Links Between Oocyte Metabolism & Oocyte Quality

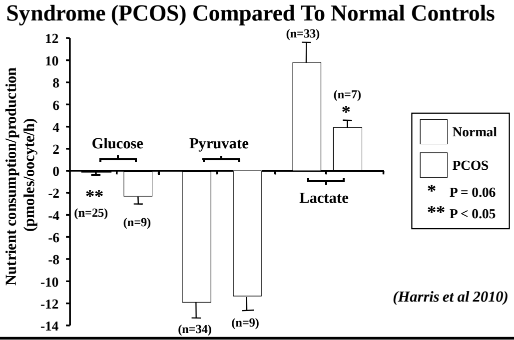
4 Species: mouse, sheep, cow & human

Pyruvate Consumption By Individual Oocytes Throughout Mouse Oocyte Development



Different letters are significantly different at p<0.05
Harris et al (2009) Mol Reprod Dev. 76:231

Energy Metabolism By Individual Human Oocytes From Infertile Patients With Polycystic Ovarian Syndrome (PCOS) Compared To Normal Controls



Metabolic Comparisons Of PCOS vs. Control Oocytes During IVM

1)No differences were detected in oocyte meiotic maturation or frequency of chromosome abnormalities (46%) (P>0.2) between 58 Control and 17 PCOS oocytes after 16-18 hrs of IVM.

2) Group G chromosomes were most likely to be involved in aneuploidy and predivision, for which there was an age-related increase (P=0.035). There was a trend for increased frequency of predivision in PCOS oocytes.

3)The PCOS aetiology did not influence oocyte pyruvate consumption but was significantly associated with increased glucose consumption and reduced lactate production.

(Harris et al 2010)

Protein Metabolism

Amino Acid Turnover

Physiological Functions Of Amino Acids

- ❖ Building blocks for protein synthesis
- ❖ Energy source
- ❖ Involved in nucleotide synthesis
- ❖ Osmolyte functions
- ❖ Antioxidant functions
- ❖ Involved in pH regulation (micro buffer function)
- ❖ Chelators- working as protection against oxidation
- ❖ Signalling molecule precursors

Measurement Of
Amino Acid Turnover By Individual Pronucleate & Cleavage Stage Embryos

Evidence from mouse, pig, cow and human embryos shows that the non-invasive measurement of the turnover of key amino acids in spent embryo culture media by HPLC is predictive of embryo development to the blastocyst stage in vitro, pregnancy in vivo, DNA damage, embryo deveopment after cryopreservation (Houghton et al 2002; Brison et al 2004; Stokes et al 2007; Sturmey et al 2010)

Furthermore, amino acid metabolism in human embryos is linked to embryo genetic health (Picton et al 2010).

Amino Acid Profiling
As The Means To Select The Best Embryo

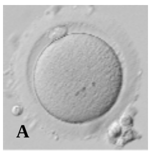
Philosophy of Approach

- The most viable preimplantation embryos are those with the lowest level of metabolism i.e. the *"quiet embryos"*

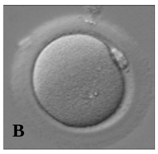
overall metabolism, aa turnover and glycolysis

- Low metabolism is achieved by reducing the concentration of nutrients in culture media to the levels measured in the female reproductive tract, this encourages the embryo to use endogenous resources.

Can Measurement Of Amino Acid Turnover Be used To Measure Oocyte Quality?

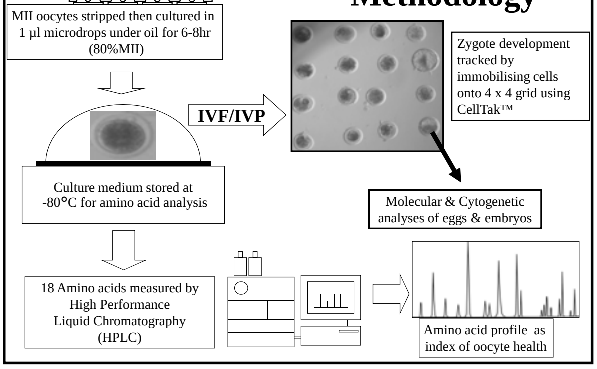


?

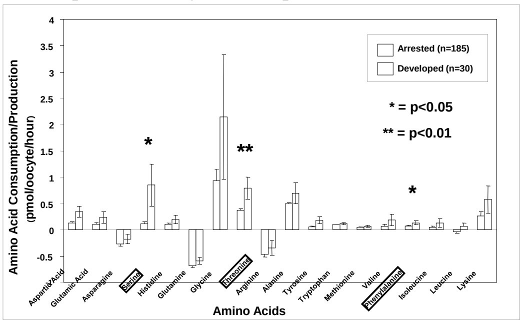


1. Do developmentally competent oocytes have a distinct metabolic finger print ?
2. Can the metabolic signature of an oocyte be linked to molecular &/or cytogenetic correlates of developmental competence ?

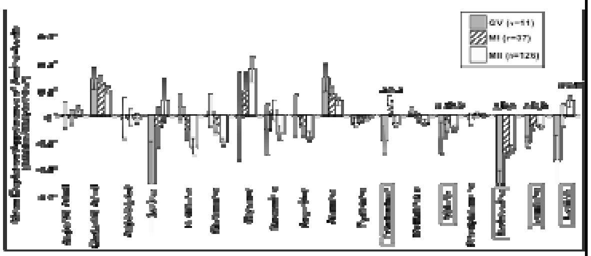
Methodology



Amino Acid Profile Of <u>Bovine</u> MII Oocytes According To Subsequent Blastocyst Development or Arrest *In Vitro*

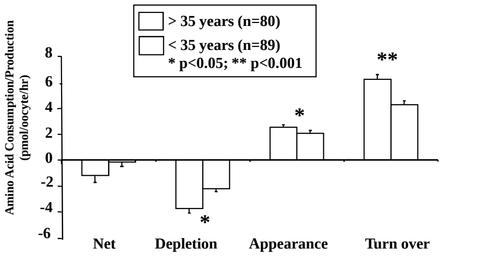


<u>Human</u> Oocytes Which Progress To MII Have a Different Amino Acid Profile To Oocytes That Fail To Complete Meiosis *In Vitro*



Picton et al., 2008

Mean Amino Acids Turnover By Individual Human Oocytes Is Related To Patient Age

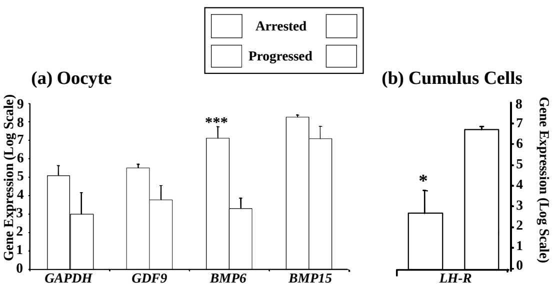


Picton et al 2008

Summary Of Metabolic Analyses of Oocytes

- Amino acid consumption/production is significantly different between individual, developmentally competent bovine MII oocytes and those which fail to fertilise and/or arrest.
- Asparagine, glutamine, serine and phenylalanine turnover are potential markers of bovine oocyte developmental competence.
- Carbohydrate and amino acid metabolism by human oocytes are significantly linked to oocyte developmental competence, patient age, aetiology and gonadotrophin dose/treatment.

Gene Expression In Individual Human Oocytes & Cumulus In Relation To Oocyte Developmental Potential



K Hemmings, HJ Leese, AH Balen, & HM Picton, Unpublished

Summary Of Molecular, Cytogenetic and Metabolic Markers Of Oocyte Quality

1. Multiple assays of oocyte quality can be conducted on the same cell which has enabled us to link molecular, cytogenetic & metabolic markers of development.

2. Oocyte quality can be quantified by non-invasive assays of metabolism & oocytes of high quality have a metabolic signature which differs significantly from oocytes of low quality.

3. Oocyte quality *in vitro* and *in vivo* is characterised by oocyte and cumulus gene expression profiles which are themselves associated with oocyte developmental competence.

4. The manipulation of these indices of oocyte competence by exposure to different gonadotrophins *in vivo* will enable us to redefine ovarian stimulation protocols & improve oocyte quality.

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Patients**…

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ESHRE Precongress Course:
Reproductive Endocrinology, Stockholm 2011

Is the oocyte the main determinant of embryo quality?

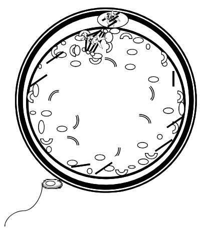
Prof. Dr. Ursula Eichenlaub-Ritter
University of Bielefelöd
Gene Technology/Microbiology
Bielefeld
Germany

ESHRE Precongress Course:
Reproductive Endocrinology, Stockholm 2011

Is the oocyte the main determinant of embryo quality?

Objectives:
1.Provide an updated overview of relative contribution of oocyte and sperm to high quality embryo and some pathologies related to reduced embryo quality
2.To evaluate the impact of age on oocyte and embryo quality
3.To discuss some options which may become relevant to improve embryo quality

Oocyte



Sperm

Pre-fertilization:

Relative Contribution and Relevance of Oocyte and Sperm for Embryo Quality:

Genome

Cytoplasm

Organelles

Epigenome

Maternal and Paternal Pathologies affecting Embryo Quality

Post-fertilization:

Suboptimal culture conditions

Sperm:

Paternal Genome

Activating Factors

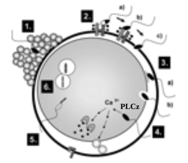
Centrosome

Sperm Genome:

Aneuploidy on average much lower in the sperm compared to the oocyte and there is no pronounced paternal age effect (average 3-4% versus 20%, e.g. Martin et al., Am J Hum Genet, 1991)

Structural aberrations are more common in sperm than oocytes and DNA damage negatively impacts embryo quality, implantation and birth rate (Speyer at al., 2010, Hum Reprod)

However, the oocyte contains DNA repair enzymes and can thereby take care of lesions in sperm chromatin and zygote (Jaroudi et al., 2009). Repair capacity within the oocyte appears induced in advanced maternal age, possibly as a result of a compensatory mechanism to cope with stress (Grondahl et al., 2010)

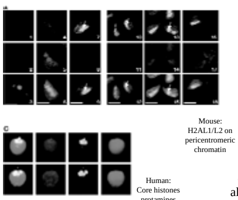


Swain & Pool, Hum Reprod Update 2008

Cytoplasmic activating factors:

PLCzeta essential for fertilization but not embryo development/quality (Taylor et al., RBM Onlne 2010,20(4))

In contrast, maternal contributions like *zona* pellucida are involved in preventing polyspermy and polyploidy in the embryo but zona morphology does not appear to be affected by maternal age (Heindryckx et al., Hum Reprod 2011)



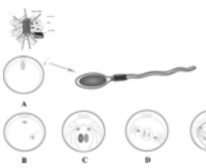
Mouse: H2AL1/L2 on pericentromeric chromatin

Human: Core histones protamines

Epigenome:

Paternal chromatin important for regulation of transcriptional activity in zygote (Bui et al., Reproduction 2011); nucleosome packaged DNA sequences important; without this, epigenetic errors may become increased that lead to non-viable embryos (Miller et al., 2010, Reproduction)

But: Bi-maternal genomic embryos of the mouse are viable and bi-maternal females have an extended lifespan (Kono et al., Nature 2004; Kawahara et al., 2010); reprogramming is not affected by maternal age (Esteves et al., Ageing Cell, 2011)

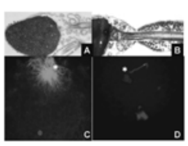


Schatten et al. Mol Hum Reprod. 2009

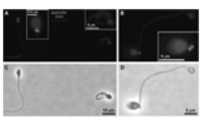
Sperm contribution: Cytoplasmic factors/Organelles:

Paternal mitochondria degraded

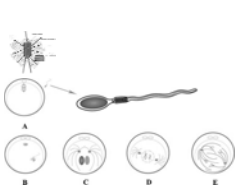
Centrosome with basal body is essential for aster formation and pronuclear apposition as well as normal zygote bipolar spindle formation (e.g. Santhananthan, Hum Rep 2004)



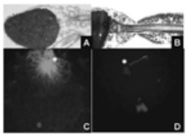
Teratozoospermia but not globozoosperia with failures; (Terada et al: Tohoku J Ex Med 2010)



Speriolin; Goto M et al. Hum. Reprod. 2010

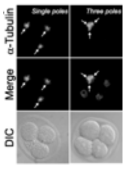


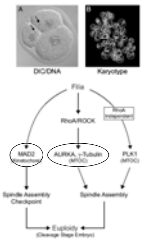
Schatten et al. Mol Hum Reprod. 2009



Teratozoospermia but not globozoosperia with failures; (Terada et al: Tohoku J Ex Med 2010)

But: maternal products like Filia essential to complement centrosomes/MTOCs and mediate bipolar spindle formation and euploidy in the embryo
(Zheng and Dean, PNAS 2009). Mitotic errors are common in blastocyst (Fragouli & Wells, Cytogenet Genome Res. 2011), which potentially could relate to altered expression of mitotic genes in aged oocytes (Grondahl et al., 2010). Genes in GO'microtubule cytoskeleton' are consistently altered with age although identity differs (e.g. Hamatami et al., 2004)





Contribution of the Oocyte to the Embryo:

Maternal Genome

Wast amount of cytoplasm containing ,activating factors' (Maternal factors/ chromatin remodelling/ zygotic gene expression/totipotency)

,House keeping' molecules: metabolism, secretion, translation

Organelles: ER, Golgi, mitochondria, ribosomes, cortical granules, zona pellucida,

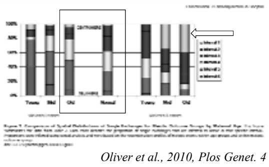
Cytoskeletal components (actin cytoskeleton/microtubules)/cell cycle regulation

membranes etc.

Maternal Genome:

,History' of oocyte is relevant: e.g. recombination at early meiosis in embryonic ovary (Pachierrotti et al., Environ. Res., 2007; Chen et al., PLOS Genet 2009):

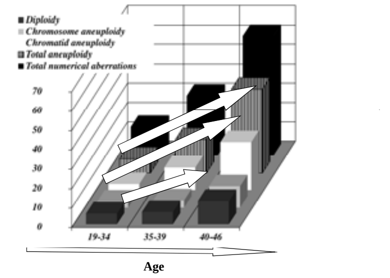
First meiotic errors giving rise to trisomy 21 involve all recombination patterns, and one distal chiasmata poses of high risk, second meiotic errors predominantly affect pericentromeric exchanges



Oliver et al., 2010, Plos Genet. 4

Recombination and number of surviving oocytes is possibly affected by exposures of primary oocytes in embryonic ovary in utero (Susiarjo et al., PLOS Genet, 2007; Rodrigues et al., Reprod Toxicol. 2010)

Errors in chromosome segregation and premature separation of chromatids are a major cause of reduced oocyte quality and developmental potential, implantation failures, spontaneous abortions and chromosomal aberrations like Down syndrome in offspring



Pellestor et al., Hum Genet. 2003; 112(2): 195-203.

Errors in chromosome segregation are related to maternal age

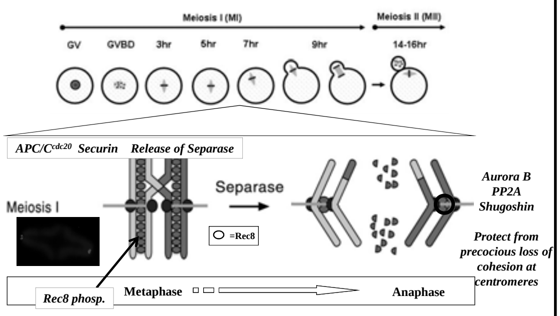
PB analysis by CGH revealed only 3% aneuploidy in oocytes of young (average 22 years) patients as compared to over 60% in oocytes of aged patients (Fragouli et al., RBM Online 2009)

Rate of aneuploidy is lower in blastocyst compared to cleavage stage (≈30% meiotic aneuploidy and 33% mitotic aneuploidy; of the latter 15% aneuploid mosaics with aneuploidy in each cell). Euploids have a better chance to develop to the blastocyst.

Errors in chromsome segregation and quality of oocyte/embryo may also relate to stimulation protocoll:

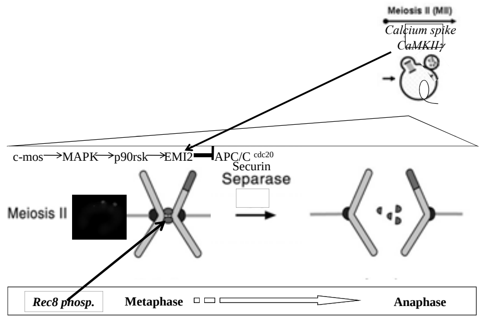
Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo (Baart et al., Hum Rep 2007)

Age-related nondisjunction may be based on loss of cohesion: Homologues are attached to each other by cohesion between sister chromatids and chiasmata; chiasmata are resolved when cohesion between arms is released by proteolysis of phosphorylated Rec8 cohesin at anaphase I

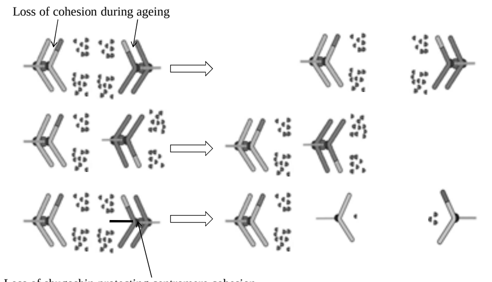


Modified from Lee & Orr-Waever, 2001

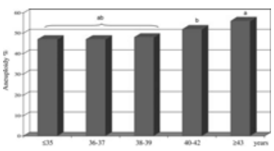
Anaphase II progression requires release from cytostatic arrest by EMI2 inhibitor downstream from fertilization triggered calcium release



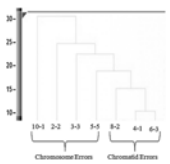
Loss of cohesion during ageing prior to anaphase I by reduced REC8, SMCs and/or Shugoshin (Lister et al., Curr Biol 2010; Chiang et al., Curr Biol.;2010 Tachibana-Konwalski et al., 2010): High risk for precocious loss of chiasmata and random segregation at meiosis I



Chromatid separation predominant cause of imballance detected by PB analysis– aneuploidy linked to generalized oocyte disorganisation not to the shape of chromosome



Gianaroli et al., Hum Reprod 2010.



Hirarchial cluster analysis of transcript patterns of human aneuploid oocytes containing either whole chromosome aberrations and chromatid aberrations suggest that there are subtle differences in expression associated with these types of errors (Fragouli ert al., Mol Hum Rep 2010).

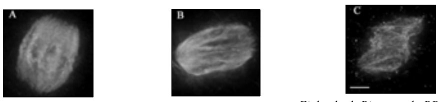
Oocyte:

Maternal Genome:

Age- and aneuploidy-related differentially expressed genes include such regulating cell cycle and spindle dynamics from the kinesin family of microtubule depolymerases.

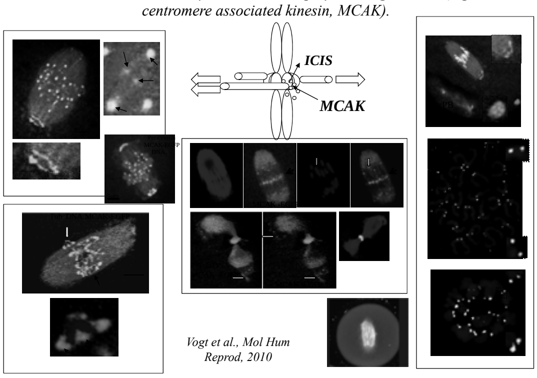
'History' of oocyte is therefore relevant with respect to spindle function:

Observations in aged oocytes: aberrat spindles, congression failure, increased univalents/ chromatids: may be related to altered expression.



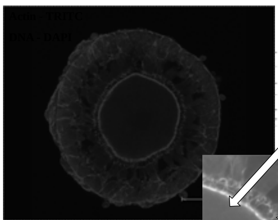
Eichenlaub-Ritter et al., RBM Online, 2004.

One example: Microtubule attachment is dynamic and aberrant (e.g. merotelic) attachments can be resolved by microtubule-depolymerising kinesins (e.g. mitotic centromere associated kinesin, MCAK).

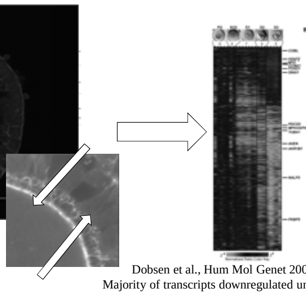


Vogt et al., Mol Hum Reprod, 2010

Gene expression in the oocyte is regulated by interactions between the oocyte and the somatic compartment (reviewed by Su et al., 2009): The majority of transcripts and protein are from the oocyte growth phase and contributed, used or degraded in early embryogenesis.
Therefore embryo quality relates to oocyte quality, which, in turn relates to follicular quality

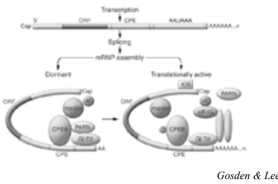


Oocyte growth and regulation of expression depend on interactions via growth factors and direct contacts via transzonal projections



Dobsen et al., Hum Mol Genet 2004: Majority of transcripts downregulated until day 3

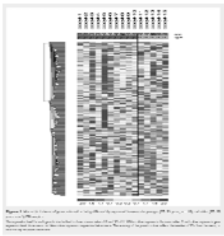
Particularly during maturation and early embryogenesis many mRNAs are recruited or degraded.



Gosden & Lee, 2010, J Clin Invest.

Ageing/ depletion of follicle pool affects gene expression at the transcriptome level

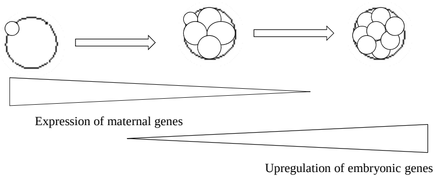

Gene expression profiles of single human mature oocytes in relation to age

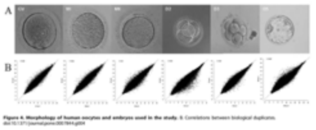


Fragouli et al. (Mol Hum Reprod 2010) detected differences between transcriptome of euploid and aneuploid human metaphase II oocytes

Cram and coworkers detected differences in abundance and also length of the poly(A) tail of mRNAs in aged human oocytes (reported at ESHRE Rome 2010)

Transcriptome profiling in human preimplantation embryos: Two major transition phases (Zhang et al., 2009, PLOS One)

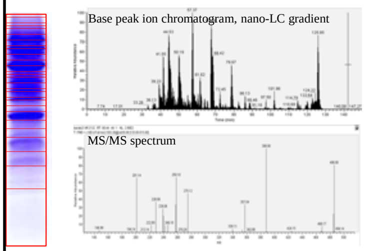




Embryo qualityis therefore dependent on maternal factors plus suitable environment (e.g. low oxygen; suitable culture conditions) for upregulation of zygotic gene expression!

Analysis of oocyte proteome (mouse):
Already at GV stage many putative or known maternal factors present
(Wang et al., PNAS 2010):

Oocyte growth phase derived factors are of relevance!



Pre-fractionation of a lysate ≈1000 murine GV oocytes on 1D SDS-PAGE and LC-MS/MS: 1123 peptids/ proteins

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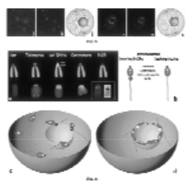
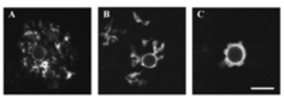
Oocytes have to become transcriptionally quiescent before resumption of maturation and this significantly affects developmental capacity of the embryo after IVM and IVF (Bellone et al. Reproduction 2009).

Gosden & Lee, 2010 J Clin Invest 120 (4)



Longo et al., Mol Rep Dev 2003



Vanhoutte et al., Hum Rep 2007

Chromatin organisation in fully mature oocytes is in the surrounded nucleolus stage (SN)

Epigenetic modifications of histones (e.g. H3K4 trimethylation) by maternal histone modifying enzymes (e.g. MLL2; Andrieu-Vieyare et al., PLOS Biol. 2010) are essential for oocyte maturation and embryonic development

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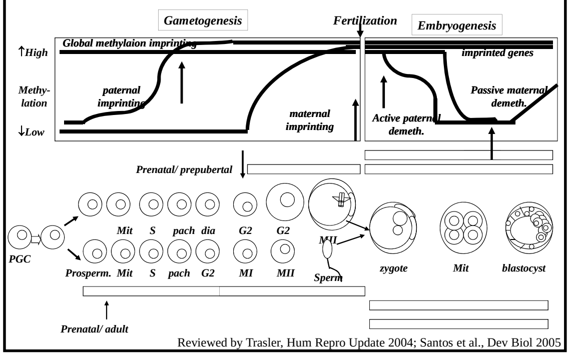
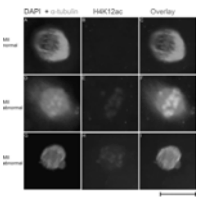
Maternal and paternal imprinting during gamete formation provides for monoallelic expression in the embryo while resetting of global imprint patterns is essential to attain a totipotent state



Reviewed by Trasler, Hum Repro Update 2004; Santos et al., Dev Biol 2005

No age-related changes in methylation of DNA of the differentially methylated regions of imprinted genes Snrpn, Kcnq1ot1, U2af1-rs1, Peg1, Igf2r and H19 were observed in embryos of aged mouse oocytes (Lopes et al., Hum Mol Genet 2009).

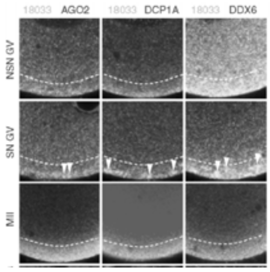
However, a significant percentage of old GV and MII mouse oocytes lacked typical histone modifications affecting chromatin conformation like H3K9me3, H3K36me2, H3K79me2 and H4K20me2 (Manosalva et al., Therigenology 2009), and defective deacetylation of histones (H4K12) during human female meiosis appears to increase with maternal age and is correlated with misaligned chromosomes (van den Berg, Hum Reprod, advanced press, 2011.



The quality of the oocyte is predetermined by processes prior to resumption of maturation. Homeostasis and follicular environment are of relevance!

Spatial deposition and accummulation of molecules and cell organelles in oocytes define ‚high quality'

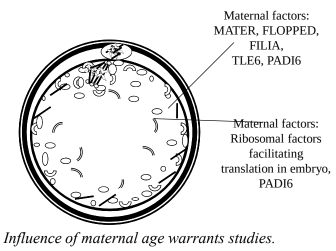
For instance, oocytes with high developmental capacity possess aggregates of RNA-binding proteins implicated in maturation and differential recruitment/degradation of RNAs.

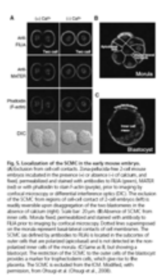


Acquisition of high developmental potential coincides with formation of RNA-binding proteins forming complexes containg RNA at the oolemma

(Flemr et al., Biol. Reprod. 2010)

Functional organization of ooplasm appears to play a role in gene expression and acquisition of high developmental potential of mammalian oocytes (subplasmalemmal maternal complex, SMC and cytoplasmic lattices, CS)

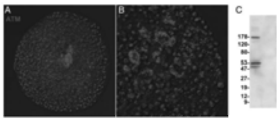




Influence of maternal age warrants studies.

(Li et al., Development 2010)

Oocyte Proteome Markers
and their spatial distribution in porcine oocytes



ATM (ataxia telancetasia mutated DNA protein kinase) in subplasmalemmal clusters in porcine oocytes

Kelch-like ECH-associated protein 1 (an adaptor for ubiquitin-ligase CUL3), nuclear export factor CRM1 and ataxia-telangiectasia mutated protein kinase appear more abundant in high quality porcine oocytes (IVM with gonadotrophin) compared to low quality oocytes (IVM without gonadotrophins).

Powell et al., Proteomics Clin. Appl. 2010

Maternal mitochondria are determinants of oocyte quality and developmental potential of the embryo

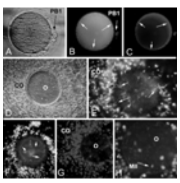
1.Numbers (DNA copy numbers) may vary greatly and mutation in mtDNA of granulosa cells appear increased with age

2. Functional status (morphological and functional alterations and mutation during ageing) rather than numbers appears affected by age (e.g. ATP production)

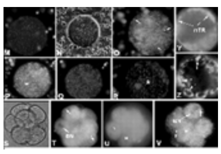
3. Distribution (e.g.alterations in local ATP supply) can impact fertilization and spindle formation, metabolism, survival/cell death etc.

Reviewed by Eichenlaub-Ritter et al., Mitochondrion, 2010;
Van Blerkom, Mitochondrion, 2010)

Functional organization of ooplasm appears also to involve mitochondrial distribution



Domains of mitochondria with high and lower inner membrane potential exist in mature oocytes and embryos

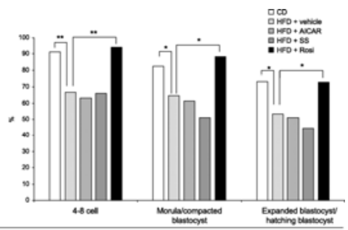


The funcional state of mitochondria is influenced by cumulus

Van Blerkom J et al. Mol. Hum. Reprod. 2008;14:431-444

Examples of how metabolism of oocyte/follicle and oocyte quality affect embryo quality and developmental potential

High fat diet in mouse affects embryo development



	4-8 cell	Morula/compacted blastocyst	Expanded blastocyst/ hatching blastocyst
CD	31/34	28/34	25/34
HFD + vehicle	30/45	29/45	24/45
HFD + AICAR	31/49	30/49	25/49
HFD + SS	31/47	24/47	21/47
HFD + Rosi	49/52	46/52	38/52

Rosiglitazone, an insulin-sentizing agent acting on expression of genes in lipid metabolism restores development of embryos from obese, high fat diet mice

Minge et al., Endocrinology 2008; 149:2646-2656

Diet affects oocyte quality (via protection from ROS?): moderate caloric restriction initiated in rodents during adulthood sustains function of the female reproductive axis into advanced chronological age (Salesniemi et al., Ageing Cell 2008).

By contrast, high fat diet causes mitochondrial damage: reduced inner mitochondrial membrane potential in oocytes

Causes stress in endoplasmic reticulum

Increases ROS and mitochondrial calcium overload

Increased apoptosis

Reduced numbers of cells in inner cell mass/trophectoderm

BIOLOGY OF REPRODUCTION 80, 622–630 (2009)
Published online before print 7 January 2009.
DOI 10.1095/biolreprod.108.072595

Metabolic and Mitochondrial Dysfunction in Early Mouse Embryos Following Maternal Dietary Protein Intervention[1]

Megan Mitchell,[2,3] Samantha L. Schulz,[3] David T. Armstrong,[3] and Michelle Lane[3,4]

Active mitochondria

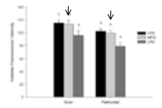


FIG. 3. The effect of maternal dietary protein supply on the distribution of active mitochondria in the outer and perinuclear regions of the 2-cell embryo. Different lowercase letters within areas are significantly different

ROS

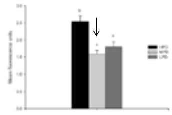


FIG. 6. ROS production by individual 2-cell, in-vivo-derived mouse embryos from females fed different levels of dietary protein. Different lowercase letters are significantly different (P < 0.001; n = 8–19 embryos/diet, n = 3 females/diet).

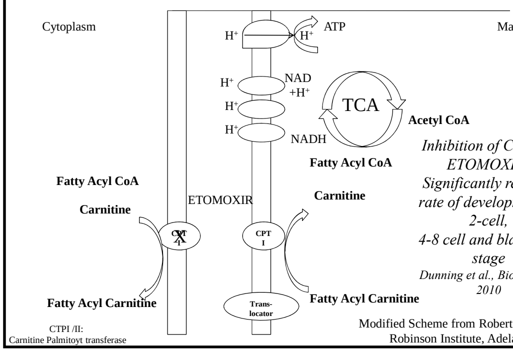
Cell numbers in ICM

TABLE 3. The influence of maternal dietary protein supply on ovulation rate, 2-cell embryo recovery, and average blastocyst cell number.

Parameter	HPD	MPD	LPD
Ovulation rate*	27.8 ± 3.05	21.3 ± 2.85	24.4 ± 2.85
Embryo recovery†			
2-Cell embryos	12.5 ± 1.83	12.9 ± 2.40	11.2 ± 1.68
Degenerate	6.8 ± 2.24	3.4 ± 2.84	8.8 ± 1.91
Blastocyst cell no.‡			
Total cell no.	48.7 ± 1.5	46.9 ± 1.9	43.7 ± 2.0
ICM cell no.	9.8 ± 0.6[c]	13.7 ± 0.7[d]	8.3 ± 0.7[c]
TE cell no.	38.9 ± 1.5[b]	33.2 ± 1.8[a]	35.4 ± 1.9[ab]
ICM/TE cell no. (%)	27.7 ± 2.1[c]	42.9 ± 2.6[d]	24.4 ± 2.7[c]

* Ovulation rate determined 15 h post-hCG administration (n = 7–8 females per diet); data expressed as mean number of oocytes ± SEM.
† The number of 2-cell embryos was determined following recovery 40 h post-hCG administration (n = 9–19 females per diet); data expressed as mean number of embryos ± SEM.
‡ Blastocysts recovered 90 h post-hCG administration (n = 21–31 blastocysts per diet, 3–4 females per diet); data expressed as mean cell number or proportion of total cell number ± SEM.
a–d Different superscripts within a row are significantly different; a,b (P < 0.05) and c,d (P < 0.001).

Lipid Metabolism and β-Oxidation in Mitochondria affect Oocyte Quality and Embryo Quality

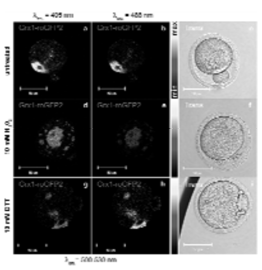


Inhibition of CTPI by ETOMOXIR: Significantly reduced rate of development to 2-cell, 4-8 cell and blastocyst stage

Dunning et al., Biol Reprod 2010

Modified Scheme from Robert Norman, Robinson Institute, Adelaide

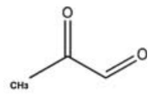
Novel method to assess redox regulation and status in mammalian oocytes



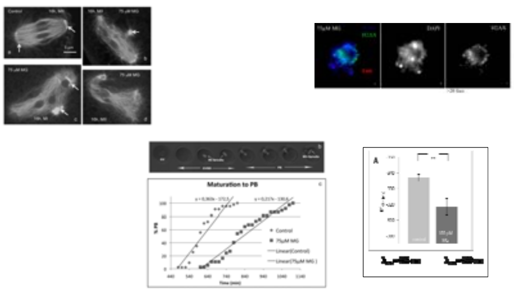
Eichenlaub-Ritter et al., Mitochondrion, 2010;
Tatone et al., Hum Reprod, 2011)

The thiol tripeptide glutathione (γ-glutamylcysteinylglycine, GSH) is the major intracellular redox buffer and represents a central element of the oocyte antioxidative system.
The redox sensitive fluorescent fusion protein Mito-Grx1-roGFP2 contains a mitochondrial signal sequence, the human glutaredoxin-1 (Grx1), an enzyme that interacts with the GSH/GSSG system, and roGFP2. Specific mRNA injection allows it to determine the mitochondrial glutathione dependent redox potential under physiological conditions.

Highly reactive carbonyl compounds like methylglyoxal from glycolysis causing increased formation of advanced glycation end products and carbonly stress are discussed in age-related accummulation of damage to DNA, membranes and mitochondria (Desai et al., Can J Physiol Pharmacol 2010; Tatone et al., Hum Reprod Update 2008).



We have shown that carbonyl stress by methylglyoxal exposure induces spindle aberrations, altered (prolonged) cell cycle kinetics, DNA damage, altered inner mitochondrial redox potential and contributes to ageing (Tatone et al., Hum Reprod 2011).



Cumulus from young females more efficiently than that from aged females protects from MG-induced meiotic arrest (Tatone et al., Hum Rep 2011)

Developing more efficient methods to obtain high quality metaphase II oocytes from IVM with ‚young‘cumulus could be useful to estblish heterologous systems in which aged oocytes mature under optimized conditions and protection from cumulus.

Is there room to improve oocyte and embryo quality?

Simulated physiological oocyte maturation (SPOM) is a novel in vitro maturation system that substantially improves embryo yield and pregnancy outcomes. Albuz et al., Hum Reprod 2010:

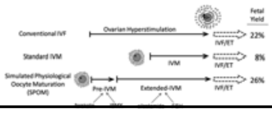
Pre-maturation period in cAMP modulator to increase cAMP in COC

IVM in presence of PDE inhibtor and FSH

Prolonged IVM

In bovine model: increased blastocyst rate and quality

In mouse model: blastocyst rate, implanation rate and fetal yield similiar to IVF of in vivo matured oocytes



Is there room to improve oocyte and embryo quality?

Improve follicular/oocyte health- by diet, anti-oxidants, etc.??

Is there room to improve oocyte and embryo quality?

Avoid age-related deterioration: social freezing- adverse influences of cryopreservation?

‚Correct' age-related deterioration: total exchange of cytoplasm, nuclear donation or cytoplasmic transfer?

Conclusions I:

Oocytes are the main determinantes of embryo quality but paternal contributions and pathologies have to be considered.

Oocyte ‚history' largely determines on its quality and developmental potential- accordingly, ovarian physiology, maternal age and some pathologic conditions are the predominant determinants of embryo quality in the human and this relates primarily to events in the follicle prior to or during resumption of maturation.

Since physiology is important, it may be improved by personalised treatment of the patient (e.g. diet, stimulation etc.).

Conclusions II:

There are novel options to improve oocyte IVM and thereby obtain good quality oocytes in animal studies producing embryos of high developmental capacity- studies in human are pending and it is unknown whether culture might improve quality of aged oocytes- loss of cohesion and aneuploidy may be inevitable.

Initial stages of embryo development appear specifically valnerable to disturbances, e.g. while chromatin remodelling, zygotic gene activation and complex alterations in cellular homeostasis are taking place but it appears to be mainly the genetic and physiological status of the oocyte that determines embryo quality.

Optimization of the culture conditions may help to improve embryo quality while there are currently few options to compensate for intrinsic aberrations, particularly chromosomal aberrations, transmitted by the oocyte.

Thank you for your attention!

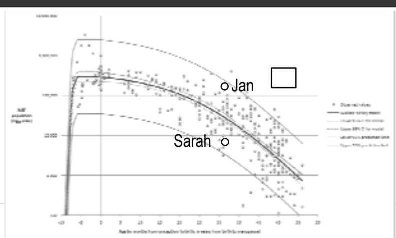
Do ovarian reserve tests correlate with oocyte quality and natural fertility or simply numbers of oocytes available during ART?

Scott Nelson
Muirhead Chair in Obstetrics & Gynaecology

Learning objectives

- Understand the role of AMH in assessing the ovarian reserve
- Appreciate the performance of AMH relative to other markers of ovarian reserve
- Be aware that AMH and age can be used together to stratify patients into prognostic groups for live birth
- Biomarkers have yet to be assessed in conjunction with novel prediction models of IVF success

Why do we need to know ovarian response?

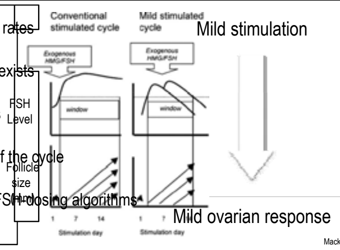


"Just do IVF and see response"

Kelsey and Wallace PLOS One 2010.

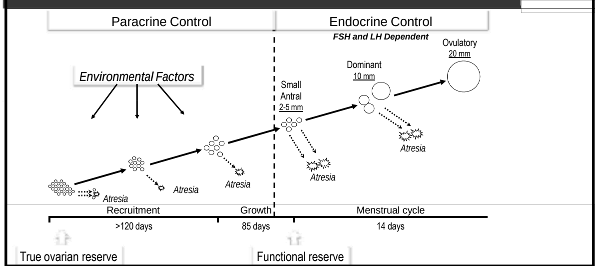
Mild stimulation: one size fits all or does it?

- Lower live birth rates
- OHSS risk still exists
- Fewer embryos
- Programming of the cycle
- Individualised FSH-dosing algorithms not available



Macklon, et al. *Endocrine Reviews*. 2006.
Fauser, et al. *Hum Reprod*. 2010.

So what options do we have?



Primary alternative are endocrine markers

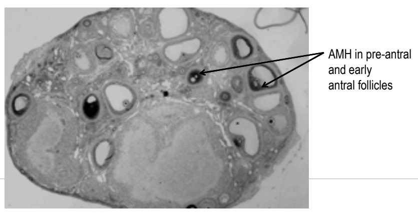
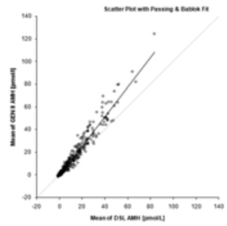


Image courtesy of Hamish Fraser, MRC.

The new AMH Gen II assay

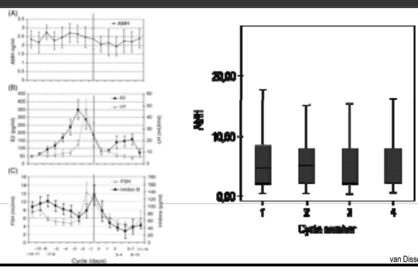
DSL antibody
Immunotech standards

Values ~40% higher



Wallace, Faye, Fleming and Nelson *Annals of Clin Biochem* 2011 (in press).

AMH is stable within and across menstrual cycles



Tsepelidis. *Hum Reprod*. 2007.
van Disseldorp, et al. *Hum Reprod*. 2010.

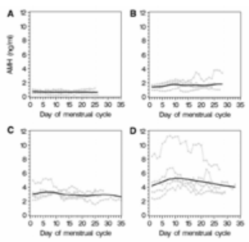
AMH menstrual cycle stability is dependent on age

4.8 ±0.7 pmol/L

12.2 ±1.5 pmol/L

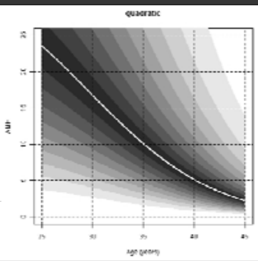
21.6 ±1.1 pmol/L

38.1 ±7.5 pmol/L



Sowers, et al. *Fertil Steril*. 2010.

Validated AMH age nomogram for DSL

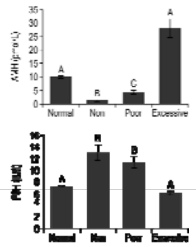


N>25,000

Nelson, et al. *Fertil Steril*. 2010, Nelson et al *RBM Online* 2011

Does AMH relate to ovarian response?

- Large prospective cohort of 340 women undergoing their first IVF cycle with a standard agonist approach: Prostap and 225IU
- AMH strongly correlates with oocyte yield
- AMH distinguishes treatment categories
- FSH does not



Nelson, et al. *Hum Reprod*. 2007

AMH correlates with oocyte yield and is better than other predictors

Author	n	R with oocytes	AMH better than				
			Ov. Vol	d3 FSH	d3 E_2	d3 inhB	age
Seifer (2002)	107	0.48		√	√		
Van Rooij (2002)	130	0.57		√	√	√	√
Fanchin (2003)	93	0.43					
Muttukrishna (2004)	69	0.69		√		√	
Hazout (2004)	109	0.38		√	√	√	√
Muttukrishna (2005)	108	0.5		√			
Elder Geva (2005)	56	0.64		√		√	
Ficicioglu (2006)	50	0.56		√	√		√
La Marca (2007)	48	0.7					
Kwee (2007)	110	0.63	√	√			√
Elgindy (2007)	33	0.88	√	√			
Nelson (2007)	340	0.71		√			√
Wunder (2008)	276	0.35		√		X	
Gnoth (2008)	132						
Nardo (2008)	165	-		√			

La Marca, et al. *Hum Reprod*. Update 2010.

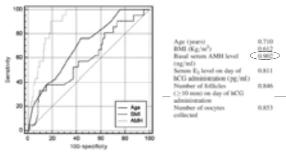
AMH for poor response: CUT-OFF values

Author	n	Study design	CUT-OFF value (ng/mL)	Sens (%)	Spec (%)
Van Rooij (2002)	119	Prosp	0.3	60	89
Muttukrishna (2004)	69	Prosp	0.1	87.5	72.2
Muttukrishna (2005)	108	Retro	0.2	87	64
Tremellen (2005)	75	Prosp	1.1	80	85
Panarrubia (2005)	80	Prosp	0.69	53	96
Ebner (2006)	141	Prosp	1.66	69	86
Ficicioglu (2006)	50	Prosp	0.25	90.9	90.9
La Marca (2007)	48	Prosp	0.75	80	93
Smeenk (2007)	80	Prosp	1.4	62	73
McIlveen (2007)	84	Prosp	1.25	58	75
Kwee (2007)	110	Prosp	1.4	76	86
Nelson (2007)	340	Prosp	0.7	75 (correctly classified)	
Gnoth (2008)	132	Prosp	1.26	97 41	
AMH <1.0pmol/L only a few pregnancies to term					

La Marca, et al. *Hum Reprod*. Update 2010.

AMH - prediction of over-response

Author	n	Study design	CUT-OFF value (ng/ml)	Sens (%)	Spec (%)	Prediction of hyper-response	Prediction of OHSS
Kwee (2007)	110	Prosp	5	53	91	√	
Nelson (2007)	340	Prosp	3.52	60	94.9	√	
Lee (2008)	262	Prosp	3.36	90.5	81.3		√



Strengths:
- High number of patients
- Optimal classification of OHSS
- Only moderate to severe OHSS considered

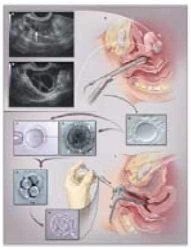
Limitations:
- Limited to Chinese population
- Not clear definition of PCOS women included

TH Lee, et al. *Hum Reprod.* 2008.

So can we use AMH to inform IVF?

AMH:
- Stable molecule
- Relatively stable across cycle
- Stable between cycles
- One assay on the market
- Age related nomogram – quadratic
- Strongly related to oocyte yield
- Can predict poor response
- Can predict excessive response
- Independent of age

- **How can we utilise this information?**

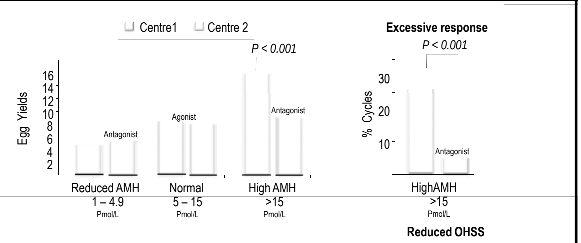


Prospective Evaluation of AMH based strategies

Centre 1 (370) Control	FSH Dose	AMH		Centre 2 (168) Control	FSH Dose
Agonist	150		High Response Risk of OHSS	Antagonist	150
		15			
Agonist	225		Normal Response	Agonist	225
		5.0			
Agonist	300		Reduced Response	Agonist	300
		1.0			
Antagonist	375		Negligible Response	Modified Natural Cycle	

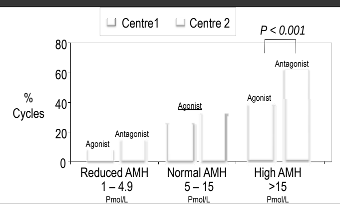
Nelson, et al. *Hum Reprod* 2009.

Lower oocyte yields in high responders



Nelson, et al. *Hum Reprod.* 2009.

Individualisation significantly improves clinical pregnancy rates



Adjusted for age and AMH antagonist protocol had substantially higher clinical pregnancy rates per cycle started [OR 4.40 (95% CI 1.95 – 9.93), p<0.001]

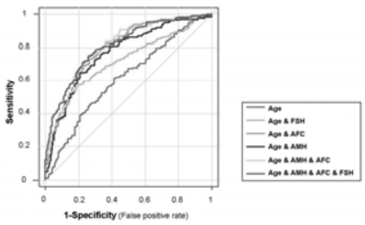
Nelson, et al. *Hum Reprod.* 2009.

AMH dictated strategic approach

AMH		Control	FSH Dose (nWt / Obese)
	High Responders (150IU daily)	Antagonist: FSH + LH	150 / 225
20	Normal Responders	Agonist: HMG or rFSH	225 / 300
10	Reduced Responders	Minimal treatment burden e.g. flare	225 / 300
4.0	Negligible response		

Composite measures incorporating AMH

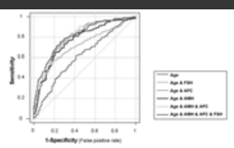
AMH and age are independent predictors of oocyte yield



IMPORT consortium submitted.

Composite measures incorporating AMH

AMH and age are independent predictors of oocyte yield



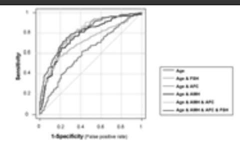
	AUROC	95% CI
Age	0.60	0.57, 0.64
AMH	0.81	0.78, 0.83
Age & FSH	0.69	0.69, 0.77
Age & AFC	0.76	0.72, 0.80
Age & AMH	0.80	0.76, 0.84
Age & AMH & AFC	0.80	0.74, 0.86
Age & AMH & AFC &FSH	0.81	0.75, 0.86

IMPORT consortium submitted.

Composite measures incorporating AMH

AMH and age are independent predictors of oocyte yield

Maximal value is from AMH



Never mind their age just measure AMH

	AUROC	95% CI
Age	0.60	0.57, 0.64
AMH	0.81	0.78, 0.83
Age & FSH	0.69	0.69, 0.77
Age & AFC	0.76	0.72, 0.80
Age & AMH	0.80	0.76, 0.84
Age & AMH & AFC	0.80	0.74, 0.86
Age & AMH & AFC &FSH	0.81	0.75, 0.86

IMPORT consortium submitted.

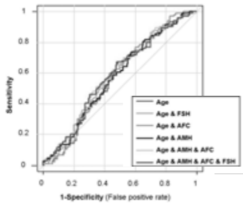
So utilising biomarkers?

- Individualise expectations of oocyte yield
- Individualise treatment strategies
- Improve safety of IVF
- Prospectively evaluate novel therapies
- A role independent of the classical biomarker date of birth?

What are our chances of having a baby?

Can biomarkers predict live birth?

- No – everything including age awful
- Limitation of ROC analysis



IMPORT consortium submitted

Can biomarkers predict live birth?

Yes if you think of "predict" in conventional terms of low medium, or high risk

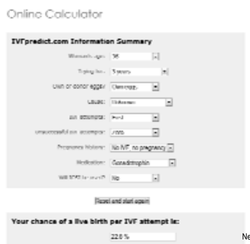
Age (years)	AMH (ng/mL)		
	<0.4	0.4 – 2.8	≥2.8
>37	0.05 (0.01 to 0.16)	0.18 (0.12 to 0.26)	0.29 (0.17 to 0.44)
31 – 37	0.09 (0.02 to 0.24)	0.27 (0.21 to 0.35)	0.40 (0.28 to 0.54)
<31	0.13 (0.04 to 0.36)	0.38 (0.26 to 0.51)	0.52 (0.38 to 0.67)

*Values in parenthesis are 95% confidence intervals

La Marca and Nelson 2011 *RBMOnline*

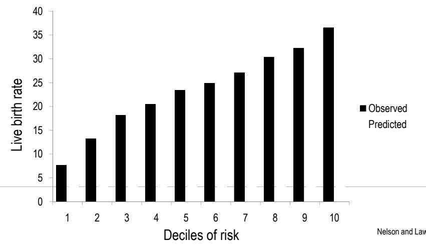
Will biomarkers add to established risk estimates?

- 144,000 fresh IVF cycles
- Baseline characteristics
- Freely available



Nelson and Lawlor *Plos Medicine* 2011.
IVFpredict.com

Accurate calibration is the key



Nelson and Lawlor *Plos Medicine* 2011.
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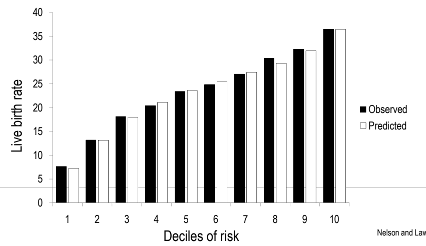


Nelson and Lawlor *Plos Medicine* 2011.
IVFpredict.com

Summary

- Just do IVF is no longer an option
- AMH is easy and relatively stable
- AMH relates strongly to oocyte yield
- Accurate prediction of live birth is feasible
- Biomarkers can individualise expectations and treatment
- Biomarkers can improve outcomes and safety of IVF

Conflicts of interest?

- no commercial conflicts of interest
- author of publications on POF
- member of ESHRE POF guideline development group

Hormone replacement therapy for premature ovarian insufficiency and menopause

Melanie Davies
Consultant Gynaecologist
University College London Hospitals

Learning objectives

- know the immediate & long-term effects of hormone deficiency
- review evidence on benefit and risk of HRT in young women
- discuss the most appropriate form of HRT
  - types of estrogen and progestogen
  - dose
  - route of administration
  - role of testosterone
- plan follow-up and duration of therapy

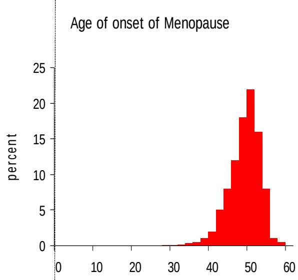
definition

loss of ovarian function:
- before the age of 40
- age > 2SDs below mean for reference population (average age for Western populations 51)

terminology:
- “ovarian insufficiency” is preferred to “failure”

prevalence

- **1% of women < 40**
- **0.1% of women < 30**
- **0.01% of women < 20**



causation

- environmental
- metabolic
- iatrogenic
- autoimmune
- genetic
- infective
- unknown

management

- make - and explain - diagnosis
- treat symptoms
- prevent long-term consequences
- address psychological needs
- treat infertility
- offer long-term follow-up and support

make the diagnosis!

- diagnosis is often delayed, even with classic symptoms of menopause

  Alzubaidi 2002

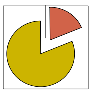
- ovarian insufficiency is often a fluctuating condition ovarian dysfunction precedes menopause

presentation

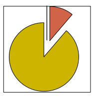
- amenorrhoea
- oligomenorrhoea
- menstrual dysfunction
- infertility
- oestrogen-deficiency symptoms

Coulam 1986, Anasti 1998

primary amenorrhoea



secondary amenorrhoea



symptoms

- flushes
- night sweats and sleep disturbance
- vaginal dryness
- loss of libido
- stiffness and muscle pain
- mood changes
- fatigue
- poor concentration and memory

diagnostic tests

- elevated FSH levels in menopausal range (usually above 40iu/l) on at least two occasions a few weeks apart
- ultrasound not required for diagnosis
- no role for ovarian biopsy — Khastgir 1994
- AMH may be useful

assessment

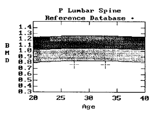
- FSH, LH, oestradiol (prolactin) (androgens) ?AMH
- thyroid function
- autoantibody screen
- karyotype (young patients)
- FRAXA screen
- pelvic ultrasound
- bone mineral density

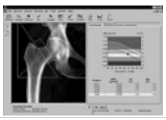
pelvic ultrasound

- ovarian activity commonly seen in POI
- may be seen in primary amenorrhoea
- associated with higher BMD
- higher chance of pregnancy Conway 2006

bone density measurement

- methods of measurement
- baseline assessment at diagnosis
- serial follow-up





long-term risks

life expectancy reduced

Rocca et al 2006

cohort of >12,000 women

2 years less life expectancy if menopause <40

- increased mortality ischaemic heart disease
- reduced uterine and ovarian cancer

Ossewarde et al 2005

long-term risks

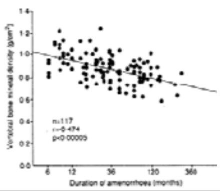
Mayo clinic cohort study – bilateral oophorectomy

- premature death
- cardiovascular disease
- cognitive impairment, dementia, parkinsonism
- osteoporosis & fractures
- ↓ psychological wellbeing
- ↓ sexual function

Shuster et al 2008

bone loss

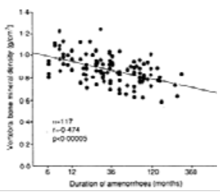
- failure to achieve peak BMD
- increased loss
- fracture rate   OR 1.5 [1.2-1.8]



Davies 1990, Notelovitz 1993, Vega 1994, Van der Voort 2003

bone loss

- failure to achieve peak BMD
- increased loss
- fracture rate   OR 1.5 [1.2-1.8]



HRT prevents bone loss
little evidence on other Rx:
bisphosphonates
calcium + vitamin D

cardiac disease

- ischaemic heart disease increased in POF Lokkegaard 2006
- subclinical coronary artery disease
  x 2 (OR2.0, 95%CI: 1.2-3.4) after TAH+BSO
  modified by HRT within 5 years of oophorectomy Allison 2008
- vascular endothelial dysfunction with oestrogen-deficiency
  improved by HRT Kalantaridou 2004, Ostberg 2007

cognitive function

oophorectomy associated with

- increased risk of dementia:
  linear trend with age at menopause
- increased risk of Parkinsonism Rocca 2008

HRT

- which type?
- what dosage?
- what duration?

there are no satisfactory RCTs to determine the ideal dose, regimen or delivery system for young women

OCP vs HRT

OCP	HRT
• synthetic	• physiological
• more potent	• may be safer for long-term use
• Pill-free week	• continuous estrogen
• like peer-group	• stigma of HRT
• reminder of infertility	• not contraceptive
• free in UK	• UK prescription charge x2

HRT type

- cyclical or “no bleed” HRT?
- choice of progestogen?

  C19 e.g. norethisterone, norgestrel and levnorgestrel
  C21 e.g. dydrogesterone and medroxyprogesterone acetate
- route of administration?

HRT dosage

- standard HRT doses may be suboptimal
- monitor by symptoms and BMD
  (oestradiol levels useful only for implants)
- urogenital symptoms may need local Rx
  (vaginal moisturisers, topical oestrogen)

testosterone

- androgen levels ↓ in POI
  (half of testosterone supply from ovaries) Hartmann 1997

- reduced libido, sexual function, ?energy ?BMD
- worse in oophorectomised women

- replacement – patches (Intrinsa), implants
  s/e excess hair growth and acne
  Braunstein 2005, Shifren 2007

alternatives to HRT

- efficacy lower than HRT:
  - serotonin and noradrenaline re-uptake inhibitors
  - clonidine
  - gabapentin
- efficacy unproven:
  - progesterone transdermal creams
  - phyto-oestrogens (soy, red clover)
  - acupuncture
- safety unproven:
  - herbal preparations (black cohosh, dong quai)

Panay and Rees 2006

benefits and risks

- Women's Health Initiative study and Million Women studies are not applicable to young women

- breast cancer: *less common in POI, ? effect of physiological HRT*
- ischaemic heart disease : *HRT may benefit*
- osteoporosis : *clear benefit*

HRT duration

until expected age of menopause

"In women who have experienced a premature menopause (due to ovarian failure, surgery or other causes) HRT may be used for treatment of menopausal symptoms and for prevention of osteoporosis until the age of 50 years. After this age, therapy for prevention of osteoporosis should be reviewed and HRT considered a second choice"

MHRA 2007

psychological needs

- counsellor is key member of clinic staff
- information
  - from health professionals
  - from support groups

http://www.pofsupport.org/ www.daisynetwork.org.uk

The International Premature Ovarian Failure Association

THE DAISY NETWORK®

fertility (1)

- HRT is not contraceptive!
- spontaneous pregnancy rate 5-10%
- miscarriage rate ? 20%

Van Kasteren 1999

- prognostic factors:
  - recent diagnosis – short period of amenorrhoea
  - fluctuating FSH
  - ovarian activity on ultrasound
  - POI due to autoimmunity or chemotherapy

fertility (2)

- treatment strategies unproven:
  - stimulation after FSH suppression (OCP, GnRH-a)
  - corticosteroids
- review of >50 case reports, > 20 studies (poor quality)
  194 patients 3 pregnancies
  conclusion: no difference from background rate

Van Kasteren 1999

HRT for egg donation

summary

- POI is under-diagnosed – need improved awareness and information
- HRT effectively treats symptoms
- HRT can prevent long-term consequences
- HRT essential for egg recipients
- paucity of research on HRT in young women – cannot apply studies in older women
- watch out for the ESHRE guidelines!

Preservation of fertility:
Oocyte or ovarian tissue freezing

Professor Dror Meirow

Fertility preservation Center,
Sheba Medical Center,
Sackler school of medicine, Tel Aviv University, Israel.

ESHRE 27th annual meeting 2011
Stockholm, Sweden

Learning Objectives

- Indications for fertility preservation.
- Fertility preservation options.
- Egg, embryo freezing; results, odds and cons.
- Ovarian tissue freezing; indications and results.
- Decision making; egg vs. ovarian tissue freezing.

Meirow D. 201

Indications for fertility preservation

In Cancer patients

- Chemotherapy that causes ovarian injury.
- Pelvic irradiation.
- Ovarian surgery.
- Genetic- hereditary cancer gene mutation (BRCA).

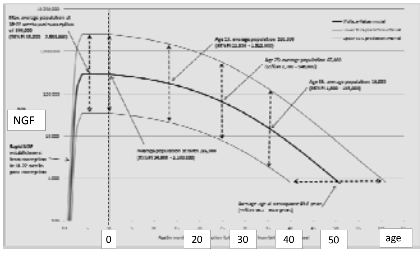
Benign non- oncologic indications

- Chemotherapy.
- Ovarian surgery.
- Endometriosis – severe, ovarian involvement.
- Genetic- fragile X, Turner syn. Mosaic.
- Family planning, age related (social preservation).

Meirow D. 201

Ovarian reserve assessment

- Age
- Hormone profile (FSH, E2) AMH
- AFC
- Previous IVF treatments

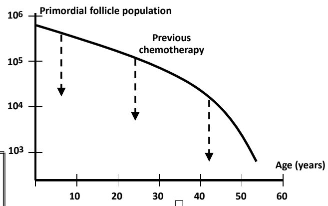


Meirow D. 201

Wallace Kelsey 2010

Consulting young female cancer patients- Risk assessment

Ovarian reserve



Toxicity risk

Pelvic Rx.	↑↑↑↑
Alkylating agents	↑↑↑
platinum agents	↑↑
Taxanes	↑↑
Plant alkaloids	↑
Anthracyclines	→
Anti metabolites	→

Assessment of an individual Patient sterilization risk

Meirow D. 201

Meirow & Wallace 2010

Evaluation of patients before Fertility preservation procedure

- Sterilization risk of future planed treatment.
- Age, family planning
- Ovarian reserve
- Time available – window for fertility preservation.
- Medical status – be aware of complications.

Only for cancer patients:

- Risk of ovarian cancer cells involvement.
- Estrogen sensitive tumors.
- Previous recent chemotherapy treatments.

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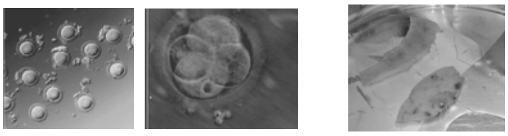
Options for fertility preservation

Egg, Embryo freezing

Ovarian tissue freezing



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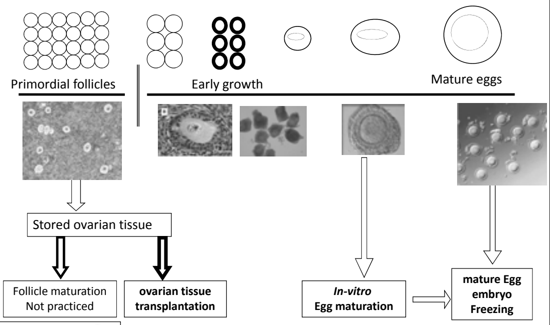
Different approaches for fertility preservation

Currently used & experimental



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Cryopreservation and Transplantation of ovarian tissue.

A realistic technique and dilemmas

Meirow D. 201

Ovarian tissue cryopreservation in cancer patients

Advantages

- **Large number PMF survive freezing / thawing.**
- **Fast fertility preservation procedure.**
- **Well-adapted to children.**
- **Can prevent mutagenic effects of chemotherapy.**
- **Can produce many cycles of mature eggs after grafting.**

Disadvantages

- **Experimental (conditions, transplantation, outcome).**
- **Not economic- many PMF are lost.**
- **Risk of cancer cells.**

Meirow D. 201

Storing ovarian tissue has been practiced during the last decade to preserve fertility.

Ovarian cryobanking as a strategy to preserve fertility
After first pregnancies in the sheep model.

Gosden, Baird, Donnez, Meirow, Oktay

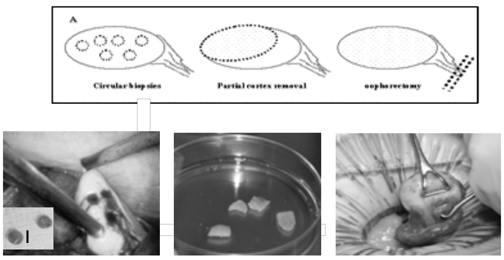
Ovarian tissue banking in patients with Hodgkin's disease. Is it safe? Meirow D *et al.* (Fertil. Steril. 1998)

A laparoscopic technique for obtaining ovarian cortical biopsies for fertility conservation in cancer patients.

Meirow D *et al.* (Fertil. Steril. 1999)

Meirow D. 201

Operation – laparoscopy/ Lap



When sterilization risk is minimal, is it justified?

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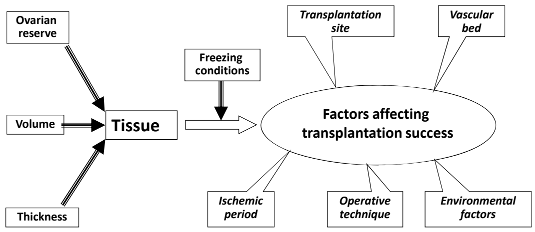
Factors affecting procedure success

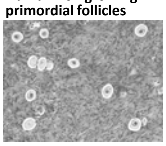


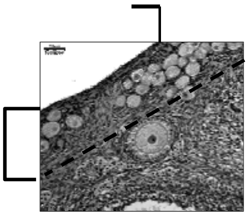
Meirow et.al.2010

Meirow D. 201

Preparation for freezing- Tissue thickness

Human non growing primordial follicles





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Freezing conditions

Slow freezing Gosden, Gook, protocols

Medium- Libovitz, oocyte,

Cryoprotectant – DMSO, PROH

Serum- autologous, comercial

Sucrose - +/-

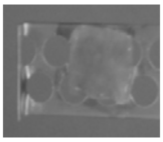
Survival of ~ 70% of follicles.

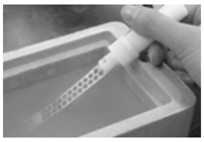
Meirow D. 201

Freezing conditions

Vitrification - Kagawa protocol

- Thin slices 1mm cryotome
- Rapid cooling
- High follicle survival
- No reports on human pregnancies





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Surgical grafting of ovarian tissue

Successful sites

- Orthotopic pelvis
- Ovary

Failures

- Arm
- Abdominal wall

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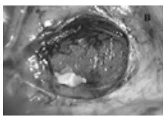
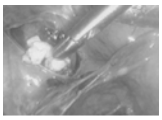
Orthotopic Surgical grafting of ovarian tissue

Publications: Radford, Oktay, Donnez, Demeestere, Silber

Indications:

- Better option ?
- No ovary
- Fibrosis vascular bed

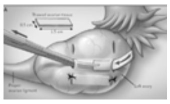




Meirow D. 201

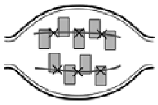
Grafting ovarian tissue to the ovary

Meirow D Dor J NEJM 2005



Donnez J. et.al. 2008

Andersen Hum Reprod. 2008



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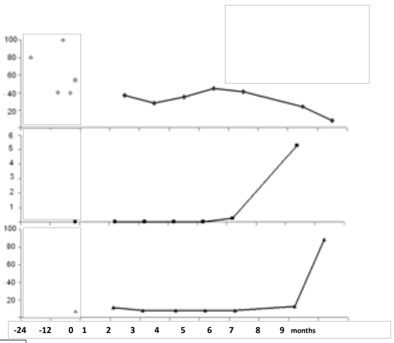
Basal hormone levels

FSH
IU/L

AMH
ng/ml

Inhibin B
pg/mL



Meirow D. 201

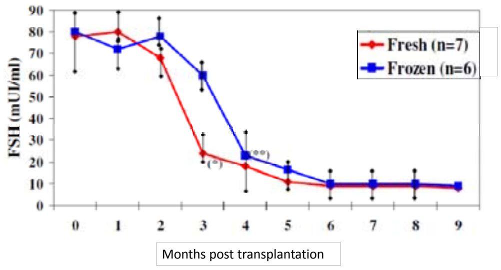
Children born after ovarian transplantation. A review of 13 live births.

- Age at tissue collection 19-36
- Previous chemotherapy 40%.
- Endocrine results.
- IVF / Spontaneous pregnancy 50%.
- Pregnancy results- normal babies 100%.

Donnez et al 2011

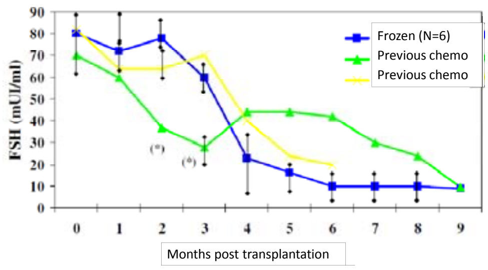
Meirow D. 201

Recovery of Endocrine function



Meirow D. 201

Recovery of Endocrine function



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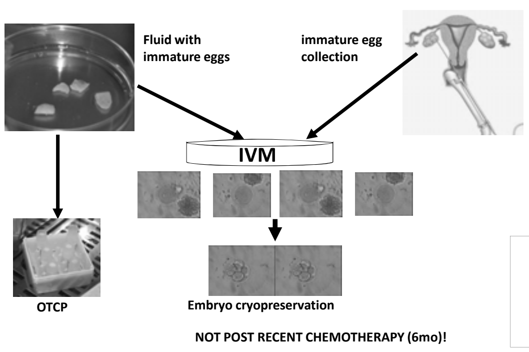
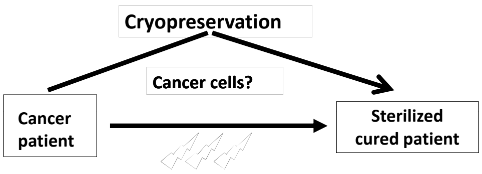
single procedure- IVM + ovarian tissue



NOT POST RECENT CHEMOTHERAPY (6mo)!

Meirow D. 201

Detection of Microscopic Metastases in Cryopreserved Ovaries.



- Use the most sensitive techniques for detection.

Meirow D. 201

EDITOR'S CORNER

FERTILITY AND STERILITY®

Ovarian tissue banking in patients with Hodgkin's disease: is it safe?

Dror Meirow, M.D., Dina Ben Yehuda, M.D.,† Diana Prus, M.D.,‡ Aaron Pollack, M.D.,† Joseph G. Schenker, M.D.,* Eliezer A. Rachmilewitz, M.D.,† and Aby Lewin, M.D.**

Hadassah University Hospital, Jerusalem, Israel

Fertil Steril 1998

FAST-TRACK

Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients

Dror Meirow[1,5], Izhar Hardan[2], Jehoshua Dor[1], Eduard Fridman[3], Shai Elizur[1], Hila Ra'anani[1], Elena Slyusarevsky[4], Ninette Amariglio[2], Eyal Schiff[1], Gideon Rechavi[2], Arnon Nagler[2] and Dina Ben Yehuda[4]

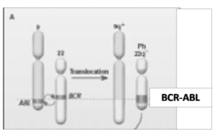
Human reproduction 2008

Meirow D. 201

Evaluation of ovarian tissue (CML)

- A 20-year-old female was diagnosed with CML.
- Ovarian tissue harvested for cryopreservation prior to bone marrow transplantation.
- Fragments of cortex were evaluated for MRD.

Philadelphia chromosome (reciprocal translocation t(9;22) is present in 95% of patients with CML.



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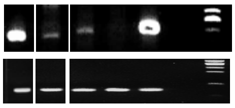
Real-time PCR to detect CML cells

1 2 4 5 6 7 8

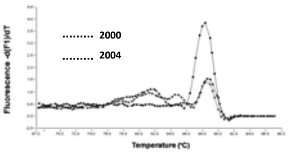


Bcr-Abl

Abl

Real time PCR products run on 2% agarose gel

Real time PCR melting curves for Bcr-Abl



1. Bone marrow
2. Ovary 2000
4. Ovary 2004
5. Control
6. Positive control
7. No template
1. marker

The tissue was not thawed.

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~~The % of ovarian metastasis was 22.4%~~

Leukemia	10.2% -7.9%
Breast ca	25.0%- 0%
Uterine ca	13.3%- 0%
Lymphoma	14.7% - 10.7%
pulmonary ca.	>24.8%,
GI ca. (gastric; colon)	54.2; 26.1%.

K. Kyono, *et.al.* Fertil Steril 2010

Meirow D. 201

Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia

Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. (26 patients)

Rosendahl, Andersen, et.al. Fertil Steril 2010

Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. (18 patients)

Dolmans, Donnez et.al. Blood 2010

Meirow D. 201

Fertility preservation using

Embryo freezing

and

Egg freezing

Meirow D. 201

Embryo freezing for fertility preservation

- Currently the most widely used method to preserve fertility worldwide.
- Cycle success rate – according with ovarian reserve.
- In cancer patients- thousands of patients worldwide.

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IVF for benign conditions - Dilemmas

- No partner – Egg freezing or Donor sperm.
- Older patients- Low ovarian reserve.
- Adolescent patients
- Time needed before treatment.
- Patient's health condition.

Meirow D. 201

Egg Freezing for fertility preservation- Justified?

Egg Freezing using vitrification method – high success rate.

	Frozen eggs 300 patients	Fresh cycles 300 patients
No of transfers	267	259
Embryos replaced	1.7 ± 0.7	1.7± 0.7
Implantation rate	39.9%	40.9%
Clinical pregnancy rate / transfer	55.4%	55.6%

A. Kobo, A. Pellicer *et.al.* Hum Reprod. 2010

Meirow D. 201

~~Egg Freezing for fertility preservation~~

- Older patients- Low ovarian reserve.
- Adolescent patients – Are ovarian stimulation & OPU justified?
- Family planning – Age 30-40y according with ovarian reserve.
- Time available before medical treatment.
- Patient's health condition.

Meirow D. 201

IVF in cancer patients - Dilemmas

- Time needed for IVF before chemotherapy.
- Age –children, aged patients- ovarian reserve.
- Patient's health condition.
- No partner –Egg freezing or Donor sperm.
- Success rate in cancer patients.
- IVF post exposure to chemotherapy.
- Hormone sensitive tumors.

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Indications for IVF In cancer patients

E. Ginsburg
Brigham & Women's Hospital, USA, 2010

Pre-therapy Diagnoses	N (%)
Breast Cancer	16 (42 .1)
Cervical Cancer	1 (2.6)
Colorectal Cancer	3 (7.9)
Endometrial Cancer	1 (2.6)
Hodgkin's Lymphoma	1 (2.6)
Leukemia, AML, ALL	3 (7.9)
Malignant Brain Tumor, Glioma	1 (2.6)
Sarcoma	1 (2.6)
Multiple Sclerosis	3 (7.9)
Myelodysplastic Syndrome	1 (2.6)
Non-Hodgkin's Lymphoma	2 (5.3)
Ovarian Epithelial Carcinoma	3 (7.9)
Systemic Sclerosis	1 (2.6)
Systemic Lupus Erythematosus	1 (2.6)

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Stimulation Protocols for cancer patients

Minimal stimulation.
- Minimizes OHSS risk
- Fewer embryos banked

"Standard Stimulation".
OHSS risk real: could delay cancer treatment
- Higher E2 levels
- More embryos banked

Special protocols for Estrogen sensitive tumors.

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Ovarian Response to Ovulation Induction In Cancer

	Cancer Cases (n=28)	Male Factor Controls (n=135)
Age (y) Range	34 ± 5.1 (20-41)	35.4 ± 3.5 (20-41)
Day 3 FSH/ E2	7.5 ± 3.2/ 35 ± 15	8.3 ± 2.6/ 35 ± 15
Gonadotropin Dose	3,507 ± 1,012	3,306 ± 1,164
Peak E2 pg/ mL	1,515 ± 712	1,393 ± 769
#oocytes	14 ± 9	12 ± 7

Mean ± SD

Knopman Fertil Steril 2009

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Ovulation Induction In Cancer Patients

	study	controls	P value
No of patients	50	50	-
age	32	32	NS
FSH	7.3	6.3	NS
eggs	13	11.5	NS
2PN	7.4	6.8	NS
Stimulation days	10.5	9.0	P<0.001
FSH dose	4174	3416	P<0.003

controls= male factor, egg donor, oocyte cryo

Retrospective cohort study.

Quintero R Fertil Steril 2010

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Embryo yield after IVF in women undergoing fertility preservation before chemotherapy

	Fertility Preservation	Controls	P value
Age (mean ± SD)	34 ± 5	35 ± 4	0.12
stimulation days	11 ± 2	11 ± 2	0.33
FSH (IU)	4,184 ± 1,791	3,487 ± 1,897	0.02
Peak E_2 (pg/mL)	1,456 ± 1,093	2,098 ± 1,037	0.001
No. of oocytes retrieved mean ± SD (range)	12 ± 8 (2–46)	14 ± 9 (0–52)	0.06
No. of mature oocytes mean ± SD (range)	9 ± 6 (2–33)	11 ± 7 (0–40)	0.04
Fertilization, %	**62**	**55**	0.14
No. of embryos (2PN) mean ± SD (range)	**6 ± 5 (1–23)**	**7 ± 6 (0–42)**	**0.64**

N= 38

A. Robertson Fertil Steril 2010

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Oocyte/ Embryo Banking: European Registry

- Retrospective cohort study, 205 women, 70 ART centers 2007-9 (FertilPROTEKT network)
- Ages 18-40, mean 30.5
- Diagnoses: breast ca, lymphoma, Gynecol. malignancies, benign disease

Results:

- No response in 0.9%, no ER in 1.5%
- 125 women inseminated all eggs. In this group:
- No of eggs: mean 11, median 10 $\pm$6.6
- Mean fertilization rate 61.3%

Lawrenz *et al* Fertil Steril 2010

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IVF for fertility preservation in cancer Patients- Results

Reference	Eggs		2PN		P
	cancer	controls	cancer	controls	
Oktay 2005	12.3		5.3		
Knopman 2009	14 ± 9	12 ± 7			NS
Quintero 2010	13	11.5	7.4	6.8	NS
Robertson 2010	12 ± 8	14 ± 9	6 ± 5	7 ± 6	NS
Lawrenz 2011	11.6 ± 7.7		7		

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IVF time frame
Luteal Stimulation with GnRH antagonist

- Embryo cryopreservation with IVF 24 pt.
- GnRH-ant concurrent with FSH simulation.
- Luteolysis within 4d (early luteal) or 2d (mid luteal).

	follicular	Luteal
Gonadotropin used		More NS
Stimulation duration	10.6	11.4
Oocytes obtained	13	10
Fertilization rate	61	76

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Von Wolff M *et al* Fertil Steril 2009

Minimal Stimulation/ IVM Protocol

	Shalom Paz 2010	Maman 2010 Luteal	Maman 2010 follicular	Strowitzki 2010
No. cycles	31	5	13	215
Oocytes /cycle	9.7 ± 6.4	12.8 ± 8.4	17.3 ± 13.5	8.9
Total MII in 48h		7.0 ± 7.6	9.5 ± 7.7	
Maturation rate		48%	58%	
Fertilization rate	77.8 %	69%	63%	
Embryo stored	4.5 ± 2.71			2.8

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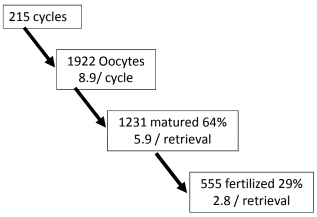
IVM results for fertility preservation



- Better results vitrifying mature rather than immature oocytes.
- Vitrification of *in vivo* mature + *in vitro* mature better results.

T. Strowitzki ESHRE 2010

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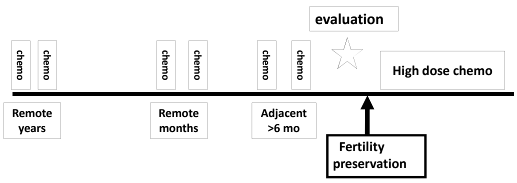
Fertility preservation post chemotherapy



IVF > 40% of patients with Hematological malignancies had previous chemotherapy.

OTCP > 50% of patients with Hematological malignancies had previous chemotherapy.

Sheba experience

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Ovarian reserve after chemotherapy for breast cancer Premenopausal survivors compared with controls.

		Mean	Min-max	P value
AFC	Controls Survivors	11 5	1 - 34 0 - 12	**0.0042**
AMH	Controls Survivors	1.8 0.6	0.3 - 6.3 <0.1 - 2.4	**0.0004**
FSH	Controls Survivors	8.0 11.6	3.1 - 17.7 3.3 - 24.5	0.02
Inh B	Controls Survivors	46.6 24.3	10.0-152.1 10.0-91.8	0.02
E2	Controls Survivors	38.8 126	12.0-89.0 14.4-806.0	0.14

20 pt. in each group

A. Partridge Fertil Steril 2010

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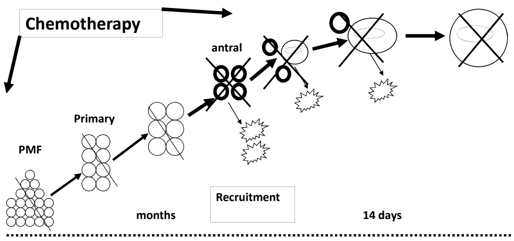
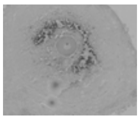
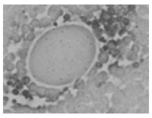


Apoptosis in large follicles
Post cyclophosphamide.





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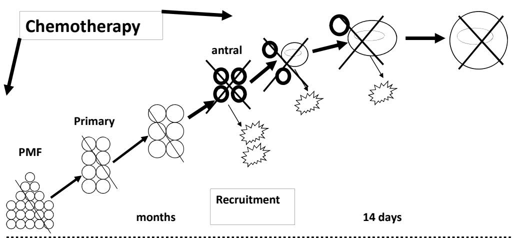
Ovarian tissue cryo-preservation post recent Chemotherapy

2-3 months post chemotherapy

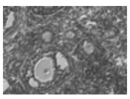
Disease	Age	IVF Eggs	Biopsy PMF
Non-Hodgkin's D	21	0	+ +
Hodgkin's D	25	0	+ + +
Hodgkin's D	25	0	+ + +

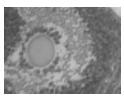
Meirow et.al. Leukemia Lymphoma 2007

Meirow D. 201



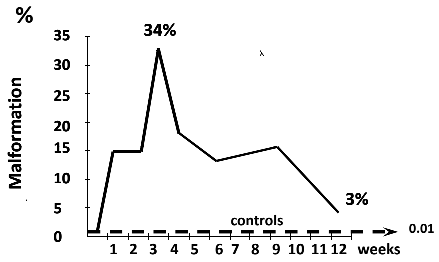
primordial follicles <<< Large follicles





Meirow D. 201

Malformation rate - time scale



weeks post cyclophosphamide

Meirow D. Gosden R. et al Hum Reprod 2000

Meirow D. 201

Conclusions
Effects of chemotherapy on large follicles

- **Immediate Apoptosis.**
- **Large follicles > > primordial follicles.**
- **DNA damage.**
- **Very low ovulation rate post exposure.**
- **High abortion and malformation rate.**

We do not collect mature or immature eggs for fertility preservation in patients recently exposed to chemotherapy (up to 6 months)

Meirow D. 201

ART and embryo / Egg freezing in breast cancer patients. Safe protocols for ovarian stimulation.

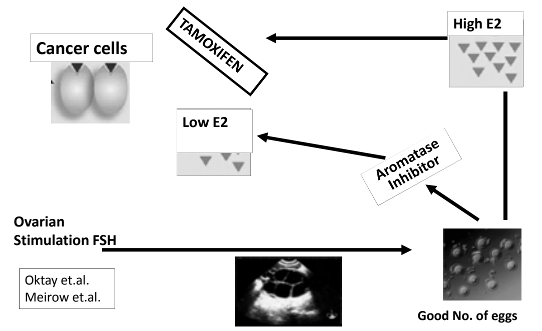
Meirow D. 201

IVF In hormone sensitive tumors

- **Normal stimulation**
- **Aromatase inhibitor (Letrazole) reduce E2 levels.**
  (Oktay *et.al.*)
- **Tamoxifen blocks Estrogen receptors.**
  (Meirow *et.al.*)

Meirow D. 201

Hormone sensitive- Breast cancer protocols



Meirow D. 201

Conclusive points for discussion

Meirow D. 201

IVF & fertility preservation research center

Effect of postponing pregnancy on society as a whole: population impact, demand for/access to infertility treatment, financial implications

Siladitya Bhattacharya
University of Aberdeen

Outline

- **Delaying pregnancy – trends**
- **Impact on total fertility rates**
- **Clinical implications**
- **Social implications**
- **Costs and consequences of ART**
- **Summary**

Age and natural livebirth rate

Length of exposure (months)	Starting age (yrs)		
	30	35	40
12	75	66	44
48	91	84	64

Leridon Hum. Reprod. 2004

Women's perceptions on delay

Perceptions of delaying childbearing.

	Subfertile (n = 362)	Pregnant (n = 362)	*P* value
When did you try for your first planned pregnancy			
<30 years	227 (62.7%)	273 (75.4%)	<.001[a]
>30 years	135 (37.3%)	89 (24.6%)	
Did you use contraception before trying for your first pregnancy?			
Yes	316 (88.3%)	284 (79.6%)	.002[a]
No	42 (11.6%)	73 (20.2%)	
How many years did you use contraception for?			
<5 years	118 (37.7%)	108 (37.5%)	
6–9 years	91 (29.1%	80 (27.8%)	.189[b]
>10 years	104 (33.2%)	100 (34.7%)	
Do you feel you postponed trying for pregnancy until your circumstances were different?			
Yes	260 (73.2%)	193 (53.8%)	
No	95 (26.8%)	166 (46.2%)	<.001[a]

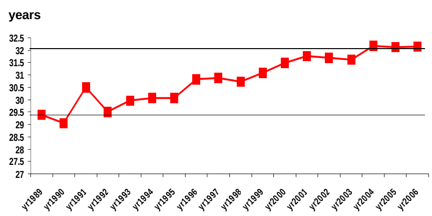
Maheshwari et al, 2007

Reasons for reproductive delay in women over 33 years

Relationships	**74%**
Other distractions	**52%**
Work or other training	**34%**

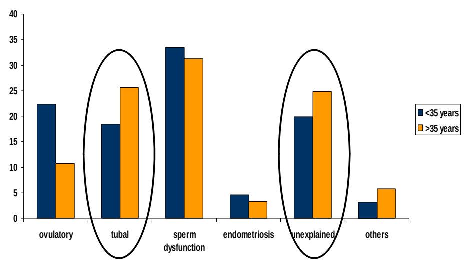
Proudfoot, et al 2009

Infertility: mean female age at referral



Maheshwari et al, Hum. Reprod. 2008

Age and the cause of infertility



Maheshwari et al, Hum. Reprod. 2008

Age and the odds of unexplained infertility

Age	Adjusted* OR (95% CI)
< 30 yrs	1
30 – 34 yrs	1.5 (1.3, 1.8)
35 – 39 yrs	1.8 (1.4, 2.2)
> = 40 yrs	1.2 (0.9, 1.6)

**Adjusted for year of diagnosis*

Maheshwari et al, Hum. Reprod. 2008

Consequences of delaying pregnancy

	24 mths spacing	Additional spacing for 1st birth	
		30 mths	69 mths
Mean fecundability	0.23	0.231	0.23
Mean age at maternity	29.1	31	32.9
Mean no of children	2.004	1.900	1.766
Mean age at first pregnancy attempt	25.1	27.6	30.8
Mean time to first conception	9.5	10.5	10.1
Infertile	6.9%	10.1%	12.6%

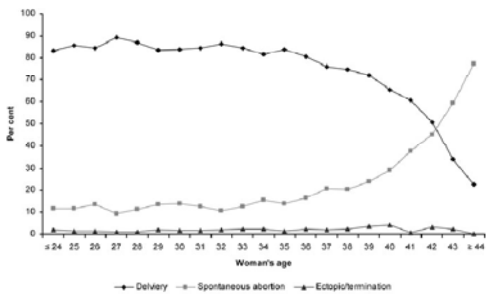
Leridon & Slama Hum. Reprod. 2008

Factors affecting the chances of live birth

	Adjusted Odds	95% CI
Previous pregnancy	1.8	1.2 – 2.7
Infertility < 3 yrs	1.7	1.1 – 2.5
Female age < 30 yrs	1.5	1.1 – 2.2

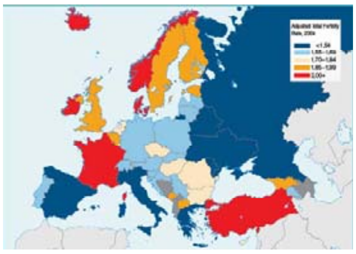
Collins et al,1995

Age and the outcome of IVF

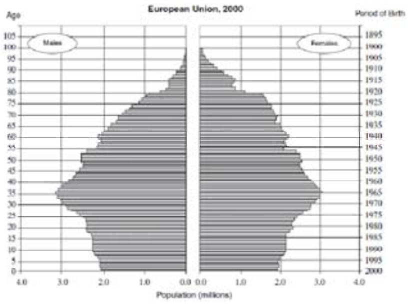


Wang et al Hum. Reprod. 2007

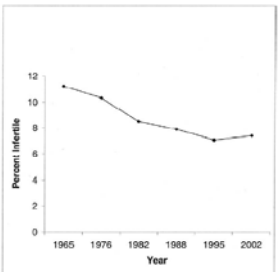
Adjusted total fertility rates in Europe



ESHRE Capri Hum. Reprod. 2010



Infertility (12 mths): married women 15-44 yrs



Stephen & Chandra Fertil Steril. 2006 Sep;86(3):516-23

Prevalence of infertility in Grampian: a comparison*

	1988 survey** Women 46-50 yrs N = 766	2007 survey Women 46 -50 yrs N = 1148
Primary infertility	7.9%	6.2%
Primary & secondary infertility	1.7%	0.3%
Secondary infertility	7.3%	2.7%
Total infertility	15.2%	9.2%

*** Templeton et al, 1990 BMJ* **P < 0.05*

Europe: predicted population 2004-50

10% decline	1-10% decline	1-10% increase	> 10% increase
Baltic Republics	Slovenia	UK	Sweden
Czeck Republic	Portugal	France	Malta
Slovakia	Greece	Spain	Ireland
Poland		Austria	Cyprus
Germany		Finland	Luxembourg
Italy		Denmark	
		Netherlands	

Rychtarikova, 2007

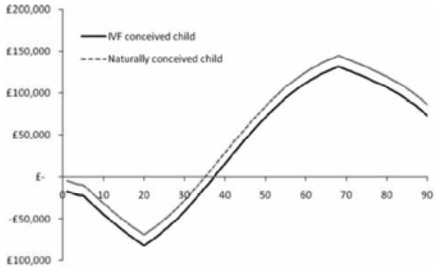
Delay vs other reasons for declining fertility

- Contraception
- Wish for smaller families
- Lack of support for child rearing
- Decreasing fecundity
- Reproductive delay
- Access to and uptake of fertility services

Interventions to increase fertility

- Income support
- Work related policies
- Access to ART

Projected value of spontaneous and IVF child born to a mother aged 35 yrs



Connolly et al Hum. Reprod. 2008

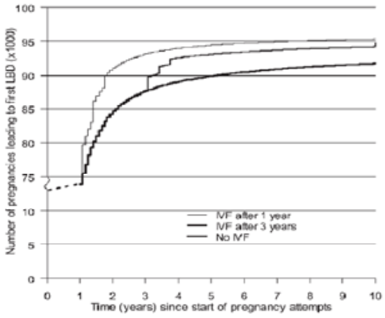
ART after reproductive delay

	Mean age at first attempt		
	25	27.5	31
Years of postponement	–	2.50	6.00
No. of naturally conceived children	2.00[a]	1.90[b]	1.77[c]
ART contribution	0.04[d]	0.05[d]	0.05[d]
Total No. of children	2.04	1.95	1.82

[a]CF, Cohort fertility.
[b]Minus 5% of CF.
[c]Minus 11% of CF.
[d]Plus 2.5% of CF.

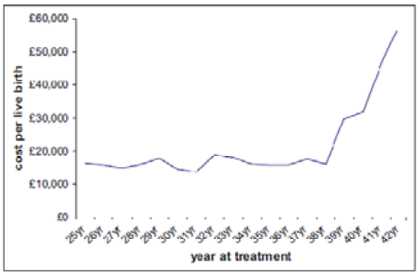
Leridon & Slama Hum. Reprod. 2008

Timing of IVF and livebirths



Habbema et al Hum. Reprod. 2009

Age & the cost of IVF live birth



Maheshwari etal, Fertil Steril. 2010

Cost of an IVF livebirth

Female age

	< 30 yrs	30–34 yrs	35–39 yrs	≥ 40 yrs
Total cost of IVF cycle	£885,802	£1,910,541	£1,753,577	£459,544
Cost per pregnancy [mean (95% CI)] (positive pregnancy test)	£7,444 (£6,579–£8,600)	£7,129 (£6,516–£7,887)	£7,624 (£6,898–£8,530)	£14,361 (£11,095–£20,792)
Total cost of early pregnancy care and OHSS	£60,400	£114,187	£99,681	£15,087
Cost per ongoing pregnancy [mean (95% CI)] (viable pregnancy at 11 wks)	£10,513 (£9,036–£12,633)	£10,330 (£9,161–£11,548)	£11,300 (£10,006–12,938)	£31,642 (£21,141–£58,979)
Total cost of antenatal care and delivery	£522,648	£1,090,603	£882,246	£96,214
Cost per live birth (mean, 95% CI)	£16,503 (£14,789–£18,866)	£16,058 (£14,836–£17,609)	£17,096 (£15,635–£18,937)	£40,320 (£27,105–£65,036)

Maheshwari etal, Fertil Steril. 2010

Reproductive delay: Summary

- Significant delay
- Social and economic reasons
- Impact on spontaneous and treatment assisted births
- Effect on total fertility rate & population growth
- Measures to address this: social and clinical
- Economic value of IVF
- Cost effectiveness of IVF in older women

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