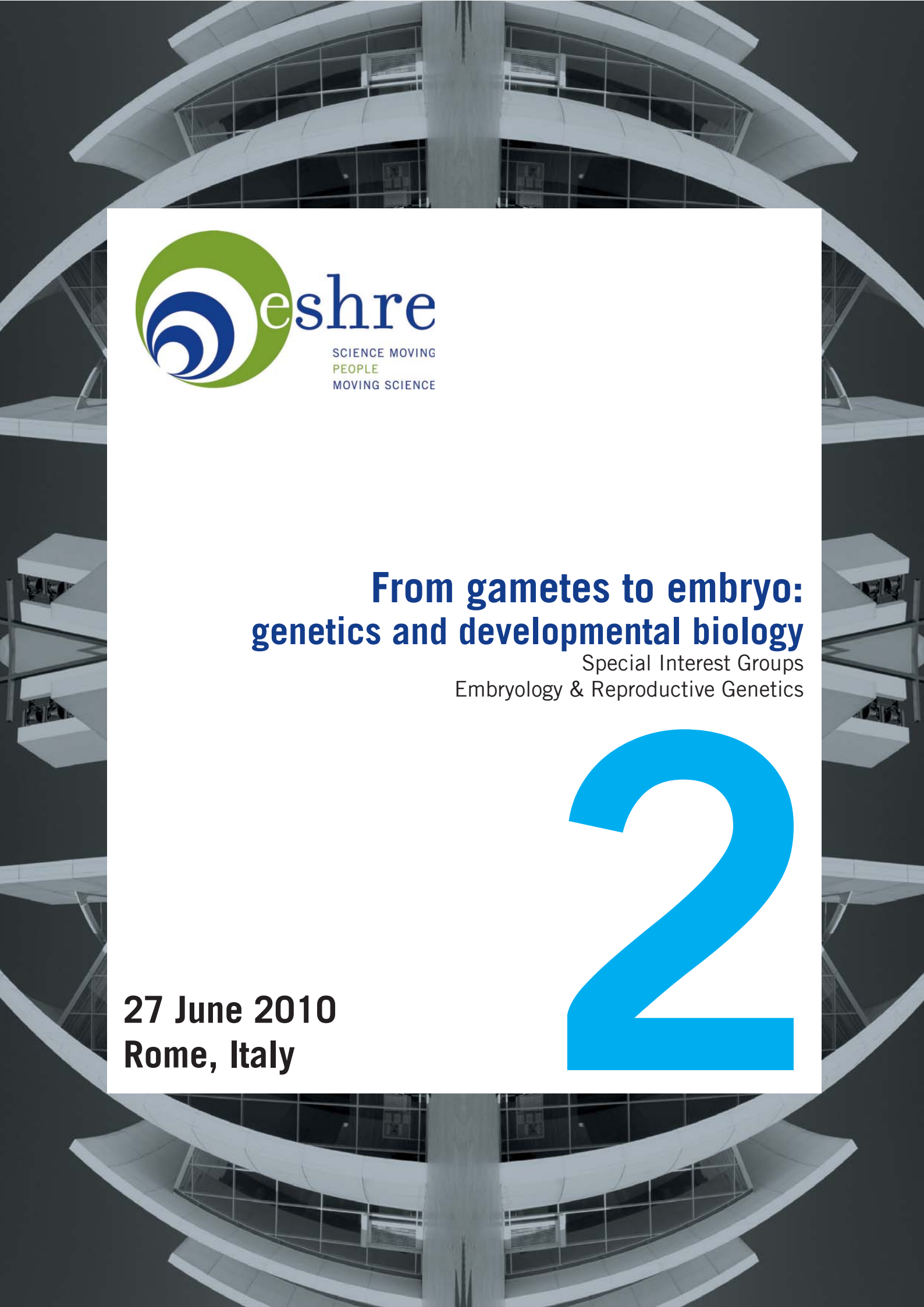


eshre

SCIENCE MOVING
PEOPLE
MOVING SCIENCE

From gametes to embryo: genetics and developmental biology

Special Interest Groups
Embryology & Reproductive Genetics

2

27 June 2010
Rome, Italy

PRE-CONGRESS COURSE 2 – Table of contents

From gametes to embryo: Genetics and developmental biology

Organised by the Special Interest Groups Embryology & Reproductive Genetics

ESHRE – European Society of Human Reproduction and Embryology

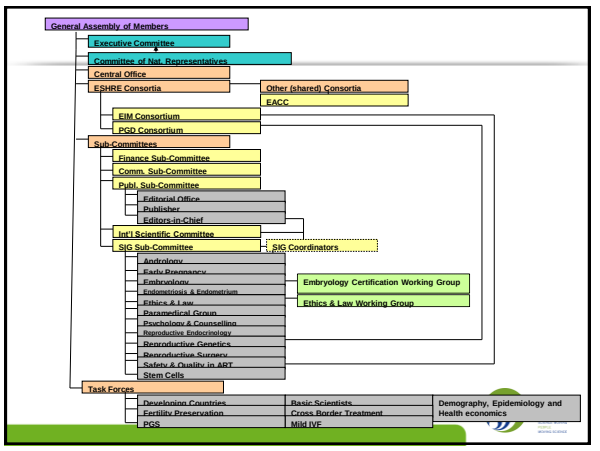
What is ESHRE?

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

- promote interest in, and understanding of, reproductive science and medicine.
- facilitate research and dissemination of research findings in human reproduction and embryology to the general public, scientists, clinicians and patient associations.
- inform politicians and 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
	Heidi Van Ranst	Belgium
	Veljko Vlaisavljevic	Slovenia
	Søren Ziebe	Denmark



ESHRE Activities – Annual Meeting

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

Track record:

ESHRE 2008 – Barcelona: 7559 participants
ESHRE 2009 – Amsterdam: 8132 participants

Future meetings:

ESHRE 2010 – Rome, 27-30 June 2010
ESHRE 2011 – Stockholm, 3-6 July 2011

ESHRE Activities – Scientific Journals

Human Reproduction with impact factor 3.773

Human Reproduction Update with impact factor 7.590

Molecular Human Reproduction with impact factor 2.537

ESHRE Activities – Campus and Data Collection

- Educational Activities / Workshops
  - Meetings on dedicated topics are organised across Europe
  - Organised by the Special Interest Groups
  - Visit: www.eshre.eu under CALENDAR
- Data collection and monitoring
  - EIM data collection
  - PGD data collection
  - Cross border reproductive care survey

ESHRE Activities - Other

- Embryology Certification
- Guidelines & position papers
- News magazine “Focus on Reproduction”
- Web services:
  - RSS feeds for news in reproductive medicine / science
  - Find a member
  - ESHRE Community

ESHRE Membership (1/3)

- ESHRE represents over 5,300 members (infertility specialists, embryologists, geneticists, stem cell scientists, developmental biologists, technicians and nurses)
- Overall, the membership is distributed over 114 different countries, with 50% of members from Europe (EU). 11% come from the US, India and Australia.

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	(€ 200)

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

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

Annual Meeting

Rome, Italy 27 June to 30 June 2010

Pre-congress courses (27 June):

- PCC 1: Cross-border reproductive care: information and reflection
- PCC 2: From gametes to embryo: genetics and developmental biology
- PCC 3: New developments in the diagnosis and management of early pregnancy complications
- PCC 4: Basic course on environment and human male reproduction
- PCC 5: The lost art of ovulation induction
- PCC 6: Endometriosis: How new technologies may help
- PCC 7: NOTES and single access surgery
- PCC 8: Stem cells in reproductive medicine
- PCC 9: Current developments and their impact on counselling
- PCC 10: Patient-centred fertility care
- PCC 11: Fertility preservation in cancer disease
- PCC 12: ESHRE journals course for authors

Annual Meeting – Scientific Programme (1/2)

Rome, Italy 27 June to 30 June 2010

- Molecular timing in reproduction
- Rise and decline of the male
- Pluripotency
- Preventing maternal death
- Use and abuse of sperm in ART
- Live surgery
- Emerging technologies in the ART laboratory
- Debate: *Multiple natural cycle IVF versus single stimulated cycle and freezing*

Annual Meeting – Scientific Programme (2/2)

- Fertility preservation
- Congenital malformations
- ESHRE guidelines
- Data from the PGD Consortium
- European IVF Monitoring 2007
- Debate: *Selection of male/female gametes*
- Third party reproduction in the United States
- Debate: *Alternative Medicine, patients feeling in control?*
- Historical lecture: "Catholicism and human reproduction"

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

Meerstraat 60, 1852 Grimbergen, Belgium

Tel: +32 (0)2 269 09 69

Fax: +32 (0)2 269 56 00

E-mail: info@eshre.eu

www.eshre.eu

PRE-CONGRESS COURSE 2 - Programme

From gametes to embryo: Genetics and developmental biology

Organised by the Special Interest Groups Embryology & Reproductive Genetics

Course coordinators: M. Cristina Magli (SIG Embryology) & Stephane Viville (SIG Reproductive Genetics)

Course description: A basic course on the events regulating gametogenesis and embryogenesis, both in vivo and in vitro

Target audience: Clinical embryologists and reproductive geneticists

Session 1 – Gametogenesis: the mechanisms underlying the development of competent gametes

09:00 – 09:30 Physiology of oogenesis, implications for oocyte competence - **Helen M. Picton (United Kingdom)**
09:30 – 09:45 Discussion
09:45 – 10:15 Physiology of spermatogenesis, implications for fertilising competence - **Dominique Royere (France)**
10:15 – 10:30 Discussion
10:30 – 11:00 Coffee break

Session 2 – The genetics of development

11:00 – 11:30 Meiosis: possible errors - **Renee H. Martin (Canada)**
11:30 – 11:45 Discussion
11:45 – 12:15 Chromatin states and lineage choice in the mouse preimplantation embryo - **Maria-Elena Torres–Padilla (France)**
12:15 – 12:30 Discussion
12:30 – 13:30 Lunch

Session 3 – Embryogenesis

13:30 – 14:00 First mitoses: principles of embryonic patterning and what can go wrong with it? – **Takashi Hiiragi (Germany)**
14:00 – 14:15 Discussion
14:15 – 14:45 Early stages or blastocysts, a critical choice for transfer - **Gayle Jones (Australia)**
14:45 – 15:00 Discussion
15:00 – 15:30 Coffee break

Session 4 – The IVF laboratory

15:30 – 16:00 How to select the best gametes? - **Sjoerd Repping (The Netherlands)**
16:00 – 16:15 Discussion
16:15 – 16:45 In–vitro culture conditions and epigenetic modifications - **Wolf Reik (United Kingdom)**
16:45 – 17:00 Discussion

Physiology of Oogenesis:
Implications For Oocyte Competence

Prof. Helen M Picton *BSc, Ph.D*

Leeds Institute For Genetics, Health & Therapeutics

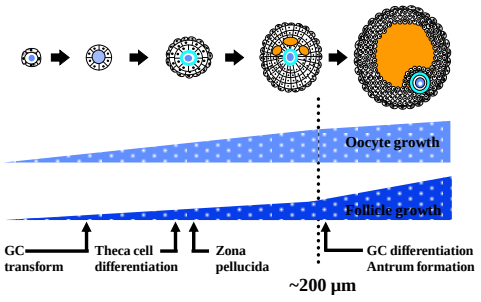
University of Leeds

UK

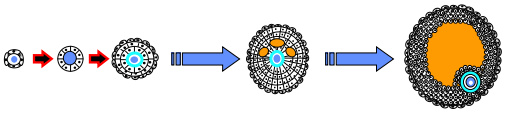
Physiology of Oogenesis
Learning Objectives

1. To map the growth and development of an oocyte from the earliest staged primordial germ cell to the production of a mature oocyte capable of undergoing fertilisation.
2. To gain insight into the relationship between somatic follicular cells and oocytes during oogenesis
3. To understand the dynamics of the nutritional environment needed to support oocyte growth and development
4. To understand the mechanisms regulating follicle and oocyte growth (drivers and moderators)
5. To provide an overview of the biology of oocyte maturation

Morphological Changes During Folliculogenesis
In Vivo

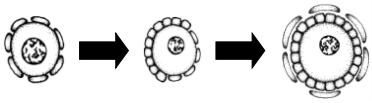


Follicle Growth Rates In Different Species
In Vivo



	Preantral Size (µm)	Growth Period (days)	Mature Size
Mouse	100-200	10-12	500-600 µm
Pig	150-300	40-50	3.0-10 mm
Sheep	180-250	40-50	3.0-10 mm
Cow	180-250	40-50	3.8- >8.5 mm
Human	180-250	≥ 90-180?	17-20 mm

Primordial To Primary Follicle Transition

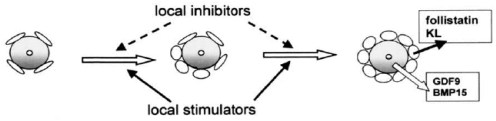


Clinical relevance:
Abnormalities lead to pathologies
eg. POF
Therapeutic targets

Model of Follicle Growth Initiation

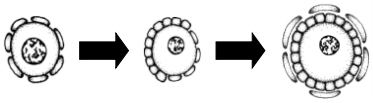
(Braw-Tal_Mol Cell Endocrinol. 2002 187:11-8.

Phase I



Phase I:-
- Slow proliferation of GCs followed by gradual transformation of cells from flattened to cuboidal.
- Under influence of locally produced inhibitory (e.g. activin A) and stimulatory signals (e.g. bFGF, KL)?

Primordial To Primary Follicle Transition



Regulators of primordial initiation		
Regulators	**Cell source**	**Site of action**
TNFa	Oocyte	Oocyte
bFGF	Oocyte	GC, theca, stroma
Kit Ligand	GC	Oocyte, GC
LIF	GC	Oocyte, GC
KGF	Theca	GC
BMP-4	Theca/ stroma	GC
BMP-7	Stroma	GC
Insulin	Endocrine	Oocyte
AMH	Antral Follicle	Primordial Follicle

Transcription factors: Arh-R, FIGa, Fox 12, NOBOX

Early Folliculogenesis

2-6 months

Follicle and Oocyte growth initiation

↓

Flattened GC become cuboidal and proliferate

↓

Oocyte grow and synthetic activity

↓

Zona pellucida forms

↓

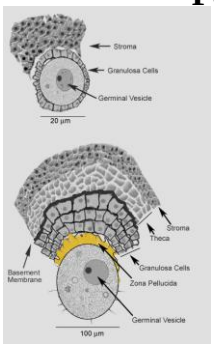
GC continue to proliferate & theca layer forms

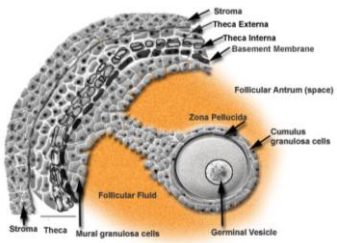
↓

Antrum forms (200-500 µm)

(rate of oocyte growth declines and rate follicle growth accelerates)

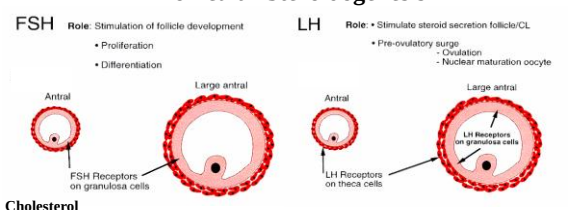
Folliculogenesis





Partial cross section of the antral follicle

Follicular Steroidogenesis

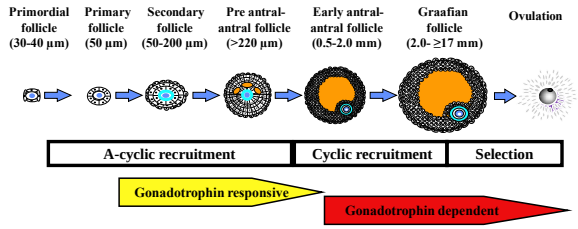


Cholesterol
↓ Cytochrome P450 Side-chain cleavage
Progestagen
↓ P450 17α-hydroxylase
Androgen
↓ P450 Aromatase
Oestrogen

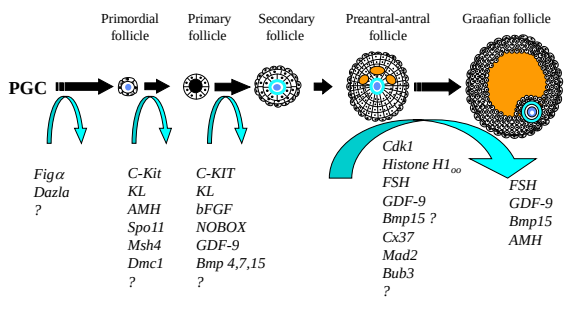
Two Cell, Two Gonadotrophin Theory of Steroidogenesis

- Theca cells synthesize androstenedione and testosterone from blood cholesterol when stimulated by binding of LH to thecal LH receptors
- Follicular oestrogen synthesis which is under the influence of FSH is due to the aromatization of androgens originating in the theca. These diffuse across the basement membrane into the granulosa cells for conversion to oestrogens

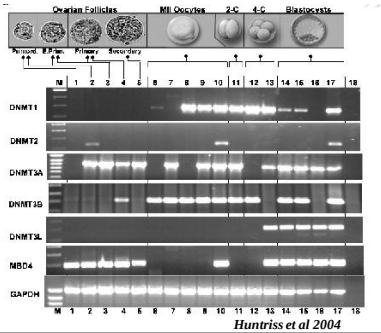
The Biology Of Follicle & Oocyte Development



Genes Involved In Regulating Oocyte and Follicle Growth In Mice and Humans

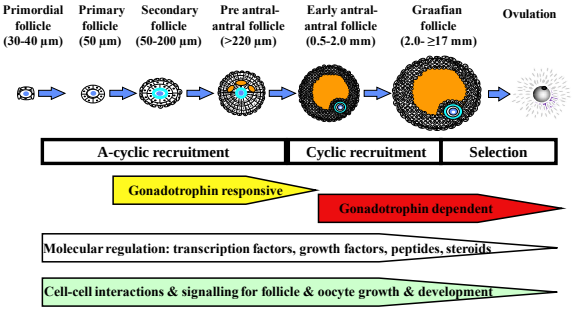


Analysis Of Imprinting and Epigenetics In Human Oocytes And Embryos

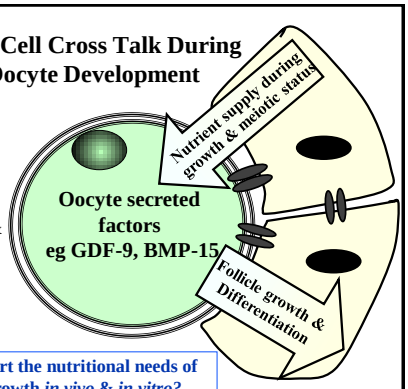


Huntriss et al 2004

The Biology Of Follicle & Oocyte Development

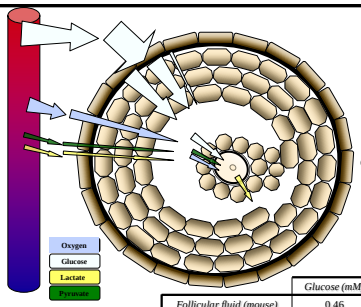


Oocyte-Somatic Cell Cross Talk During Follicle & Oocyte Development



Bidirectional communication between the oocyte & GCs via gap junctions

Gap junctions support the nutritional needs of follicle & oocyte growth *in vivo* & *in vitro?*

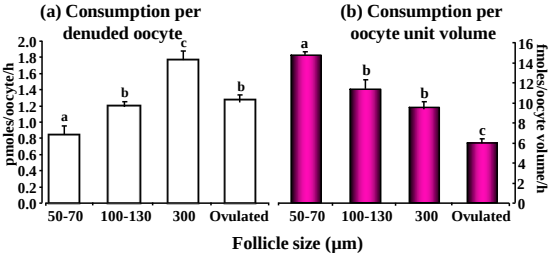


Nutrient Origin & Consumption During Follicle & Oocyte Growth *In Vivo*

	Glucose (mM)	*Pyruvate (mM)*	*L-Lactate (mM)*
Follicular fluid (mouse)	0.46	0.38	17.3
Oviduct fluid (mouse)	1.09 (+ *CCs*)	0.37 (+ *CCs*)	10.9 (+ *CCs*)
Plasma (mouse)	11.7	0.16	4.8
Follicular fluid (human)	3	0.26	6.06
Oviduct fluid (human)	0.5-3.11	0.24-0.32	4.87-10.55
Plasma (human)	5	0.1	0.6

Adapted from: Harris & Picton, 2007

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

Mouse Follicle Metabolism *In Vivo*

<18 µm

Small follicles use a combination of glycolytic & aerobic metabolism of glucose

Diffusion of nutrients across small distances: primordial follicles utilise a variety of carbohydrate energy substrates

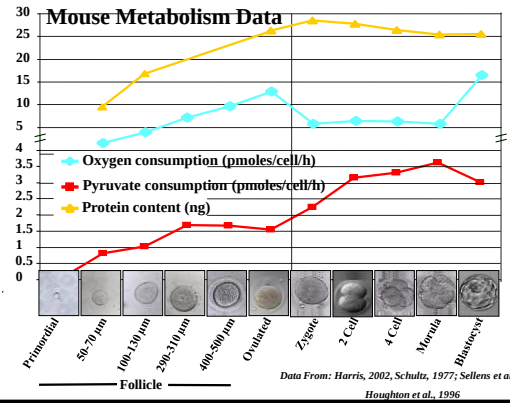
Large follicles become almost totally reliant on glycolytic glucose consumption

~400 µm

Primordial – Graafian follicle
Mouse: >30,000 –fold increase in volume
Human: >91,000,000 –fold increase in volume

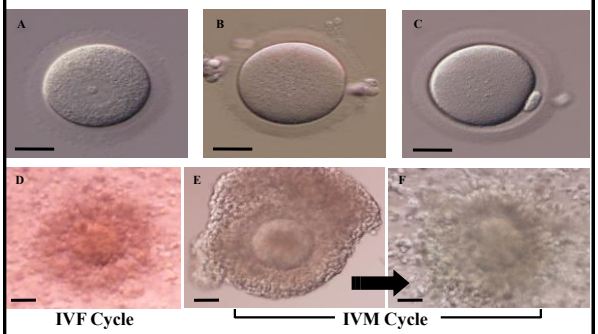
Harris 2002,
Harris et al., 2007)

Mouse Metabolism Data



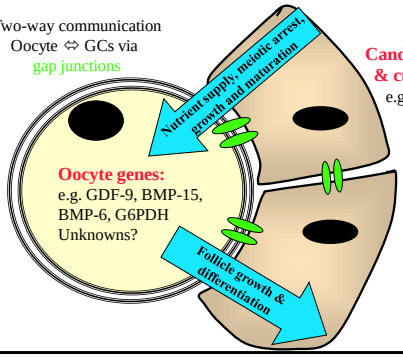
Data From: Harris, 2002, Schultz, 1977; Sellens et al., 1981; Houghton et al., 1996

Human Oocyte Maturation *In Vivo* and *In Vitro*



Molecular Regulation Of Follicle & Egg Development

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?

What Is Oocyte Maturation?

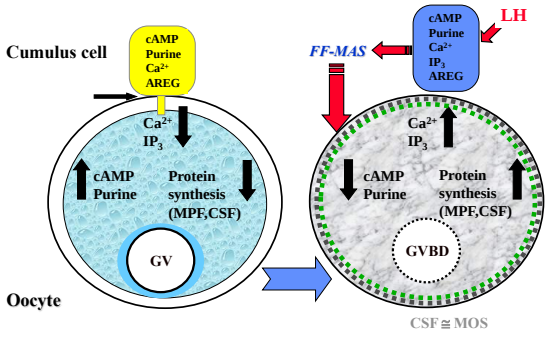
1. Nuclear Maturation

Resumption of the first meiotic division at the germinal vesicle stage (diplotene) to produce a metaphase-II gamete

2. Cytoplasmic Maturation

Changes in molecules/ organelles/ membranes needed for successful fertilization and embryo viability

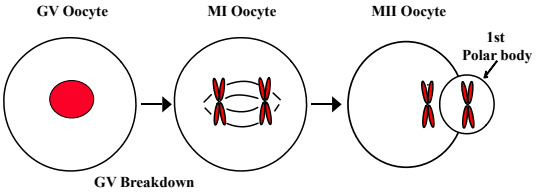
The Biology Of Oocyte Maturation



Cytoplasmic Maturation

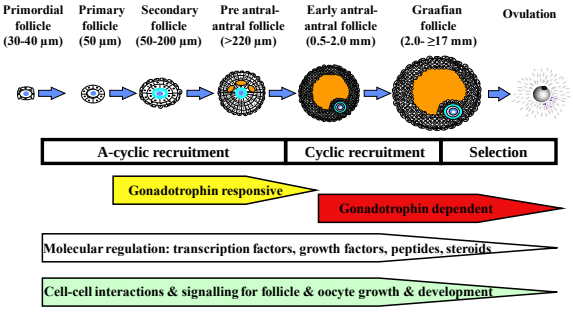
- Organisation/replication of the cytoplasmic organelles
- Most RNA is synthesized and accumulated during oocyte growth
- Transcription is suspended from Germinal Vesicle Break Down (GVBD) to Embryonic genome activation (EGA)
- Protein synthesis increases before GVBD in both cumulus-intact and -free human oocytes
- Protein synthesis in cultured human oocytes is modified by cumulus cells
- Newly synthesized protein may be important for fertilization and early embryo development

Nuclear Maturation -Resumption of Meiosis



- Assembly/ disassembly of spindles
- Prophase I (GV) ⇒Metaphase II
- No sister chromatid separation (Ana I)
- No intervening S-phase
- Checkpoints?

The Biology Of Follicle & Oocyte Development



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Telfer EE, McLaughlin M (2007). Natural history of the mammalian oocyte. Reprod Biomed Online. 2007 Sep;15(3):288-95.

Physiology of spermatogenesis: implications for fertilising competence

D Royère, Médecine et Biologie de la Reproduction, CHU Bretonneau, UMR6175 Inra / Cnrs / Haras / Université de Tours, France

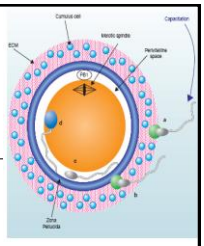
Disclosure of commercial and/or financial relationships

- I have no commercial interest with any pharmaceutical industry and other commercial industry
- I have no financial relationship with any pharmaceutical industry and other commercial industry

INTRODUCTION

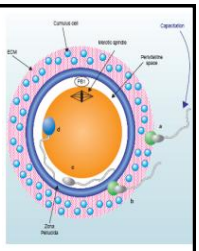
How to define fertilizing competence?

- A highly polarized cell
  - with a head region containing a nucleus with a haploid number of chromosomes
  - A single enlarged secretory granule = acrosome in the apical region
  - A flagellum containing a 9+2 array of microtubules and associated fibrous sheath proteins
- Additional biochemical and functionnal changes during epididymal transit, storage in cauda epididymis



How to define fertilizing competence?

- "Competent" spermatozoa are able
  - to undergo capacitation during migration through the female genital tract
  - To penetrate the cumulus oophorus, fix on zona pellucida, then undergo acrosome reaction = Ca dependent exocytotic event
  - To penetrate the zona pellucida, then contact and fuse with plasma membrane of the oocyte
  - Finally to induce oocyte activation, pronuclear formation and syngamy



Learning objectives

- A comprehensive approach of all mechanisms underlying the fertilising competence of spermatozoa looks like "Annapurna"
- Otherwise it might lead to an ever lasting and fastidious list
- Therefore the aims of this presentation, on a voluntary basis will describe

Learning objectives

- Several features relating experimental data to clinical observations
- As an attempt to give evidence based relevance of physiological data on gametic interaction and its disorders

From experimental date to clinical observations

- Spermatogenesis / Spermiogenesis
- Capacitation / Gametic interaction
- Meiosis resumption / Embryo development

From experimental data to clinical observations

- Spermatogenesis / Spermiogenesis
  - Aberrant DNA methylation in oligospermic patients
  - Mutations in dynein genes
  - Mutations in protamine genes and spermatogenic failure
  - Mutation in *SPATA16* in infertile men

From experimental data to clinical observations

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  - Mutations in protamine genes and spermatogenic failure
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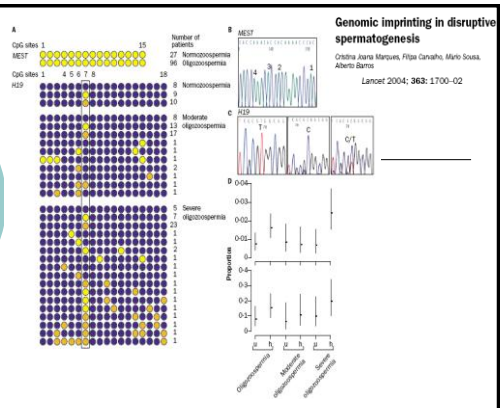
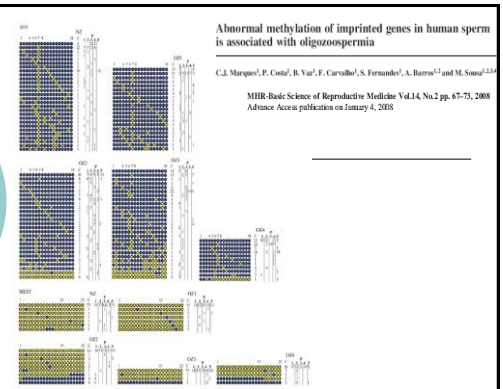


Genomic imprinting in disruptive spermatogenesis

Cristina Joana Marques, Filipa Carvalho, Mário Sousa, Alberto Barros

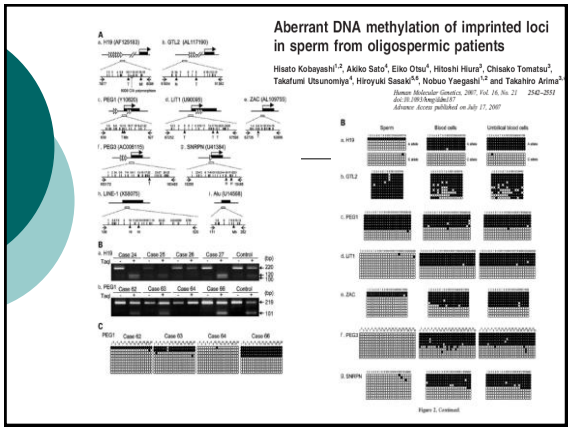
Lancet 2004; **363:** 1700–02



Abnormal methylation of imprinted genes in human sperm is associated with oligozoospermia

C.J. Marques[1], P. Costa[1], B. Vaz[1], F. Carvalho[1], S. Fernandes[1], A. Barros[1,2] and M. Sousa[1,2,3,4]

MHR: Basic Science of Reproductive Medicine Vol.14, No.2 pp. 67–73, 2008
Advance Access publication on January 4, 2008



Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients

Hisato Kobayashi[1,2], Akiko Sato[1], Eiko Otsu[1], Hitoshi Hiura[3], Chisako Tomatsu[3], Takafumi Utsunomiya[4], Hiroyuki Sasaki[5,6], Nobuo Yaegashi[1,2] and Takahiro Arima[3]

Human Molecular Genetics, 2007, Vol. 16, No. 21 2542–2551
doi:10.1093/hmg/ddm157
Advance Access published on July 17, 2007

From experimental data to clinical observations

- Spermatogenesis / Spermiogenesis
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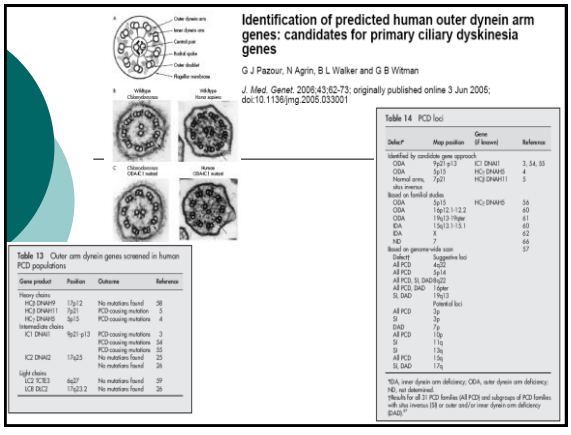
  - Mutations in dynein genes
  - Mutations in protamine genes and spermatogenic failure
  - Mutation in *SPATA16* in infertile men



Identification of predicted human outer dynein arm genes: candidates for primary ciliary dyskinesia genes

G J Pazour, N Agrin, B L Walker and G B Witman

J. Med. Genet. 2006;43;62-73; originally published online 3 Jun 2005; doi:10.1136/jmg.2005.033001

Table 13 Outer arm dynein genes screened in human PCD populations

Gene product	Position	Outcome	Reference
Heavy chains			
$HC\beta$ DNAH9	17p12	No mutations found	58
$HC\delta$ DNAH11	7p21	PCD-causing mutation	5
$HC\gamma$ DNAH5	5p15	PCD-causing mutations	4
Intermediate chains			
IC1 DNAI1	9p21-p13	PCD-causing mutations	3
		PCD-causing mutations	54
		PCD-causing mutations	55
IC2 DNAI2	17q25	No mutations found	25
		No mutations found	26
Light chains			
LC2 TCTE3	6q27	No mutations found	59
LC8 DYC2	17p13.2	No mutations found	26

Table 14 PCD loci

Defect*	Map position	Gene (if known)	Reference
Identified by candidate gene approach			
ODA	9p23-p12	IC1 DNAI1	3, 54, 55
ODA	5p15	$HC\gamma$ DNAH5	4
Normal arms, situs inversus	7p21	$HC\delta$ DNAH11	5
Based on familial studies			
ODA	5p15	$HC\gamma$ DNAH5	56
ODA	16p12.1-12.2		60
ODA	19q13-19qter		61
IDA	15q13.1-13.1		60
IDA	X		62
MD	?		66
Based on genome-wide scan			57
Defect†	Suggestive loci		
All PCD	6q22		
All PCD	5p14		
All PCD, SL DAD	8p22		
All PCD, DAD	16pter		
SL DAD	19q13		
	Potential loci		
All PCD	3p		
SI	3p		
DAD	7p		
All PCD	10p		
SI	11q		
SI	13q		
All PCD	15q		
SL DAD	17q		

*IDA, inner dynein arm deficiency; ODA, outer dynein arm deficiency; MD, not determined.
†Results for all 31 PCD families (All PCD) and subgroups of PCD families with situs inversus (SI) or outer and/or inner dynein arm deficiency (DAD).[57]

Mutations in dynein genes in patients affected by isolated non-syndromic asthenozoospermia

D. Zuccarello[1], A. Ferlin[1], C. Cazzadore[1], A. Pepe[1], A. Garolla[1], A. Moretti[1], G. Cordeschi[2], S. Francavilla[2] and C. Foresta[1,3]

Human Reproduction Vol.23, No.8 pp. 1957–1962, 2008
Advance Access publication on May 20, 2008

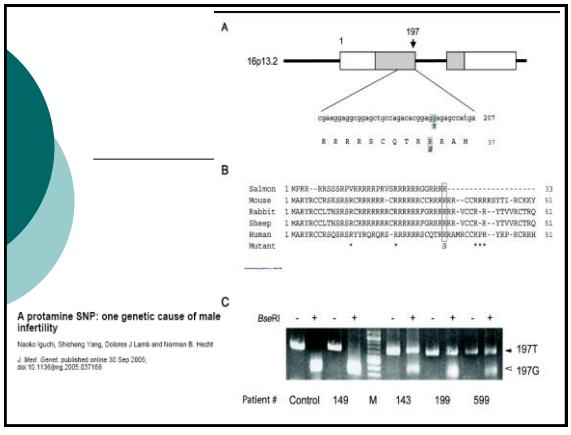
Table I. Summary of sequence variants found in 90 AZS patients.

Exon	Base	Amino acid	Type	Frequency in patients	Frequency in controls
DNAI1 gene					
19	CGC>TGC	**R663C**	unknown transversion	3/90	0/200
1	GCT>TCT	A8S	known SNP	7/90	19/200
11	GTC>ATC	V335I	known SNP	3/90	12/200
DNAH5 gene					
32	GAA>AAA	**E1756K**	unknown transition	1/90	2/200
48	GAG>GAT	**E2666D**	unknown transversion	1/90	0/200
28	CGG>CGT	R1458R	known SNP	8/90	np
32	GCC>GCT	A1724A	known SNP	3/90	np
53	ACG>ACA	T2966T	known SNP	14/90	np
62	ACA>ACG	T3491T	known SNP	11/90	np
75	GCC>GCT	A4357A	known SNP	4/90	np
DNAH11 gene					
55	ATT>GTT	**I3040V**	unknown transition	3/90	0/200
55	CGG>CAG	R3004Q	known SNP	3/90	4/200

In bold are novel mutations detected in this study. np, not performed.

From experimental data to clinical observations

- Spermatogenesis / Spermiogenesis
  - Aberrant DNA methylation in oligospermic patients
  - Mutations in dynein genes
  - Mutations in protamine genes and spermatogenic failure
  - Mutation in *SPATA16* in infertile men



A protamine SNP: one genetic cause of male infertility

Naoko Iguchi, Shicheng Yang, Dolores J Lamb and Norman B. Hecht

J. Med. Genet. published online 30 Sep 2005; doi:10.1136/jmg.2005.037168

Mutations in the protamine locus: association with spermatogenic failure?

L. Imken[1,5], H. Rouba[1], B. El Houate[1], N. Louanjli[2], A. Barakat[1], A. Chafik[3], and K. McElreavey[4]

Molecular Human Reproduction, Vol.15, No.11 pp. 733–738, 2009
Advanced Access publication on July 14, 2009 doi:10.1093/molehr/gap056

Table III Summary of results obtained by direct sequencing of PCR products encompassing the *PRM1* gene

SNP	AA change	Fertile (160) Genotype	Allele	Infertile (135) Genotype	Allele	P-value
c.−191C>A		CC (113) 0.71 CA (42) 0.26 AA (5) 0.03	C = 0.84 A = 0.16	CC (85) 0.63 CA (45) 0.33 AA (5) 0.04	C = 0.8 A = 0.2	NS
c.−107G>C		GG (160) 1.00	G = 1.00 C = 0.00	GG (134) 0.99 GC (1) 0.01	G = 0.996 C = 0.004	NS
c.54G>A	p.Gln18Gln	GG (155) 0.97 GA (5) 0.03	G = 0.985 A = 0.015	GG (133) 0.99 GA (2) 0.01	G = 0.99 A = 0.01	NS
c.65G>A	p.Ser22Asn	GG (160) 1.00	G = 1.00 A = 0.00	GG (134) 0.99 GA (1) 0.0	G = 0.996 A = 0.004	NS
c.139C>A	p.Arg47Arg	CA (16) 0.1 CA (74) 0.46 AA (70) 0.44	C = 0.33 A = 0.67	CC (16) 0.12 CA (55) 0.41 AA (64) 0.47	C = 0.32 A = 0.68	NS

Genotypic and allelic frequencies are indicated.

From experimental data to clinical observations

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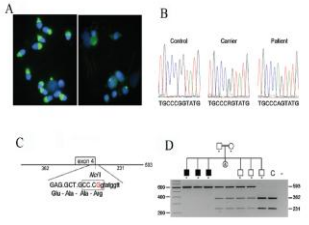
  - Mutation in *SPATA16* in infertile men

Homozygous Mutation in *SPATA16* Is Associated with Male Infertility in Human Globozoospermia

Anika H. D. M. Dam,* Isabelle Koscinski,* Jan A. M. Kremer, Céline Moutou, Anne-Sophie Jaeger, Astrid R. Oudakker, Herman Tournaye, Nicolas Charlet, Clotilde Lagier-Tourenne, Hans van Bokhoven, and Stéphane Viville

The American Journal of Human Genetics Volume 81 October 2007

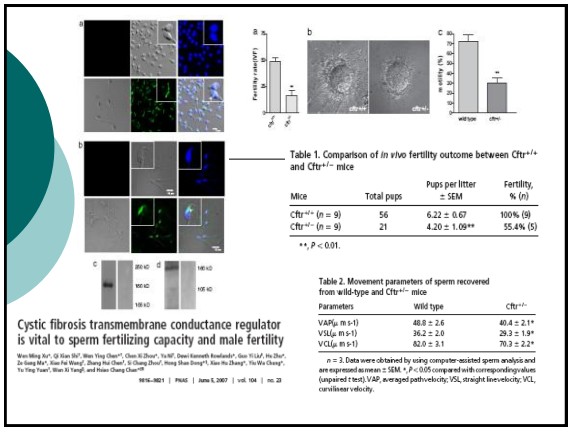


From experimental date to clinical observations

- Spermatogenesis / Spermiogenesis
- Capacitation / Gametic interaction
- Meiosis resumption /Embryo development

From experimental date to clinical observations

- Capacitation / Gametic interaction
  - CFTR involvement in sperm fertilizing capacity
  - Ca channels : a long story
  - Proton channel : a new story
  - PLCζ



Cystic fibrosis transmembrane conductance regulator is vital to sperm fertilizing capacity and male fertility

Table 1. Comparison of *in vivo* fertility outcome between $Cftr^{+/+}$ and $Cftr^{+/-}$ mice

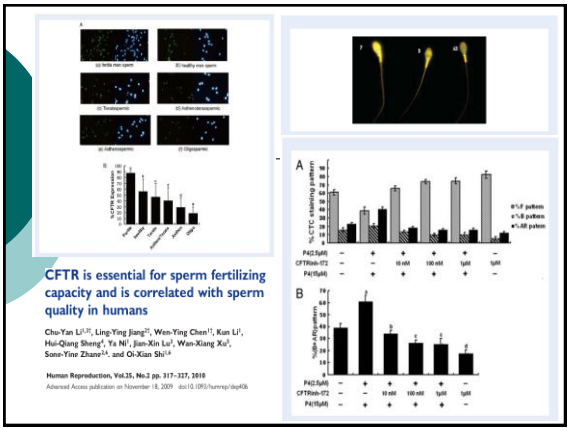
Mice	Total pups	Pups per litter ± SEM	Fertility, % (*n*)
$Cftr^{+/+}$ (*n* = 9)	56	6.22 ± 0.67	100% (9)
$Cftr^{+/-}$ (*n* = 9)	21	4.20 ± 1.09**	55.4% (5)

**, $P < 0.01$.

Table 2. Movement parameters of sperm recovered from wild-type and $Cftr^{+/-}$ mice

Parameters	Wild type	$Cftr^{+/-}$
VAP(μ m s-1)	48.8 ± 2.6	40.4 ± 2.1*
VSL(μ m s-1)	36.2 ± 2.0	29.3 ± 1.9*
VCL(μ m s-1)	82.0 ± 3.1	70.3 ± 2.2*

n = 3. Data were obtained by using computer-assisted sperm analysis and are expressed as mean ± SEM. *, $P < 0.05$ compared with corresponding values (unpaired *t* test). VAP, averaged path velocity; VSL, straight line velocity; VCL, curvilinear velocity.



CFTR is essential for sperm fertilizing capacity and is correlated with sperm quality in humans

Chu-Yan Li[1,2], Ling-Ying Jiang[2], Wen-Ying Chen[1], Kun Li[1], Hui-Qiang Sheng[1], Ya Ni[1], Jian-Xin Lu[1], Wan-Xiang Xu[1], Sone-Yew Zhang[1,3], and Qi-Xian Shi[1,3]

Human Reproduction, Vol.25, No.2 pp. 317–327, 2010
Advanced Access publication on November 18, 2009 doi:10.1093/humrep/dep406

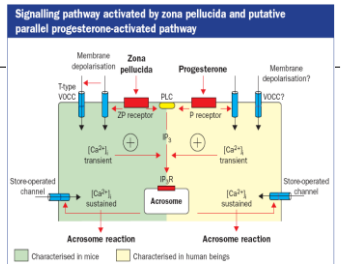
From experimental date to clinical observations

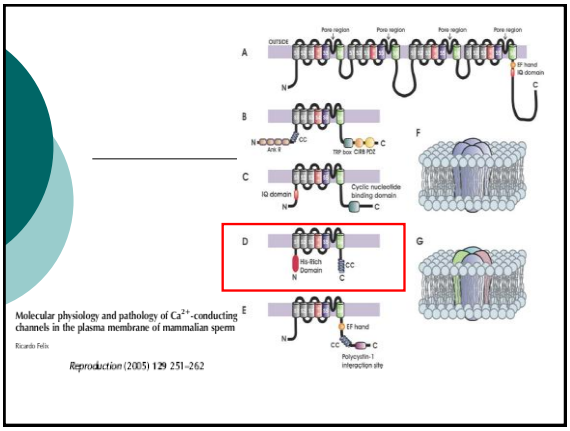
- Capacitation / Gametic interaction
  - CFTR involvement in sperm fertilizing capacity
  - Ca channels : a long story
  - Proton channel : a new story
  - PLCζ

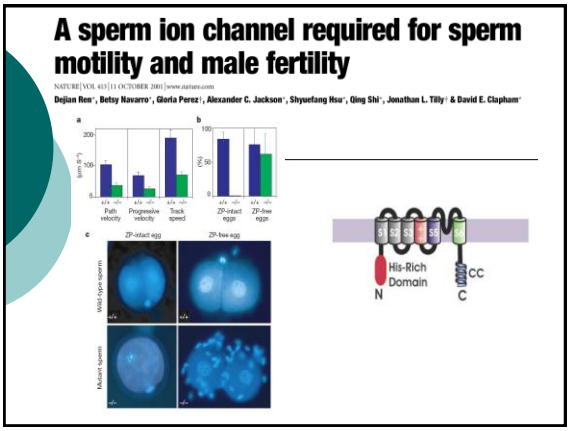
Interaction between sperm and zona pellucida in male fertility

*Christopher L R Barratt, Stephen J Publicover

THE LANCET • Vol 358 • November 17, 2001




Molecular physiology and pathology of Ca^{2+}-conducting channels in the plasma membrane of mammalian sperm
Ricardo Felix
Reproduction (2005) 129 251–262


A sperm ion channel required for sperm motility and male fertility
NATURE | VOL 413 | 11 OCTOBER 2001 | www.nature.com
Dejian Ren*, Betsy Navarro*, Gloria Perez†, Alexander C. Jackson*, Shyuefang Hsu*, Qing Shi*, Jonathan L. Tilly† & David E. Clapham*
His-Rich Domain
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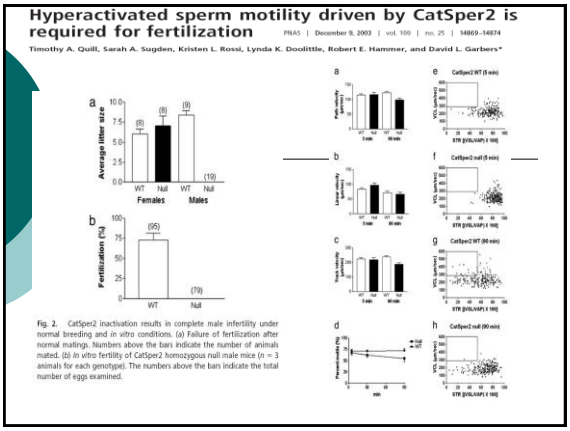
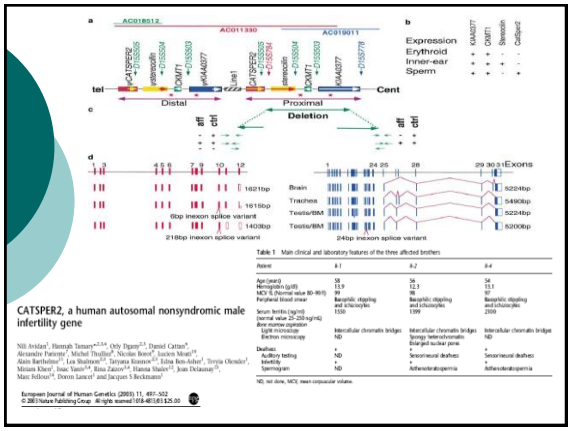

Hyperactivated sperm motility driven by CatSper2 is required for fertilization
PNAS | December 9, 2003 | vol. 100 | no. 25 | 14869–14874
Timothy A. Quill, Sarah A. Sugden, Kristen L. Rossi, Lynda K. Doolittle, Robert E. Hammer, and David L. Garbers*
Females
Males
WT
Null



CATSPER2, a human autosomal nonsyndromic male infertility gene

Nili Avidan[1], Hannah Tamary[1,2,3,4], Orly Dgany[2,3], Daniel Cattan[4], Alexandre Pariente[4], Michel Thuilliez[4], Nicolas Bowe[4], Jacleen Moati[4], Alain Barthelme[1,5], Lea Shalmon[1,2], Tatyana Krasnov[1,2], Edna Ben-Asher[1], Tsvyia Olender[1], Miriam Khen[1], Isaac Yanai[1,6], Rina Zaizov[2,3], Hanna Shalev[1,2], Joan Delaunay[7], Marc Fellous[7], Doron Lancet[1] and Jacques S Beckmann[1]

European Journal of Human Genetics (2003) 11, 497–502

Table 1 Main clinical and laboratory features of the three affected brothers

Patient	II-1	II-2	II-4
Age (years)	38	36	34
Hemoglobin (g/dl)	13.9	12.3	13.1
MCV fl (Normal value 80–96 fl)	99	98	97
Peripheral blood smear	Basophilic stippling and ichinocytes	Basophilic stippling and ichinocytes	Basophilic stippling and ichinocytes
Serum ferritin (ng/ml) (normal value 25–250 ng/ml)	1350	1399	2000
Bone marrow aspiration			
Light microscopy	Intercellular chromatin bridges	Intercellular chromatin bridges	Intercellular chromatin bridges
Electron microscopy	ND	Spongy heterochromatin Enlarged nuclear pore	ND
Deafness			
Auditory testing	+	+	+
Infertility	ND	Sensorineural deafness	Sensorineural deafness
Spermiogram	+	+	+
	ND	Asthenoteratospermia	Asthenoteratospermia

ND, not done; MCV, mean corpuscular volume.

From experimental date to clinical observations

- Capacitation / Gametic interaction
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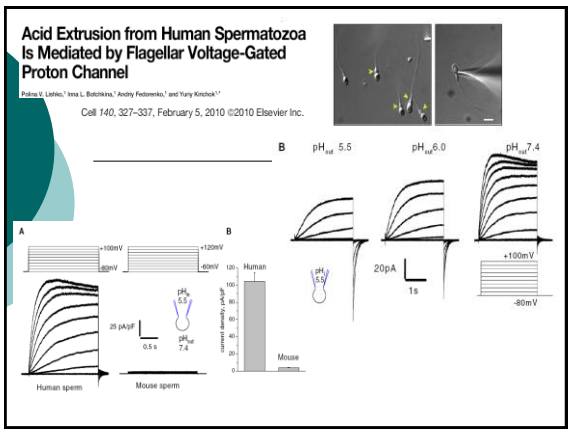
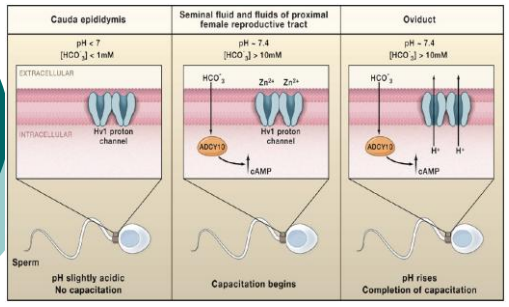
  - Ca channels : a long story
  - Proton channel : a new story
  - PLCζ



Acid Extrusion from Human Spermatozoa Is Mediated by Flagellar Voltage-Gated Proton Channel

Polina V. Lishko,[1] Inna L. Botchkina,[1] Andriy Fedorenko,[1] and Yuriy Kirichok[1,*]

Cell 140, 327–337, February 5, 2010 ©2010 Elsevier Inc.



Shedding Light on Sperm pHertility

Harvey M. Florman,[1,*] Melissa K. Jungnickel,[1] and Keith A. Sutton[1]
[1]Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA 01655, USA
[*]Correspondence: harvey.florman@umassmed.edu
DOI 10.1016/j.cell.2010.01.035

From experimental date to clinical observations

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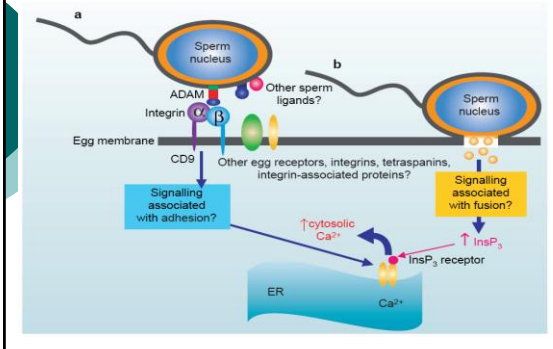


ESHRE EMBRYO-GEN_PCC2ROME

From experimental date to clinical observations

- Spermatogenesis / Spermiogenesis
- Capacitation / Gametic interaction
- Meiosis resumption / Embryo development
  - PLCζ
  - Centrosome

Reduced amounts and abnormal forms of phospholipase C zeta (PLCζ) in spermatozoa from infertile men

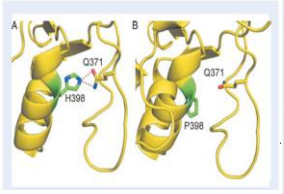
E. Heytens[1,†], J. Parrington[2,†], K. Coward[2,3,†], C. Young[2], S. Lambrecht[3], S.-Y. Yoon[4], R.A. Fissore[4], R. Hamer[3], C.M. Deane[4], M. Ruas[2], P. Grasa[2], R. Soleimani[1], C.A. Cuvelier[2], J. Gerris[1], M. Dhont[1], D. Deforce[3], L. Leybaert[8,‡], and P. De Sutter[1,2,9]

Human Reproduction, Vol.24, No.10 pp. 2417–2428, 2009
Advanced Access publication on July 7, 2009 doi:10.1093/humrep/dep207

Table I Patient characteristics

	Age (year)	Semen parameters				MOAT (%)
		Morphology (% ideal forms)	Round-headed morphology (%)	Motility[a] (%)	Concentration (10^6/ml)	
A[§]	38	0	100	32	32	0
B[§]	42	0	100	8	17	4
C	33	0	100	36	38	11
D	34	0	100	37	21	6
E	33	0	100	33	38	10
F	38	3	0	48	170	0
G	45	7	0	46	54	19
H	36	0	0	0	0.1	40
I	29	0	20	0	0.3	50

MOAT, mouse oocyte activation test.
[a]Percentage of progressive motile sperm cells.
[§]These two patients are brothers.



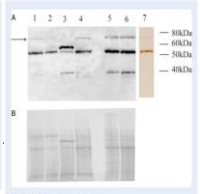


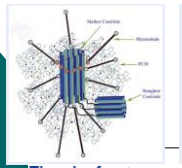
Figure 2 PLCζ immunoblotting.
(A) PLCζ immunoblotting of semen samples from globozoospermic men (lanes 1–4, patient A, B, C and D, respectively), fertile controls (lanes 5 and 6) and non-globozoospermic patient F (lane 7). The arrow indicates the full length PLCζ protein predicted MW. (B) Ponceau S staining to confirm equal loading.

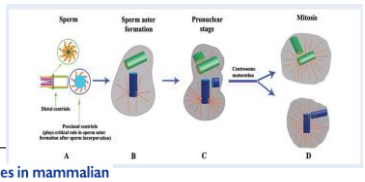
Human Reproduction, Vol.24, No.10 pp. 2417–2428, 2009

Table IV Oocyte activation and calcium dynamics in mouse oocytes injected with human sperm

	No. of injected oocytes	No. of oocytes with 2PB*, 2.5 h after ICSI (% of oocytes)	No. of oocytes showing oscillations (% of 2PB)	No. of 2-cells (% of oocytes)
Fertile sperm	38	19 (50%)[a]	17 (89%)[a]	30 (79%)[a,b]
Globozoospermia sperm	12	0 (0%)[c]	0 (0%)[c]	0 (0%)[c]
Globozoospermia sperm + AOA				
Patient A	23	14 (61%)[b]	0 (0%)[c]	19 (83%)[b]
Patient B	26	21 (81%)[a,b]	0 (0%)[c]	22 (85%)[b]
Patient C	24	20 (83%)[a,b]	0 (0%)[c]	19 (79%)[b]

*Two polar bodies.
[a]No oscillations were observed during the 2 h recording period in oocytes that did not extrude second polar body 2.5 h after ICSI. Activation in these oocytes was delayed.
[b]Significantly different from oocytes injected with fertile sperm ($p = 0.05$, Fisher's exact test).
[c]Significantly different from oocytes injected with globozoospermic sperm ($p = 0.05$, Fisher's exact test).

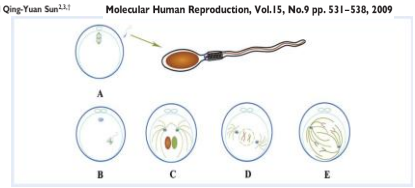


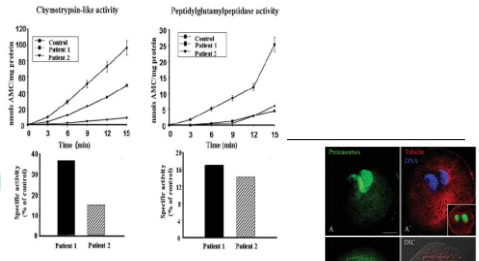


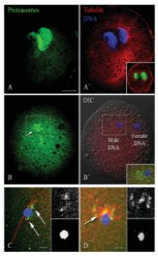
The role of centrosomes in mammalian fertilization and its significance for ICSI

Heide Schatten[1,3] and Qing-Yuan Sun[2,3,†]

Molecular Human Reproduction, Vol.15, No.9 pp. 531–538, 2009







The role of sperm proteasomes during sperm aster formation and early zygote development: implications for fertilization failure in humans

Human Reproduction Vol.23, No.3 pp. 573–580, 2008
Advance Access publication on December 18, 2007

CONCLUDING REMARKS PERSPECTIVES

Physiology of spermatogenesis : implications for fertilising competence

- Spermatozoa generated in the testis are immature and incompetent for (natural) fertilization
- They need to be modified all along the male and female genital tracts to acquire fertilising capacity
- Cellular and molecular mechanisms that underpin that capacity are myriad and species specific

Physiology of spermatogenesis : implications for fertilising competence

- Understanding these cellular and molecular mechanisms has implications for
  - diagnosis of the aetiology of human infertility
  - Development of new therapeutics, and novel targets of fertility regulation

Meiosis: Possible Chromosome Errors Rome, 2010

Renée Martin, Ph.D., FCCMG
Professor, Dept. of Medical Genetics
University of Calgary

Meiosis: Possible Chromosome Errors

No commercial relationships.

No conflict of interest.

Meiosis: Possible Chromosome Errors

Learning Objectives

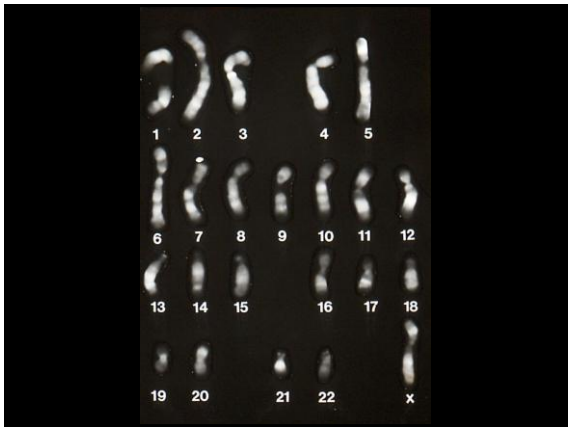
1) To appreciate the differences in the frequency and type of chromosomal errors in males and females
2) To understand the effect of maternal or paternal age on the chromosome abnormalities
3) To appreciate the similarities and differences in the distribution of aneuploidy in oocytes and sperm
4) To understand the relationship between meiotic recombination errors and aneuploidy in humans

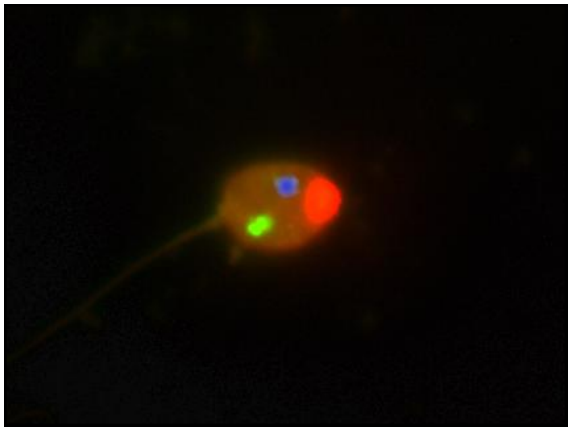
Chromosome Abnormalities

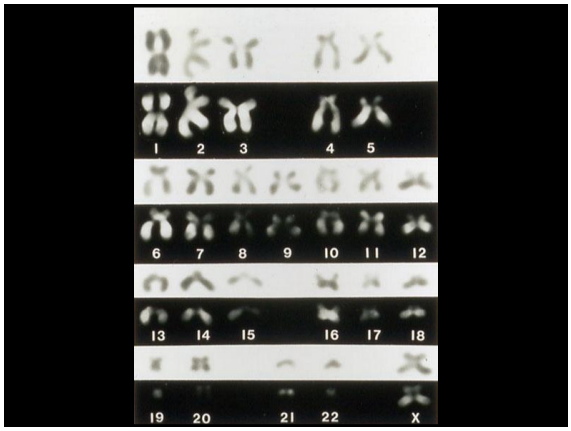
- very common in humans
  - .6% newborns
  - 6% stillborns
  - 60% spontaneous abortions
  - estimates at conception: 20 - 50%

Cause of Chromosomal Abnormalities

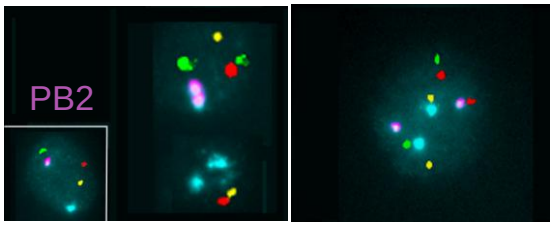
- very little information
- produced in eggs and sperm (mainly meiotic errors), but most die as embryos - information lost
- need to study chromosome abnormalities in human eggs and sperm







PB1 Blastomere



Chromosomal Abnormalities in Human Gametes

	numerical	structural	total
sperm	1-2	7	9
oocytes	20	1	21

- Martin, 2008

Parental Origin of Aneuploidy

- molecular studies of trisomic spontaneous abortions
- autosomes >90% maternal
  - Hassold and Hunt, 2001

Most Sex Chromosomal Aneuploidies Result from Paternal Nondisjunction

paternal:

- 47,XYY 100%
- 45,X 80% Jacobs *et al.,* 1990
- 47,XXY 50% MacDonald *et al*., 1994
- 47,XXX 7% MacDonald *et al*., 1994

De Novo Structural Aberrations

- >90% paternal origin
  - Olson and Magenis, 1988
  - Thomas *et al*., 2010

Effect of Parental Age on the Frequency of Chromosome Abnormalities in Gametes

Oocytes

- Most studies show an increase in the frequency of aneuploid oocytes with maternal age
- No evidence on age and structural abnormalities

Sperm

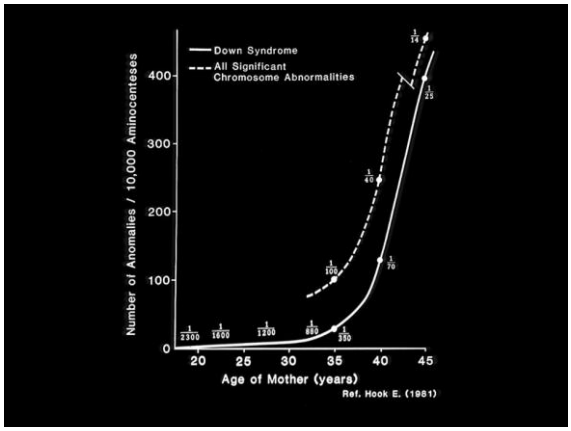
- Slight increase in the frequency of sex chromosome abnormalities with paternal age (~2x)
- Significant increase in the frequency of structural chromosomal abnormalities with donor age

Non-disjunction or Predivision in Oocytes

Non-disjunction: homologous chromosomes do not disjoin at Meiosis I or sister chromatids do not separate at Meiosis II

Predivision: premature division of centromeres at Meiosis I, resulting in single chromatids in metaphase II oocytes.

- Angel, 1991, 1997 - predivision in older females predominant error
- Garcia-Cruz *et al.,* 2010 – most errors from predivision even in younger females (18-35 years)



FISH Studies on the Effect of Paternal Age

- Griffin *et al.,* 1995
  - 24 men 18 - 60 years
  - significant increase for XX, YY, XY disomy
- Robbins *et al.,* 1995
  - 14 men – 2 age groups
  - significant increase for XX, YY disomy
- Martin *et al.,* 1995
  - 18 men in 6 age groups, 20-60 years
  - significant increase for YY disomy

Effect of Age on Structural Chromosomal Abnormalities in Sperm

Age Group	% Structural Abnormalities
20-24	2.8
25-29	2.2
30-34	3.3
35-39	7.8
40-44	7.7
45+	13.6

anova p=.007

- Martin and Rademaker, 1987

Structural Abnormalities and Paternal Age

- increased exposure to mutagens and clastogens with age may increase the risk of chromosome breaks
- continued cell divisions may lead to accumulation of risk for structural abnormalities with age

Distribution of Aneuploidy Among Chromosome Groups

- clues about etiology of aneuploidy
  - all chromosomes equal frequency?
  - certain chromosomes predisposed

Aneuploidy in Humans

newborns: trisomy 13
18
21
sex chromosomes

susceptible to nondisjunction
or
compatible with survival

Aneuploid Gametes

- hyperhaploidy in all chromosome groups for sperm and oocytes
- appears all chromosomes susceptible to nondisjunction

Hyperhaploid Oocytes

- Significant increase for chromosome groups D, F &G
- Most frequent individual chromosomes: 16, 21, 22,
  - Kuliev *et al*., 2002
  - Pellestor *et al*., 2002
  - Rosenbusch, 2004

11,615 Sperm Karyotypes

- aneuploidy in all chromosome groups
- significant increase for chromosome 21,22 and sex chromosomes (p=.0001)
  - Martin *et al*., 1991

Mean Disomy Frequency in Sperm

Chromosome	% Disomy
1	.09
2	.08
4	.11
9	.14
12	.16
13	.19
15	.11
16	.11
18	.11
20	.12
21	.29* p < .001
22	1.21* p < .001
sex	.43* p < .001

***= significant**

- Spriggs *et al.,* 1996

Nondisjunction in Individual Chromosomes

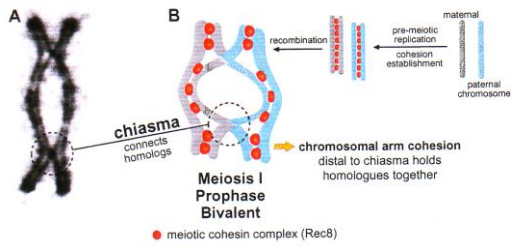
- FISH results corroborate results from sperm karyotypes
- increased frequency of aneuploidy for G group chromosomes (21 & 22) and sex chromosomes in human sperm

Other FISH Studies

- increased frequency of disomy for sex chromosomes
  - Williams *et al.,* 1993
  - Spriggs *et al*.,1995
  - Scarpato *et al.,* 1998
- increased frequency of disomy 21
  - Spriggs *et al.,* 1996
  - Blanco *et al.,* 1998

Increased Susceptibility to Nondisjunction

- G-group (21 and 22) and X-Y bivalent have only one crossover
- if recombination absent or reduced, may increase the chances of nondisjunction



Aneuploidy and Meiotic Recombination

Recent studies have linked meiotic recombination errors to aneuploid gametes and offspring in both females and males.

Meiotic Recombination and Aneuploidy in Females

- Altered recombination is associated with maternally-derived cases of trisomy 15, 16, 18, 21, sex chromosomes.
- A reduction in recombination may lead to unpaired homologues that lose the ability to segregate normally.
- For some chromosomes, the location of recombination sites confer an extra risk for an aneuploid gamete (e.g., chromosome 21)
  - Hassold *et al*., 1995
  - Robinson *et al*., 1998
  - Lamb *et al*., 1997

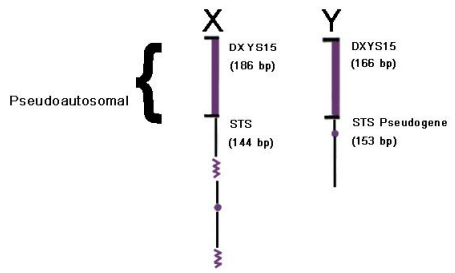
Meiotic Recmbination and Aneuploidy in Males

- 49 cases of paternally-derived 47,XXY
  - Hassold *et al.,*1991 – *39*
  - Lorda-Sanchez *et al.,* 1992 – *10*
    - **Both studies : reduced recombination in pseudoautosomal region of sperm that led to 47,XXY**

Single Sperm Typing

- to determine if there is a relationship between recombination in the pseudoautosomal region and nondisjunction
- compared frequency of recombination between STS/STS pseudogene (sex specific locus) and DXYS15 (pseudoautosomal locus) in unisomic *vs* disomic sperm

Heterozygous Male



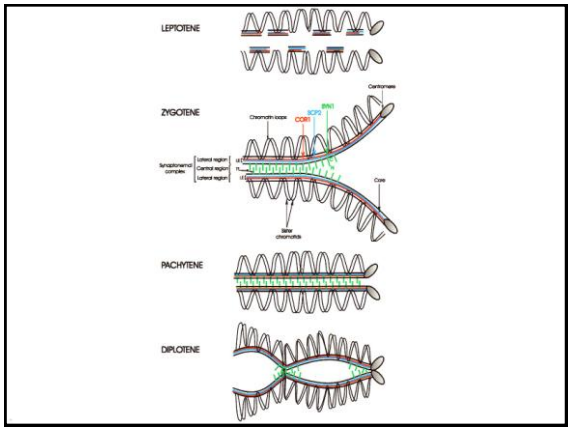
Results of Single Sperm Typing

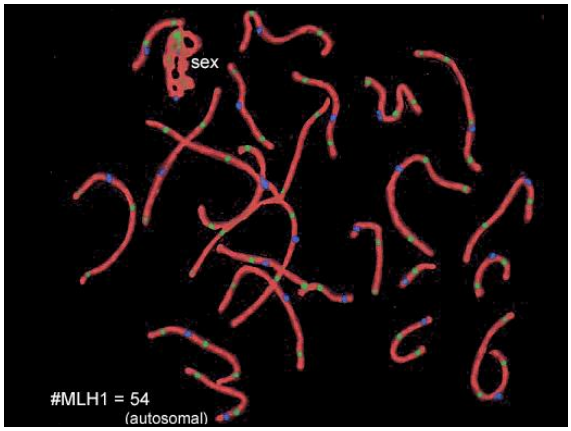
- 329 unisomic sperm - 38% recombination
- 150 disomic (24,XY) - 25% recombination
- significant decrease in recombination in XY sperm (p=.001)
- lack of recombination directly linked to nondisjunction

  - Shi *et al.,* 2001

Immunofluorescence Methods to Study Meiosis

- allows study of recombination in all chromosomes
- analysis of chromosome pairing by visualization of synaptonemal complex
- antibodies to:
  - synaptonemal complex (SCP1/SCP3)
  - recombination foci (MLH1)
  - centromeres (CREST)





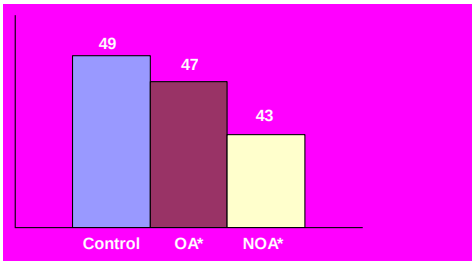
Analysis of Synaptonemal Complexes – 27 Normal men

- testicular samples from vasectomy reversals (15) and cancer patients (12)
- recombination foci - mean 48.5/cell
- 90% cells in pachytene
- 5% cells have at least 1 bivalent with no recombination foci
- no significant difference in vasectomy reversals *vs* cancer patients

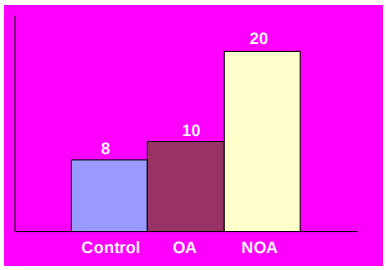
Infertile Men with Pachytene Cells

- 6/6 men with obstructive azoospermia (OA) had pachytene cells
  - mainly congenital absence of the *vas deferens* (CF)
- 14/29 men with nonobstructive azoospermia (NOA) had pachytene cells
  - 13 men with no meiotic cells
  - 1 man with a block at zygotene
    - Sun *et al.*, 2007

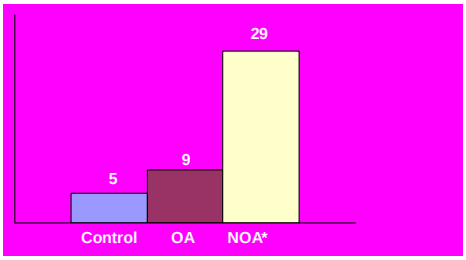
Mean Frequency of Recombination

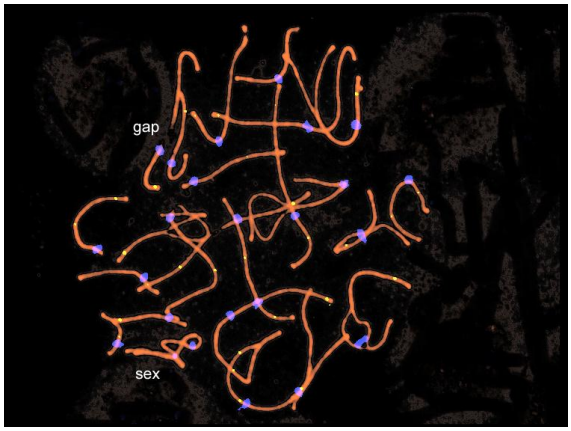


% Cells with Unsynapsed Regions



% Cells with at Least 1 Bivalent with no Recombination

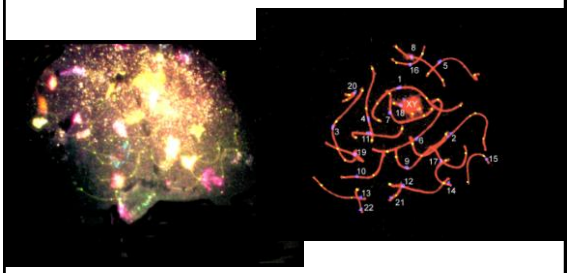


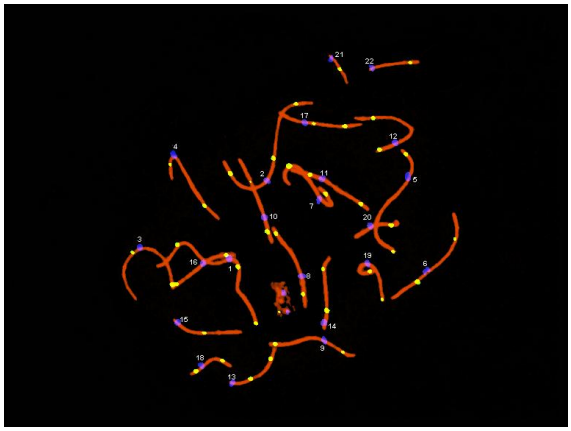


Meiotic Defects in Infertile Men

- In nonobstructive azoospermia, abnormalities in:
  - chromosome pairing
  - decreased frequency of recombination
  - increased frequency of bivalents with no recombination foci
- could lead to meiotic arrest or increased frequency of aneuploid sperm

CenM-FISH / Karyotyped SC





Non-Crossover Bivalents in Sperm

- 10 normal men studied
- cenM FISH on 886 pachytene cells (19,492 bivalents)
  - 27% - sex chromosome univalents (no c/o)
  - 60 autosomal non-crossovers
    - **significant increase for chromosomes 21,22**
  - sex chromosomes & G group chromosomes most susceptible to no recombination foci
  - consistent with sperm aneuploidy data (karyotypes and FISH)
    - Sun *et al.*, 2006

Meiotic Recombination and Sperm Aneuploidy in Infertile Men with Nonobstructive Azoospermia (NOA)

- 7 infertile men with NOA
- 6 controls (vasectomy reversal)
- meiotic recombination and FISH sperm aneuploidy for chromosomes 9, 21, X, Y
  - Sun *et al*., 2008

Recombination and Sperm Aneuploidy in Infertile Men - Results

- infertile men
  - significant increase in pachytene cells with achiasmate bivalents
  - significant increase in sperm aneuploidy
  - significant correlation between meiotic cells with no recombination in sex body and sex chromosome aneuploidy in sperm
- may contribute to elevated frequencies of chromosome abnormalities in ICSI offspring

Meiotic Recombination Errors in Oocytes

- Oocytes from 16-19 week fetuses from pregnancy terminations
- Pachytene cells with defective synapses or fragmentation 16-29%
- Abnormal pachytene cells had significantly fewer recombination foci than normal cells (49 *vs* 70)
- 8% cells with normal synapses had no recombination foci
- Errors could lead to high frequency of aneuploidy in human oocytes
  - Tease et al., 2006

Non-Cross-over Bivalents in Human Oocytes

Cheng *et al*., 2009:

chromosome 13	1%
chromosome 16	0%
chromosome 18	3%
chromosome 21	5%
chromosome 22	6%

Garcia-Cruz *et al*., 2010:

chromosome 16	7%

In general, higher % achiasmate bivalents in females with higher risk of segregation error.

Acknowledgements

- Qinghua Shi
- Maria Oliver-Bonet
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- Marv Fritzler
- Terry Ashley

Canadian Institutes of Health Research,
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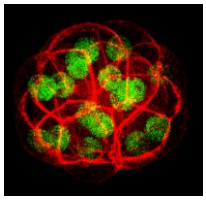
Chromatin states and lineage choice in the mouse preimplantation embryo ?



Maria Elena Torres-Padilla
metp@igbmc.fr
IGBMC, Strasbourg

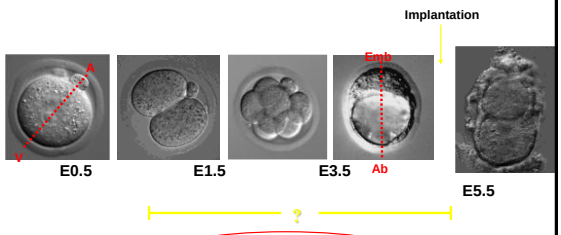
1. Introduction to the system: why, when and what to approach experimentally in the mouse embryo?
2. Epigenetic asymmetries at the beginning of development
3. Lineage choice: when and how do cells start to differ from each other?

How can one single cell generate all different cell types in the organism?

?

Thus, development is, by definition, epigenetic

Early mouse development and patterning



Transcriptional regulation ?
Chromatin?

Signal transduction?

Post-translational modifications?

Zygotic genome activation (ZGA) initiates at the late zygote stage and occurs in two phases

Maternal transcripts

Minor (first) ZGA

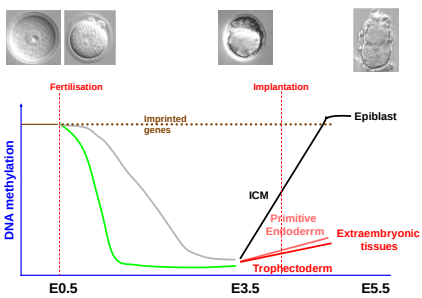
Major (second) ZGA

Changes in Chromatin Structure 'reprogramming'

- Protamine replacement
- Lysine acetylation and methylation of histones
- Recruitment of HP1 proteins
- Hyperphosphorylation of RNA Pol II

Initiation of embryonic transcription

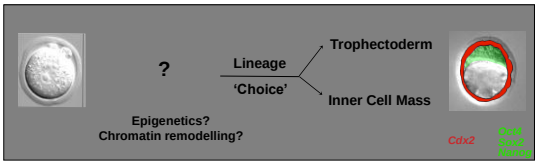
The mouse preimplantation embryo undergoes epigenetic reprogramming



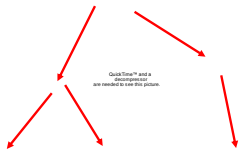
Adapted from Reik et al 2001, Li 2002

The embryo undergoes the first cell fate decision

(First overt sign of differentiation in the mammalian embryo)



Waddington's epigenetic landscape



What is epigenetics?

Information that is 'independent' of DNA sequence that is imposed on the chromatin and regulates downstream events such as gene expression, it is heritable

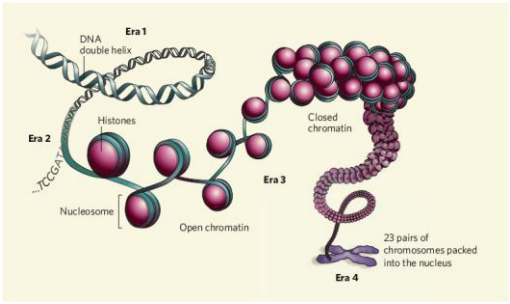
DNA methylation (imprinting), covalent modifications of histones Chromatin remodelling, histone composition (histone variants)

Epigenetic events control the transcriptional program of each cell by regulating *chromatin structure*

Dynamic programs of gene expression are required for both, the maintenance of a *pluripotent* state and differentiation of *pluripotent* stem cells into specific tissue lineages

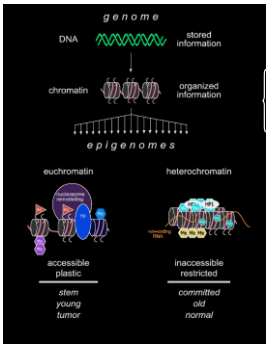
Development in multicellular organisms is, by definition, epigenetic

The epigenomics (?) era



Every level of organisation is an opportunity for regulation

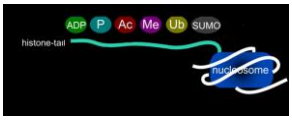
Genomes and Epigenomes

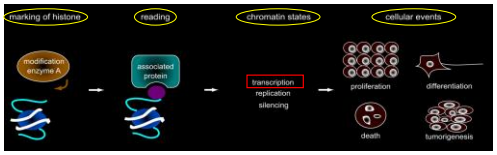


Histone content
Histone modifications
DNA methylation
Chromatin remodellers

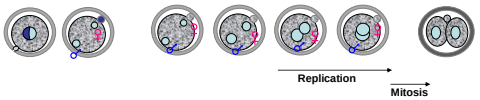
Adapted from Kubicek, et al., 2006

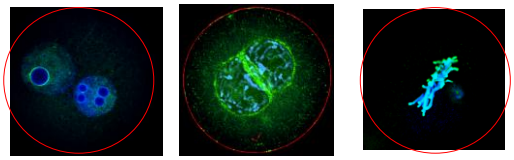
Regulation of cellular events through chromatin



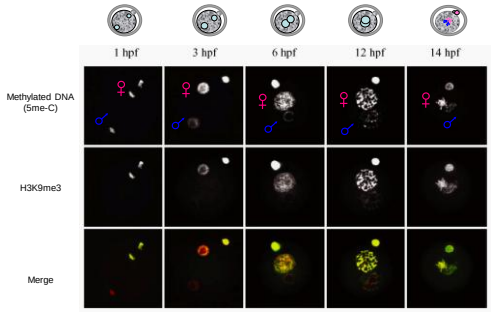


Wandering of the pronuclei in the female cytoplasm: non-mixing of the parental chromatin before the first mitosis



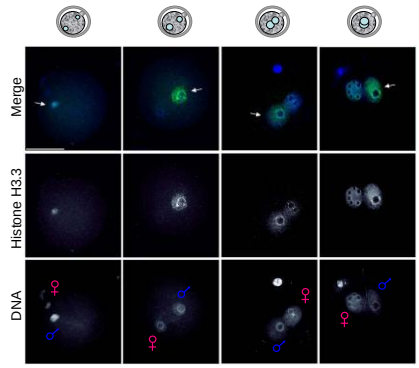


Epigenetic asymmetry of the male and female pronuclei is reflected by differences in DNA and histone methylation

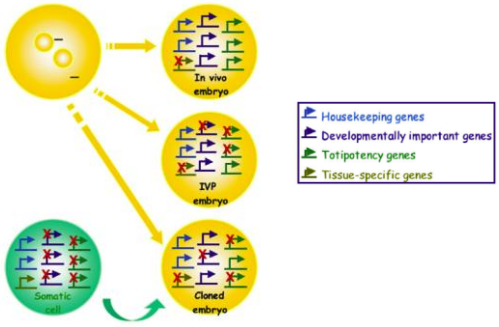


(From Reik et al 2003)

Globally, the chromatin composition is also difference between the parental chromatin



This epigenetic marking must be important for gene expression in development as both are altered in embryos derived from SCNT



Could chromatin states potentially impact on lineage choice?

Can we learn from gene expression profiles?

First mitoses: principles of embryonic patterning and what can go wrong with it

Takashi Hiiragi, M.D.,Ph.D.

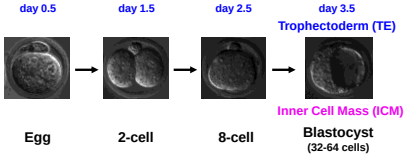
Mammalian Development Laboratory
Max-Planck Institute for Molecular Biomedicine, Münster

Learning Objectives

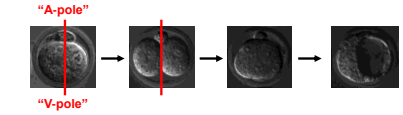
1. What are the principles of patterning mammalian embryos?

2. How is a mouse like a human?

Mouse pre-implantation development



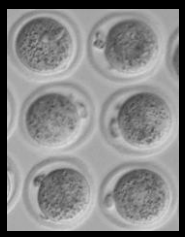
Principle 1. Dynamic and random process



Egg | 2-cell | 8-cell | Blastocyst (32-64 cells)

Pre-patterning?

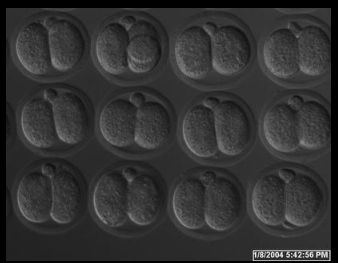
Mouse egg

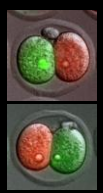


No "Animal-" or "Vegetal-" pole

(Hiiragi and Solter, 2004)

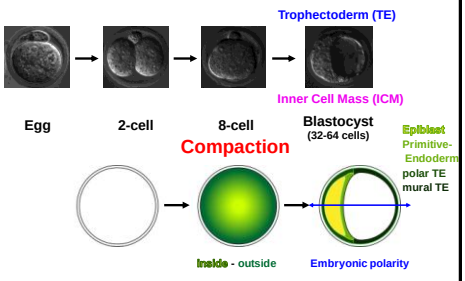
Mouse pre-implantation development
- from 2-cell to blastocyst





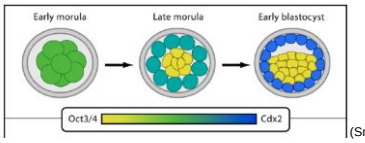
(Motosugi et al., 2005)

Mouse pre-implantation development



(Motosugi et al. 2005)

Molecular mechanism of the initial lineage specification

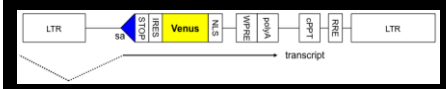


(Smith, 2005)

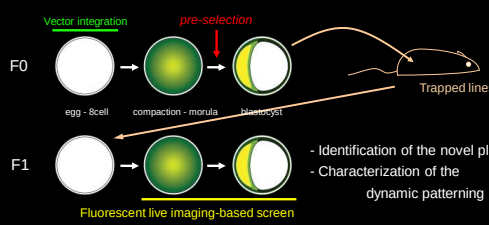
Interaction between Oct3/4 and Cdx2 determines ICM vs. TE differentiation. (Niwa et al., 2005)

A limited number of molecular players available !

Promoter Venus-trap screen



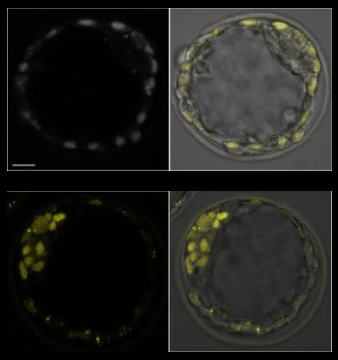
Lentiviral vector



- Identification of the novel players
- Characterization of the dynamic patterning process

(Dietrich and Wennekamp; in collaboration with Trono lab)

Venus-trapped mouse lines



TE-specific

fJAV 17

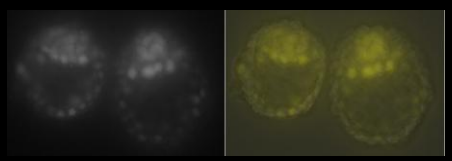
ICM-specific

JAV 53-4

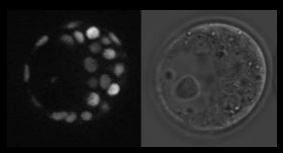
Venus-NLS

(Dietrich and Wennekamp; in collaboration with Trono lab)

Venus-trapped mouse lines

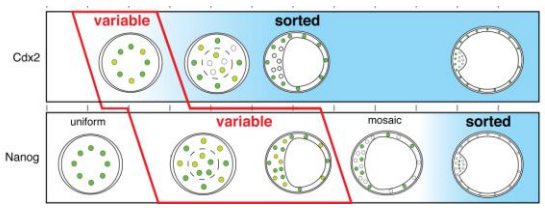


ICM-*enhanced* : fJAV12-3



(Dietrich and Wennekamp; in collaboration with Trono lab)

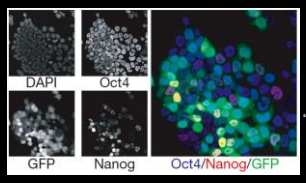
Principle 2. Stochastic processes



(Dietrich and Hiiragi, 2007)

Two patterning phases: 1. heterogeneity – stochastic patterning
2. sorting

Stochastic emergence of asymmetry



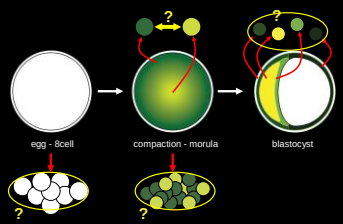
"Nanog fluctuates in mouse ES cells"
(Chambers et al. 2007)

"Dynamic equilibrium and heterogeneity of mouse ES cells" – Stella
(Hayashi et al. 2008)

"Subpopulations in undifferentiated ES cell culture" – Rex1
(Toyooka et al. 2008)

Single-blastomere gene expression profile

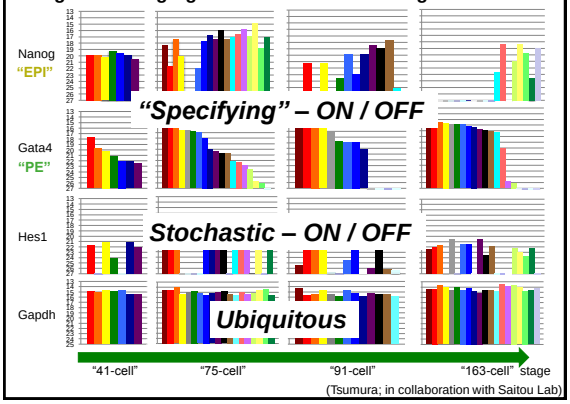
Are the cells "same" or "different"?



How many distinct populations?

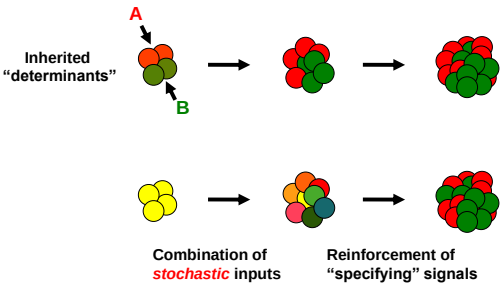
(Tsumura; in collaboration with Saitou lab)

Progressive segregation of EPI and PE lineages in the ICM

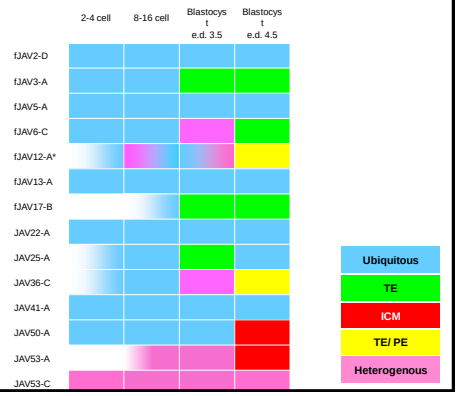


(Tsumura; in collaboration with Saitou Lab)

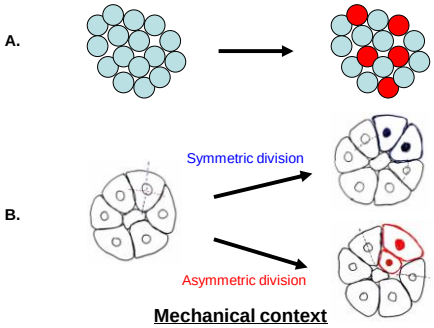
Emergence of asymmetry



Molecular dynamics identified by Venus-trap mice



1. Stochastic emergence of asymmetry

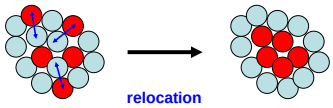


Mechanical context

(Dietrich and Hiiragi, 2008)

2. Sorting out …

A.



relocation

B.

change in gene expression

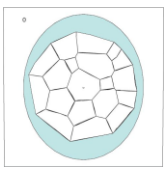
(Dietrich and Hiiragi, 2008)

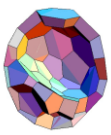
Principle 3. Mechanical context plays a key role

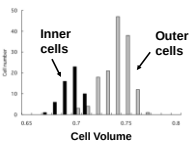
Cellular structural and mechanical context

Computer simulation of the blastocyst morphogenesis

- 40 equivalent cell aggregate
- expanding one blastocyst cavity







(Honda et al., 2008)

In-Out difference may emerge autonomously in the equivalent population.
– no need for *intrinsic* bias (localized *determinants)*

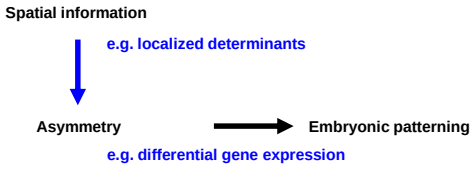
What are the principles of patterning mammalian embryos?

1. Dynamic and random process

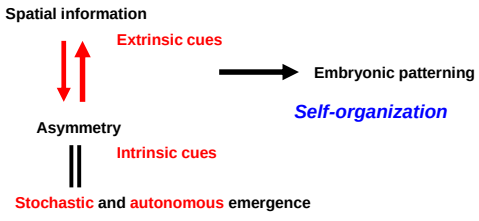
2. Stochastic processes

3. Mechanical context plays a key role

Principle underlying embryonic patterning



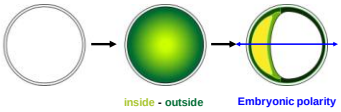
***Unique principles* in early mammalian development**



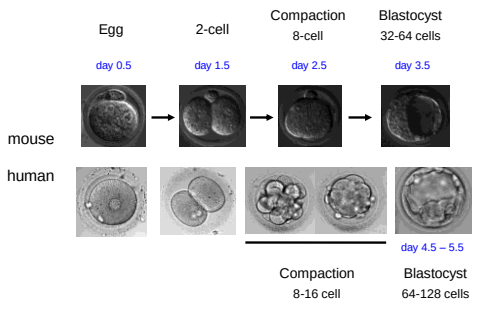
Robustness = Regulative capacity

Random process → **?** **Self-organization**

In a certain physical and temporal context

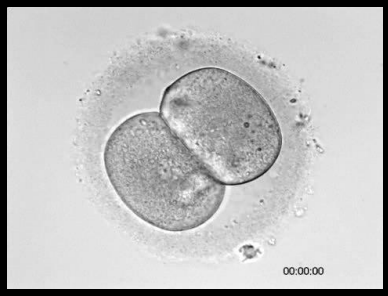


How is a mouse like a human?



(Mio and Maeda, 2008)

Human pre-implantation development



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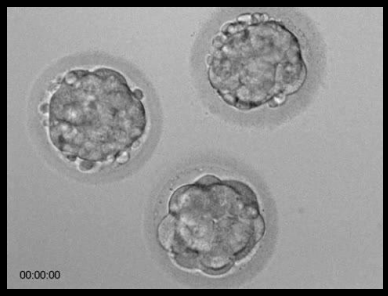
Human pre-implantation development



(Mio and Maeda, 2008)

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Early Stages or Blastocysts, A Critical Choice for Transfer

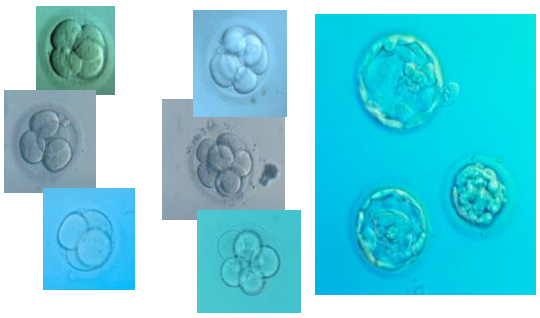
Pre-Congress Symposium
"From Gametes to Embryo: Genetics and Developmental Biology"
26th Annual Meeting of ESHRE
Rome, Italy
June 27th, 2010

Dr Gayle M. Jones, Ph.D.
Director of Research
Centre for Human Reproduction,
Genesis Athens Clinic, Athens, Greece

Learning Objectives

- Selection criteria available for day of transfer
- Laboratory considerations governing the choice of day of transfer
- Cycle considerations governing the choice of day of transfer
- How ART interventions such as PGD/PGS may dictate the day of transfer
- Patient factors that may affect choice of day of transfer
- Are there any negative outcomes associated with delaying embryo transfer to the blastocyst stage

Which Day to Transfer?



Embryo Transfer

- The majority of embryo transfers these days are performed to the uterus via the cervix despite the day of transfer
- *In vivo* the embryo would not normally enter the uterus until Days 4-5 at the morula-blastocyst transition
- Transfer of early cleavage stage embryos to the uterus of laboratory and domestic animal species results in failure or severely compromised implantation

Factors Governing Choice of Day of Transfer

- What selection criteria are available and their predictive value in identifying the single most viable embryo for transfer
- Number of embryos available for transfer
- Quality of embryos
- Quality of laboratory culture techniques
- Laboratory workload
- Efficiency of available cryopreservation techniques
- Impact of Other ART interventions i.e. PGD/PGS
- Patient factors

Selection Criteria

Day 2 Transfers

Very little available as selection criteria

- Undergone very few cleavage divisions since fertilization
- Development still under maternal genomic control
- Morphologic features usually used and this alone very poorly predicts ongoing viability
- Predictive value may be improved by combining with Day 0 or Day 1 parameters

Embryo Selection Criteria for Day 2 Transfers

Embryo Quality classification systems vary:

- Size of pronuclei
  - Irregularities in size result in arrest, multinucleation & mosaicism

Sadowy et al., 1998

- Position of 1st & 2nd PB relative to PN

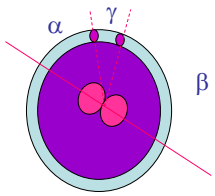
Garello et al., 1999

- Pattern and polarity of NPB

Scott & Smith, 1998; Tesarik & Greco, 1999

Pronuclei Relative to Polar Bodies

Garello et al., 1999



- α & γ not associated with viability
- β increases as embryo quality decreases
  - probably a measure of degree of rotation of pronuclei

Embryo Selection Criteria for Day 2 Transfers

Embryo Quality classification systems vary:

- Size of pronuclei
  - Irregularities in size result in arrest, multinucleation & mosaicism

Sadowy et al., 1998

- Position of 1st & 2nd PB relative to PN

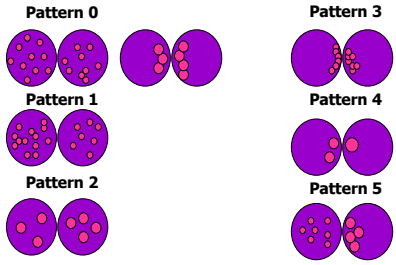
Garello et al., 1999

- Pattern and polarity of NPB

Scott & Smith, 1998; Tesarik & Greco, 1999

Scoring System 12 - 20 h Post-Injection

Tesarik and Greco, 1999



Pattern 0 associated with implantation

Embryo Selection Criteria for Day 2 Transfers

Embryo Quality classification systems vary:

- Timing of entry into syngamy/early cleavage
  - 15-16% of embryos enter early cleavage before 25h

Shoukir et al., 1997; Sakkas et al., 2001; Salumets et al., 2003

- Cleavage kinetics on Day 2
  - 4-cell on Day 2 has double the implantation potential as <4-cell or >4-cell on Day 2*

Edgar et al., 2007

- Morphology on Day 2
  - Regularity of blastomere shape and size*
  - Absence of or little fragmentation
  - Absence of cytoplasmic granularity
  - Absence of multinucleation*

Scott et al., 2007

Embryo Selection Criteria for Day 2 Transfers

- Double the implantation rate when two single parameters, entry into syngamy at 23-24h and 4-cell stage at 42h post-insemination used
- However this cohort represents approximately 44% of the embryo population so selection of the single most viable embryo remains difficult

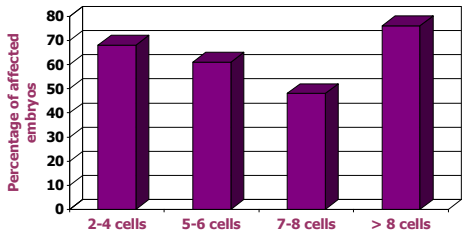
Lawler et al., 2007

Day 3 Transfers

More selection criteria available

- Better opportunity to evaluate cleavage rate as more cleavage divisions since fertilization
- Demonstrated ability to continue development under embryonic genomic control
- Selection of embryos showing normal cleavage rate on Day 3 (6- to 8-cells) have a lower incidence of chromosomal aneuploidy than embryos showing a more rapid or slower cleavage rate
- Predictive value may be improved by combining with Day 0/Day 1/Day 2 parameters

Distribution of Chromosomally Abnormal Embryos According to the Cell Number at 62 Hours Post-Insemination



Magli et al., 1998

Embryo Selection Criteria for Day 3 Transfers

Embryo Quality classification systems vary:

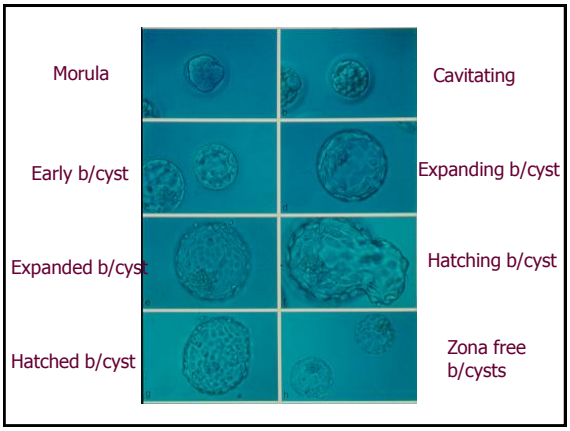
- Morphology on Day 3
  - 6- to 8-cell
  - Regularity of blastomere shape and size
  - Absence of or little fragmentation
  - Absence of multinucleation

Scott et al., 2007

Day 5 Transfers

More selection criteria available

- Better opportunity to evaluate embryos capable of complete preimplantation development in vitro
- Cleavage kinetics and morphology alone better predictor of viability than on preceding days of development
- Predictive value may be improved by combining with Day 0/Day 1/Day 2/Day 3 parameters



Blastocyst Scoring

Gardner et al., 2000

1. Early Blastocyst - cavity < 50% volume of embryo
2. Blastocyst - cavity > 50% volume of embryo
3. Full Blastocyst - cavity completely fills the embryo
4. Expanded blastocyst - cavity now larger than that of the early embryo and zona pellucida is thinning
5. Hatching blastocyst - trophectoderm herniates through zona
6. Hatched blastocyst - blastocyst completely free of zona

Blastocyst Scoring

Gardner et al., 2000

ICM Grading

A. Tightly packed, many cells
B. Loosely grouped, several cells
C. Very few cells

Trophectoderm Grading

A. Many cells forming a tightly knit epithelium
B. Few cells
C. Very few cells forming a loose epithelium

Blastocyst Scoring

Gardner et al., 2000

- Transfer of at least one grade 3AA or 4AA blastocyst is associated with very high pregnancy and implantation rates

Embryo Metabolism

- Non-invasive metabolomics can now be applied clinically to assist in selecting the most viable embryos for transfer whether on Day 2/Day3/Day5
- Presently used as an adjunct to morphological parameters to increase the power to predict viability

Factors Affecting Day of Transfer Decision Making

Number of Embryos Available for Transfer

- Greater the number of embryos available in the cohort for transfer the more difficult it is to select the single most viable embryo for transfer
- Large numbers of good quality embryos suggest that there might be a selection advantage for continued culture to the blastocyst stage of development
- Small numbers of good quality embryos run the risk of failure to reach the blasctocyst stage of development and therefore failure to transfer
  - in a general population, there is a 3 times greater risk of failure of embryo transfer if transfer is delayed to the blastocyst stage
- Recent evidence has suggested that even when only one embryo is available the implantation rate is higher when transferred at the blastocyst stage than on Day 2

Vlaisavljevic et al., 2007

Quality of Embryos Available for Transfer

- Several studies have indicated that the quality of embryos on Day 3 positively correlates to blastocyst development
  Jones et al.1998; Schoolcraft et al., 1999; Racowsky et al., 2000; Papanikolaou et al., 2005
- Many clinics adopt a protocol of continued culture only if 3-4 good quality 8-cell embryos are present on Day 3 to minimise the likelihood of cancellation of embryo transfer

Quality of Laboratory Culture Techniques

If transfer is to be delayed until the blastocyst stage:

- Culture techniques must be employed to ensure that all embryos can reach their full developmental potential
  - Quality sequential culture medium
  - Type and number of incubators
  - Gas phase
  - Quality culture ware
  - Quality control procedures
- Recommended that only laboratories capable of 45-50% of all zygotes developing to the blastocyst stage should consider extended culture and transfer at the blastocyst stage of development

Laboratory Workload

If transfer is to be delayed until the blastocyst stage:

- Significant increase in workload involved in extended culture with weekend work often involved
- Sufficient laboratory space to house additional incubators required for extended culture
- Depending on when the decision is made as to which day to transfer embryos, some flexibility in laboratory work approach needs to be adopted
- Concomitant reduction in the number of embryos requiring cryopreservation

Efficiency of Cryopreservation Programs

- Higher number of cleavage stage embryos available for cryopreservation compared to blastocyst stage
- Limited number of cumulative pregnancy studies comparing fresh and frozen transfers for randomised cleavage stage versus blastocyst stage transfers
  - van der Auwera et al., 2002 reported NSD
  - Rienzi et al., 2002 reported NSD provided one thaw cycle was undertaken for early cleavage embryos
  - Emiliani et al., 2003 reported higher cumulative pregnancy rates for transfer of early cleavage stage embryos compared to blastocysts but blastocyst cryosurvival was low
- Introduction of blastocyst vitrification and high post-warming survival may in the future favour blastocyst transfers

Impact of ART Interventions on Day of Transfer

- Although it is possible to perform early cleavage stage transfers following PGS on biopsied polar bodies, this only provides the maternal contribution to aneuploidy rates
- If PGS is to assess both the meiotic and mitotic contributions to aneuploidy then biopsy must be performed on either Day 3 or Day 5
- PGD in most instances is also required to assess both the maternal and paternal contributions
- Depending on the degree of complexity of the molecular analysis, embryo transfer may have to be deferred by at least one day or more following biopsy

Patient Factors

- Superovulation Regime

  Schoolcraft & Gardner, 2001
- Sperm Quality
  - Poor sperm quality results in a reduction in blastocyst numbers with no impact on viability

  Jones, 2000
- Maternal Age
  - Reduced ovarian reserve resulting in fewer embryos capable of development to the blastocyst stage
  - Increase in aneuploidy

  Jones, 2000

Advantages of Blastocyst Transfer

- Self-selection of most viable embryos
- Significant reduction in uterine contractility under the influence of higher progesterone by Day 5 therefore less likely that embryos expelled from the uterus following transfer

  Fanchin et al., 2001
- Better synchrony between uterine epithelium and embryo on Day 5 particularly for those patients showing premature luteinisation on the day of hCG (>1.5ng/ml progesterone)

  Papanikolaou et al., 2009

Advantages of Blastocyst Transfer

- Greater chance of selecting a normal embryo in the absence of PGS
  - 61% compared to 49% for early cleavage embryos

    Fragouli et al., 2008
  - Monosomy and complex aneuploidies can persist through to blastocyst stage

    Magli et al., 2000; Sandalinas et al., (2001); Fragouli et al., 2008
  - Decreased level of mosaicism in ICM compared to early embryos

    Evsikov & Verlinsky, 1998

Disadvantages of Blastocyst Transfer

- 3x greater chance of failure to have embryo transfer in general population
- Greater risk of cycle cancellation if culture conditions are sub-optimal
- Reports of increase in monozygotic twinning following blastocyst transfer but not confirmed in recent large cohort study

  Papanikolaou et al., 2010
- Altered sex ratio in favour of males

Meta-Analysis of Day2/3 versus Day 5 Transfers

Blake et al., 2007

- 3 times more likely to have a transfer cancelled if transfer delayed to Day 5 (not significantly different when only good prognosis patients considered)
- Live birth rate higher following blastocyst transfer (36% versus 29%)
- Higher pregnancy rate per couple following blastocyst transfer despite a higher incidence of failure to have a transfer
- Cryopreservation rates higher following early cleavage stage transfer vs. blastocyst transfer

Summary

To be a successful alternative to transfers of early cleavage stage embryos the following must be true

- those embryos which are developmentally competent need to be cultured in conditions which allows this developmental potential to be realized
- blastocysts must develop in sufficient numbers to allow the majority of patients to have an embryo transfer
- blastocysts which develop must be viable

Summary

Blastocyst versus Early Cleavage Stage Transfers

- The benefit is likely to be due to the element of selection of embryos that have demonstrated ability for complete developmental competence
- The benefit may also be due to the elimination of grossly chromosomally abnormal embryos
- Blastocyst transfer is unlikely to offer any selection advantage for those patients who produce very few embryos or very few chromosomally normal embryos
- Uterus may be more relaxed from extended exposure to progesterone which may improve ease of transfer and outcomes for certain populations of patients

Conclusions

- Advisable for all IVF programs to have a flexible approach to choice of day of transfer in order to optimise outcomes for all IVF patients

Mark your calendar for the upcoming ESHRE campus workshops!

- **Basic Genetics for ART Practitioners**
  organised by the SIG Reproductive Genetics
  16 April 2010 - Porto, Portugal

- **Array technologies to apprehend developmental competence and endometrial receptivity: limits and possibilities**
  organised by the Task Force Basic Science in Reproduction
  22 April 2010 - Brussels, Belgium

- **The management of infertility – training workshop for junior doctors, paramedicals and embryologists**
  organised by the SIG Reproductive Endocrinology, SIG Embryology and the Paramedical Group
  26-27 May 2010 - Kiev, Ukraine

- **Preimplantation genetic diagnosis: a celebration of 20 years**
  organised by the SIG Reproductive Genetics
  1 July 2010 - Rome, Italy

- **EIM 10 years' celebration meeting**
  organised by the European IVF Monitoring Consortium
  11 September 2010 - Munich, Germany

- **The determinants of a successful pregnancy**
  organised by the SIGS Reproductive Surgery, Early Pregnancy and Reproductive Endocrinology
  24-25 September 2010 - Dubrovnik, Croatia

- **Basic training workshop for paramedics working in reproductive health**
  organised by the Paramedical Group
  6-8 October 2010 - Valencia, Spain

- **Forgotten knowledge about gamete physiology and its impact on embryo quality**
  organised by the SIG Embryology
  9-10 October 2010 - Lisbon, Portugal

www.eshre.eu
(see "Calendar")

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Keep an eye on our calendar section for more information on

Upcoming events

- **Female and male surgery in human reproductive medicine**
  8-9 October 2010 - Treviso, Italy
- **Promoting excellence in clinical research: from idea to publication**
  5-6 November 2010 - Thessaloniki, Greece
- **"Update on pluripotent stem cells (hESC and iPS)" and hands on course on "Derivation and culture of pluripotent stem cells"**
  8-12 November 2010 - Valencia, Spain
- **Women's health aspects of PCOS (excluding infertility)**
  18 November 2010 - Amsterdam, The Netherlands
- **Endoscopy in reproductive medicine**
  24-26 November 2010 - Leuven, Belgium
- **Fertility and Cancer**
  25-26 November 2010 - Bologna, Italy
- **The maternal-embryonic interface**
  2-3 December 2010 - Valencia, Spain
- **GnHR agonist for triggering of final oocyte maturation – time for a paradigm shift**
  3 December 2010 - Madrid, Spain
- **Raising competence in psychosocial care**
  3-4 December 2010 - Amsterdam, The Netherlands

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