PRE-CONGRESS COURSE 2

Treating the man with evidence based medicine

SIG Andrology
Munich - Germany, 29 June 2014

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PEOPLE
MOVING SCIENCE

Treating the man with evidence based medicine

Munich, Germany
29 June 2014

Organised by
The ESHRE Special Interest Group Andrology

Contents

Course coordinators

Sheena E.M. Lewis (United Kingdom) and Rafael Oliva (Spain)

Course description

This course will
i) present the latest research on male reproductive health from the Reprotrain Consortia
ii) give overviews of endocrine disruption and male reproduction
iii) consider the latest evidence for genetic tests-how does male karyotyping impact on ART outcomes

Target audience

Clinicians, paramedical staff, embryologists and andrologists with an interest in extending their knowledge of male reproduction and the training of research andrologists

Scientific programme

Chairman: Sheena E. M. Lewis - Ireland

09:00 - 09:30	Training tomorrows research andrologists to embrace 21st century investigative techniques: the promise of the Reprotrain network ***Rafael Oliva - Spain***
09:30 - 09:45	Discussion
09:45 - 10:15	Sperm RNA as a diagnostic resource; what can it tell us that a standard test cannot and does it matter? ***David Miller - United Kingdom***
10:15 - 10:30	Discussion
10:30 - 11:00	Coffee break

Chairman: Rafael Oliva - Spain

11:00 - 11:30	Molecular messages in the ejaculate remain an underestimated resource for understanding male fertility ***Sophie Pison - Rousseaux - France***
11:30 - 11:45	Discussion
11:45 - 12:15	Steroidogenesis in the fetal testis and its susceptibility to disruption- the latest advances ***Richard Sharpe - United Kingdom***
12:15 - 12:30	Discussion
12:30 - 13:30	Lunch

Chairman: Jackson Kirkman-Brown - United Kingdom

13:30 - 14:00	Antiestrogens for treatment of male infertility or hypogonadism ***Michael Zitzmann - Germany***
14:00 - 14:15	Discussion
14:15 - 14:45	Genetic tests-how does male karyotyping impact on ART outcomes? ***Elsbeth Dul - The Netherlands***
14:45 - 15:00	Discussion
15:00 - 15:30	Coffee break

Chairman: Willem Ombelet - Belgium

15:30 - 16:00	Dietary supplements- are they any help? ***Jackson Kirkman-Brown - United Kingdom***
16:00 - 16:15	Discussion
16:15 - 16:45	Preserving fertility before puberty: what should the clinician know? ***Herman Tournaye - Belgium***
16:45 - 17:00	Discussion
17:00 - 18:00	SIG Andrology Annual General Meeting

reprotrain
Reproductive Biology Early Research Training

Training tomorrows research Andrologists to embrace 21st century investigative techniques: the promise of the Reprotrain network

Rafael Oliva
Human Genetics Laboratory, Faculty of Medicine and Hospital Clínic
University of Barcelona, Barcelona, Spain. roliva@ub.edu

Pre-congress Course. 30th Annual Meeting of ESHRE
Munich, Germany, 29 June July 2014

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I have no conflict of interest on any potential commercial relationships or other activities related to the current talk.

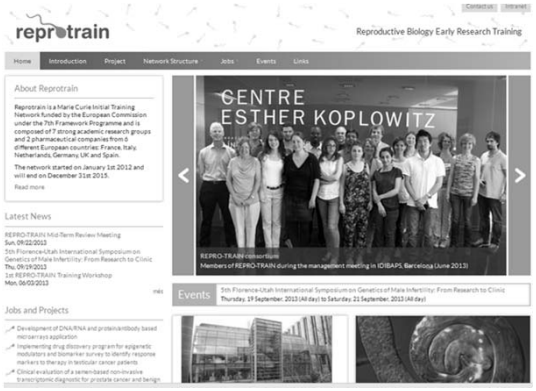
PCC Learning objectives. Attendant to the course will be expected to be learn:

- **About current European training initiatives in andrology.**
- **Frontier knowledge and research on components of the sperm cell** (sperm RNA, epigenetics, **proteome**) **and their potential involvement in male infertility or usefulness as diagnostic tools.**
- The potential threads of endocrine disruptors to male fertility and related pathogenic mechanisms in the testis.
- Therapeutic strategies (pharmacological) for the treatment of infertility or hypogonadism.
- Potential benefits of dietary supplements in male fertility.
- Relevance of genetic testing and its impact on ART outcomes.
- Controversial detrimental effects and the consequences of ICSI.

Training tomorrows research Andrologists to embrace 21st century investigative techniques: the promise of the Reprotrain network

- **Reprotrain training network**
- Methodological approaches to study the sperm cell proteome

www.reprotrain.eu



Reprotrain:

Reproductive Biology Early Research Training Network

EU FP7 Mari Curie Early Research Training Network
2012-2014

3,6 Million Euro

Train 10 Early Stage Researchers (PhDs)
4 Experienced Researchers (early postdocs)
Develop joint Reproductive Biology projects and objectives in reproductive biology

Reprotrain idea:

Idea started in 2009

Motivated by the lack of projects on reproductive biology funded by the EU

Follow up of the FP7 calls evidenced a total lack of reproduction, andrology, fertility/infertility as priority areas.

The only chance was to apply for non-directed (bottom-up) calls for collaborative research:

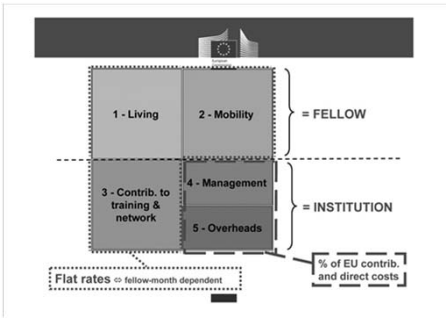
Marie Curie Initial Training Networks:

- Joint project common to different labs
- Mainly funds salaries of ESRs and ERs
- Some funds for training and laboratory expenses

Mari Curie InitialTraining Networks funding rate: 7,4%

We applied 3 times. Successful in our third application.

Kick-off meeting (march 2012):



The **overall objectives** of Reprotrain are the following:

•To provide an interdisciplinary training programme for ESRs in state-of-the-art male Reproductive Biology and Andrology allied to Medicine.

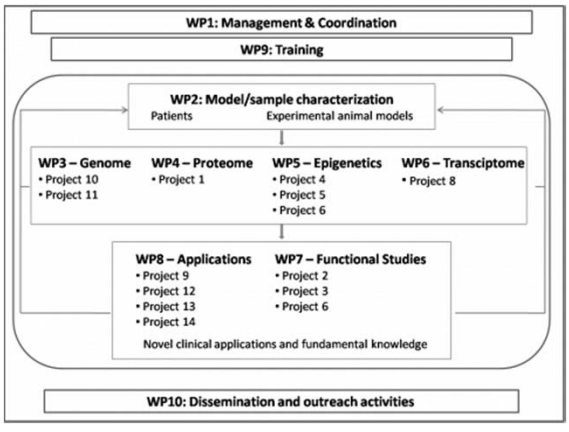
•To overcome historical fragmentation in the field of spermatogenesis and Andrology research by integrating and implementing different disciplines in our ongoing research projects.

•To develop and implement systems biology based approaches (genomic, proteomic, transcriptomic, epigenetic and metabolomic) to boost the acquisition of fundamental knowledge in the field of male Reproductive Biology and Medicine.

•To develop novel applications of this knowledge by potentiating the synergies between consortium members and private sector partners.

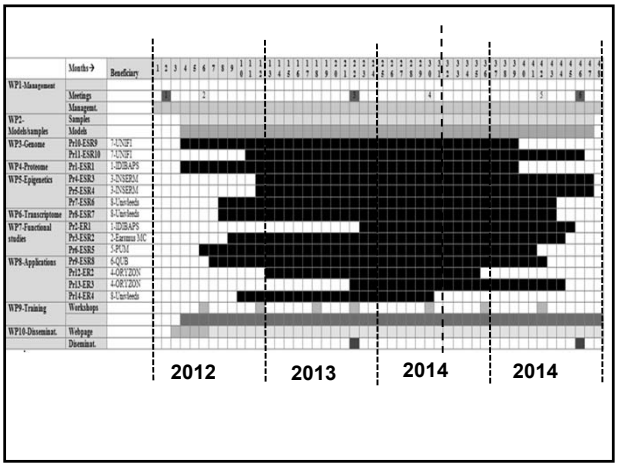
•To consolidate (or initiate) scientific collaborations among groups and to potentiate our respective synergies.

•Set up the basis for subsequent collaborative EU funded projects.



Specific projects:

Fellow	Project title
ESR1	Identification of the conserved core sperm nuclear proteome and identification of abnormalities in infertile patients
ER1	Genomics and epigenomics of male infertility and identification of the function novel genes and proteins
ESR2	Meiotic and postmeiotic chromatin remodeling and its relevance for early embryonic development in mouse
ESR3	Male genome reprogramming by histone variants and histone modifications during spermatogenesis in the mouse
ESR4	Mature sperm nuclear epigenome characterization and epigenetic potential
ESR5	Synthesis, its control and function of sperm proteins in *Drosophila*
ESR6	Investigating the relationship between sperm chromatin domains and fertility
ESR7	Mapping of gene networks involved in deregulated spermatogenesis by transcript profiling of semen from fertile and infertile men
ESR8	Characterisation of the relationships between human sperm DNA fragmentation, DNA adducts, protamines and proteomic profiles and male infertility through assisted conception outcomes
ESR9	High resolution X chromosome array-CGH study in azoospermic men and functional study of the genes involved
ESR10	Y chromosome-linked CNVs and their biological consequences
ER2	Development of DNA/RNA and protein/antibody based microarrays application
ER3	Implementing drug discovery program for epigenetic modulators and biomarker survey to identify response markers to therapy in testicular cancer patients
ER4	Clinical evaluation of a semen-based non-invasive transcriptomic diagnostic for prostate cancer and benign prostatic hyperplasia



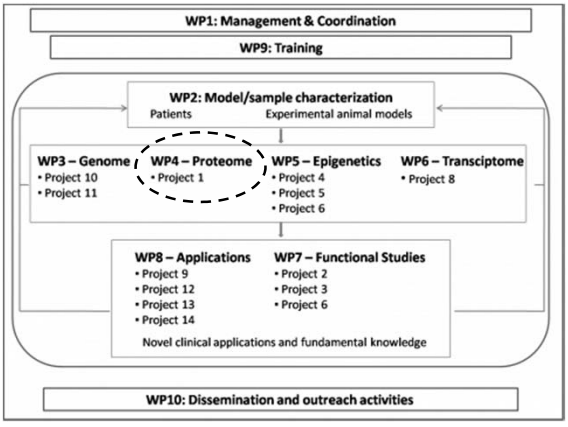
Sperm Phenotypic assessment, Proteomics, Transcriptomics, Epigenetics

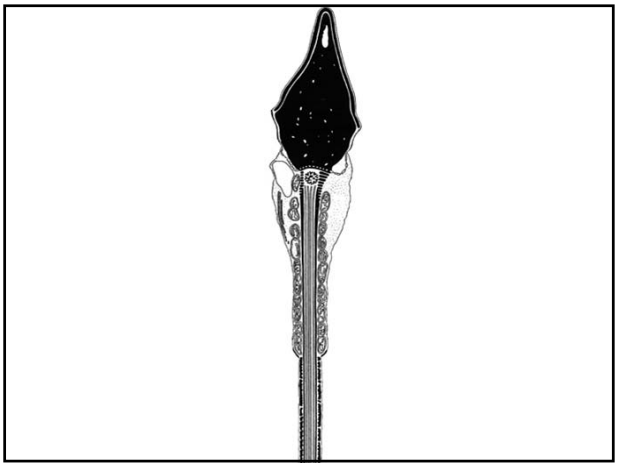
Setting up the basis for subsequent collaborative EU funded projects

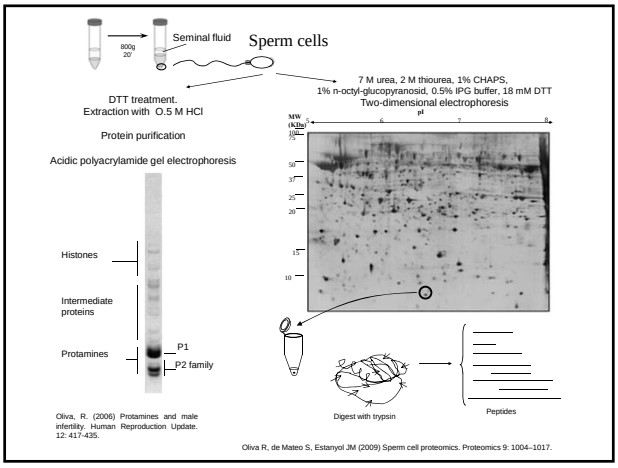
Training tomorrows research Andrologists to embrace 21st century investigative techniques: the promise of the Reprotrain network

•Reprotrain training network

•**Methodological approaches to study the sperm cell proteome**







Oliva, R. (2006) Protamines and male infertility. Human Reproduction Update. 12: 417-435.

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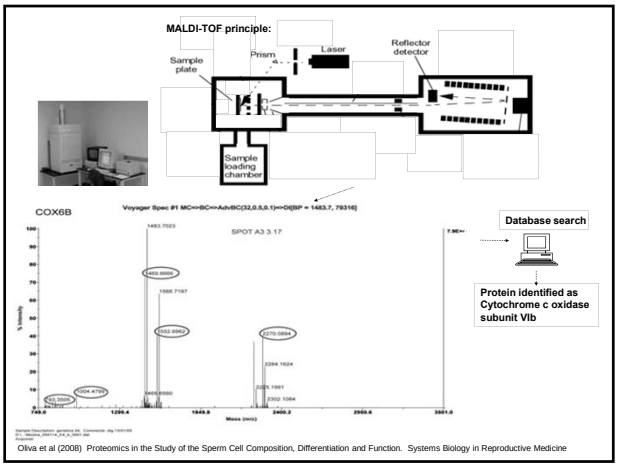
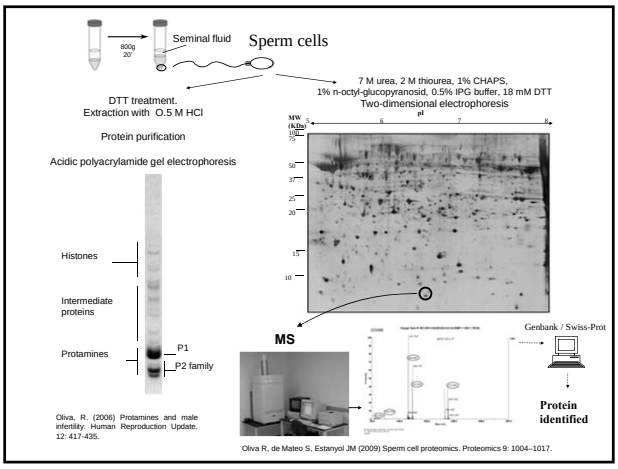


Oliva et al (2008) Proteomics in the Study of the Sperm Cell Composition, Differentiation and Function. Systems Biology in Reproductive Medicine



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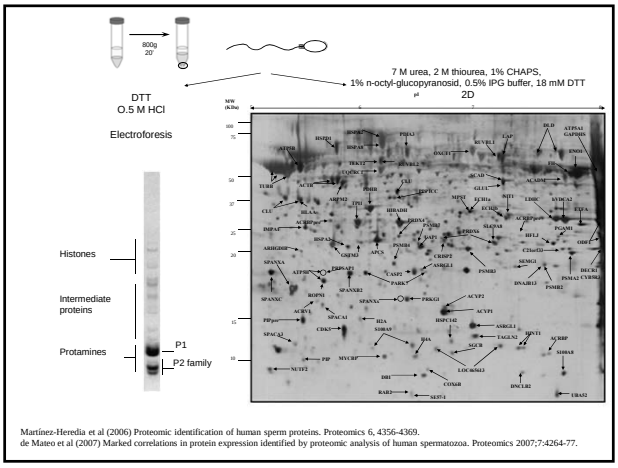
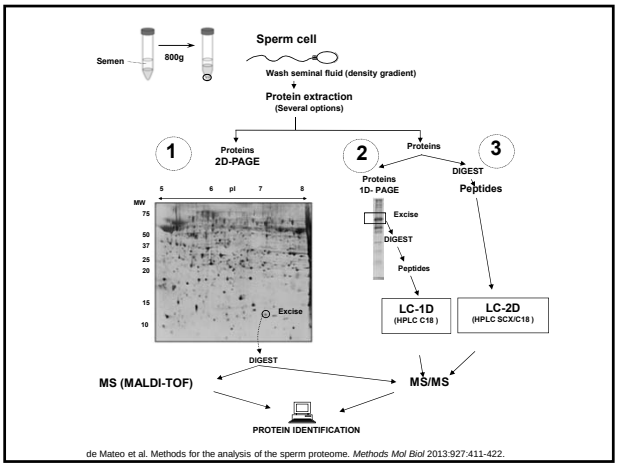


Martínez-Heredia et al (2006) Proteomic identification of human sperm proteins. Proteomics 6, 4356-4369.
de Mateo et al (2007) Marked correlations in protein expression identified by proteomic analysis of human spermatozoa. Proteomics 2007;7:4264-77.

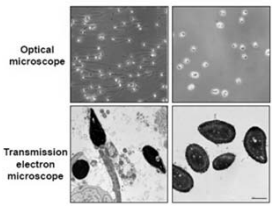


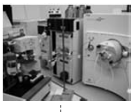
de Mateo et al. Methods for the analysis of the sperm proteome. *Methods Mol Biol* 2013:927:411-422.

Sperm cell nuclear proteome

Dr. Sara de Mateo

Sperm cell → Nuclei → Protein extraction → Peptides → LC-MS/MS



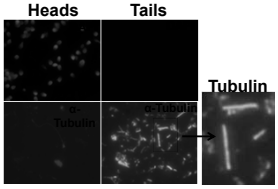


408 proteins identified

de Mateo et al. Proteomic characterization of the human sperm nucleus. Proteomics. 2011 Jul;11(13):2714-26

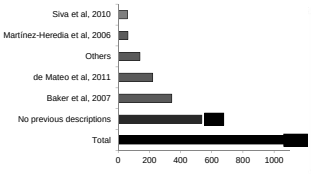
Sperm tail proteome

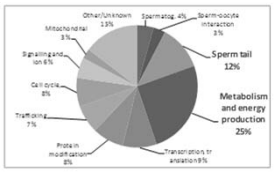
Dr. Alexandra Amaral



LC-MS/MS

1049 proteins identified





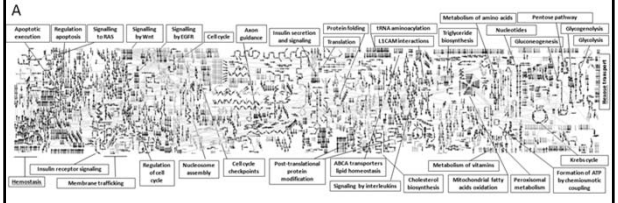
Amaral et al. Human sperm tail proteome suggests new endogenous metabolic pathways. Mol Cell Proteomics. 2013 Feb;12(2):330-42

How many proteins in the mature normal human sperm cell?

Dr. A. Amaral

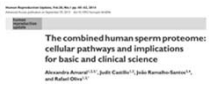
Compilation of published proteomes: 6198 proteins

A



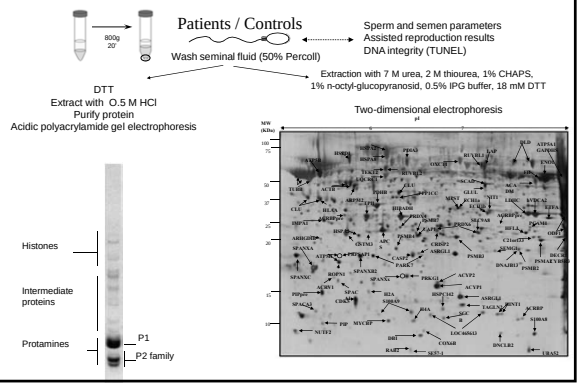
Predicted to exist from the pathway analysis: ~1800 proteins

Estimation of the complete proteome : ~8000 proteins



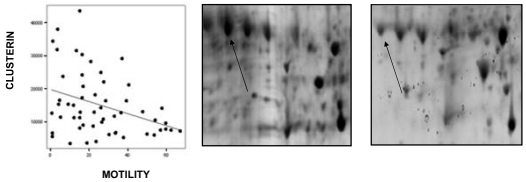
The combined human sperm proteome: cellular pathways and implications for basic and clinical science

Protein abundance increased or decreased in patients?



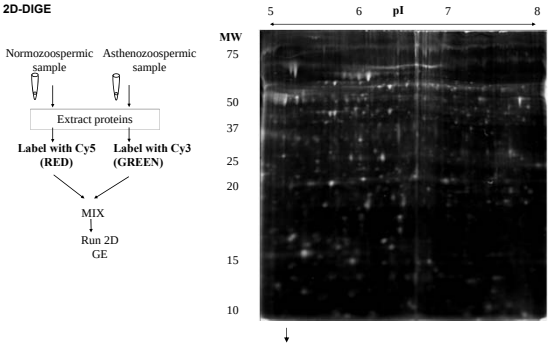
Motility

Astenozoospermia:

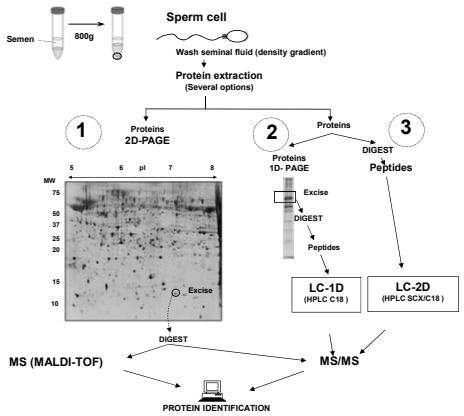


Martínez-Heredia et al (2008) Identification of proteomic differences in asthenozoospermic sperm samples. Human Reproduction 23(4):783-91

2D-DIGE



MS → Protein identification



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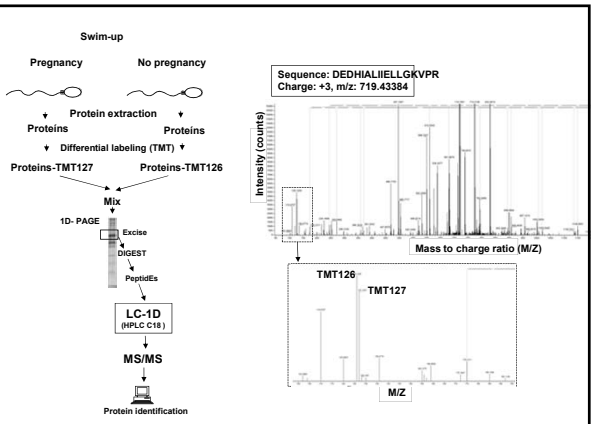


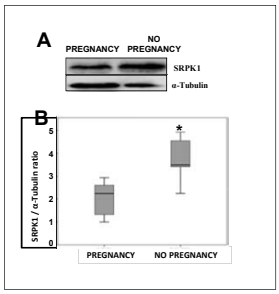
Azpiazu et al. Human Reproduction, in press

1717 proteins identified

In the "No pregnancy" group as compared to the "pregnancy" group:

- **35 increased**
- **31 decreased**

Azpiazu et al. Human Reproduction, in press

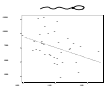


Azpiazu et al. Human Reproduction, in press

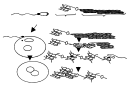
Summary

•**We are developing the European Reprotrain collaborative ITN with the goals to train next generation of researchers in reproductive biology while developing joint collaborative projects.**

•**In the Proteomic analysis of the sperm cell we are identifying proteins increased or decreased in different types of infertile patients and in different conditions in animal models, with a potential to be useful as diagnostic or prognostic markers.**



•**The mature sperm cell delivers to the oocyte chromatin associated proteins in addition to protamines and histones, with the potential of delivering a wealth of epigenetic information.**



•**The analysis of the sperm proteome and the compilation has resulted in the identification of 6198 proteins, and from pathway analysis we predict that the complete human sperm proteome will be composed of around 8000 proteins.**

•**A lot still needs to be done: Relationship between the sperm proteome, transcriptome, chromatin structure, epigenome, metabolome in health and disease. Exploitation of the synergies among labs and collaboration necessary. Future joint projects in the context of the EU Horizon 2020 calls needed.**

Selected references related to the proteomic study of the sperm cell (from recent to oldest):

Azpiazu R, Amaral A, Castillo J, Estanyol JM, Guimerà M, Ballescà JL, Balasch J, Oliva R. High throughput sperm differential proteomics suggest that epigenetic alterations contribute to failed assisted reproduction. Human Reproduction 2014, in press.

Amaral A, Castillo J, Ramalho-Santos J, Oliva R. 2014. The combined human sperm proteome: cellular pathways and implications for basic and clinical science. Human Reproduction Update 2014 Jan-Feb;20(1):40-62.

Jodar M, Oliva R. Protamine alterations in human spermatozoa. Adv Exp Med Biol. 2014;791:83-102.

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Castillo J, Amaral A, Oliva R. Sperm nuclear proteome and its epigenetic potential. Andrology. 2013 Dec 10.

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Oliva R. SBiRM: Focus on proteomics and reproduction. Preface. Syst Biol Reprod Med. 2012 Aug;58(4):177-8.

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Oliva R and Casillo J (2011) Sperm Nucleoproteins. In: A. Zini and A. Agarwal (eds.), Sperm Chromatin: Biological and Clinical Applications in Male Infertility and Assisted Reproduction, DOI 10.1007/978-1-4419-6857-9_3.

Oliva R, de Mateo S, Castillo J, Azpiazu R, Oriola J, Ballesca JL. Methodological advances in sperm proteomics. Human Fertility, December 2010; 13(4): 263–267.

Oliva R, de Mateo S, Estanyol JM (2009) Sperm cell proteomics. Proteomics 9: 1004–1017.

Martínez-Heredia J, de Mateo S, Vidal-Taboada JM, Estanyol JM, Ballescà JL and Oliva R (2008) Identification of proteomic differences in asthenozoospermic sperm samples. Human Reproduction 23(4):783-91.

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de Mateo S, Martínez-Heredia J, Estanyol JM, Domíguez-Fandos D, Vidal-Taboada JM, Ballescà JL and Oliva R (2007) Marked correlations in protein expression identified by proteomic analysis of human spermatozoa. Proteomics 2007;7:4264-77.

Martínez-Heredia J, Estanyol JM, Ballescà JL and Oliva R (2006) Proteomic identification of human sperm proteins. Proteomics 6, 4356-4369.

For more information see: www.reprotrain.eu and www.ub.edu/humangen

The author and presenter confirms that he has no conflict of interest with regard to collaborations with any industrial or pharmaceutical organisation

David Miller (University of Leeds, UK)

Sperm RNA as a diagnostic resource; what can it tell us that a standard test cannot and does it matter?

David Miller, BSc, PhD
University of Leeds

Learning Outcomes

At the end of this lecture, you should be more aware of the following:

- The presence of RNA in sperm.
- The unexpected complexity of sperm RNA.
- Sperm RNA as a non-invasive proxy for testicular gene expression.
- The relationship between sperm RNA and sperm phenotypes.
- Comparison with proteomics.
- Targeted approaches to using sperm RNA as a predictor of phenotype and of fertility.
- Microarray and sequencing based approaches to investigating sperm RNA.
- Existing and potential clinical applications.
- Ongoing research into understanding why sperm RNA exists.
- Overcoming barriers to using sperm RNA diagnostically.

Male Infertility: the scale of the problem.

1 in 6 couples experience infertility problems

Estimates of male involvement range from 30-50% with ~10% understood cause

Obstructive azoospermia ~ 5%

Non obstructive azoospermia / severe oligozoospermia ~ 5%

Structural and numerical chromosomal abnormalities ~15%

Deletions in the Y ~ 15%

Rare metabolic disorders (Spino-Bulbar, PAI etc) < 5%

Unknown others > 50%

All other infertility / subfertility ~90%

Abnormal semen profiles ~ 40%

Apparently normal semen profiles ~ 60%

Environmental impact?

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LIGHT Leeds Institute of Genetics, Health and Therapeutics

UNIVERSITY OF LEEDS

Identifying genetic/epigenetic effects linked to male fertility

Traditional gene discovery strategies? Because different mutations may cause similar effects, TGCS's are unsuitable.

Stigma of male infertility makes recruitment of consanguineous subjects very difficult.

Testicular biopsy? Only reasonable with clear phenotypes (azoospermia / severe oligozoospermia)

Spermatozoa as a proxy of the testis?

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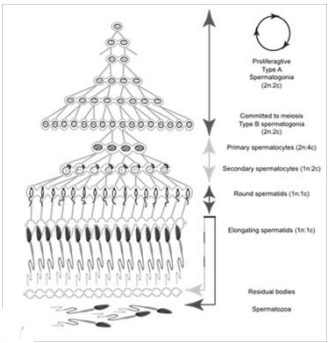
LIGHT Leeds Institute of Genetics, Health and Therapeutics

UNIVERSITY OF LEEDS

Spermatogenic Developmental Programme



64 Days in Humans

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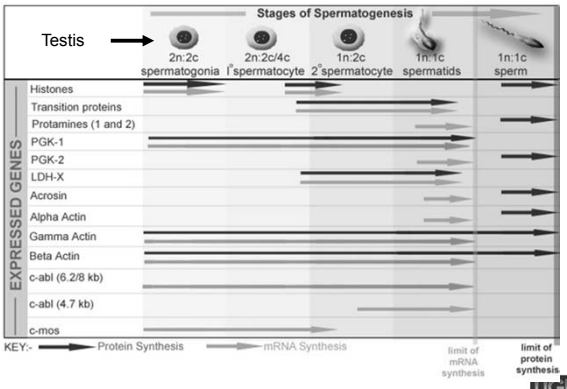
LIGHT Leeds Institute of Genetics, Health and Therapeutics

UNIVERSITY OF LEEDS

Control of gene expression in spermatogenesis



Diagnostic potential of of existing sperm function applications

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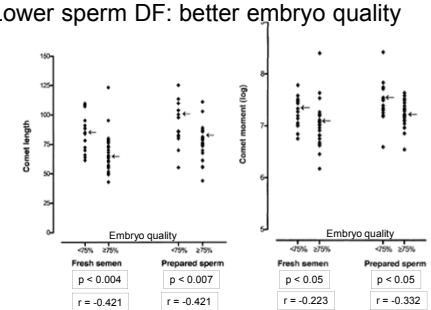
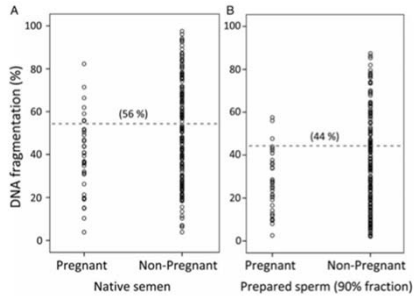
DNA Fragmentation Lower sperm DF: better embryo quality



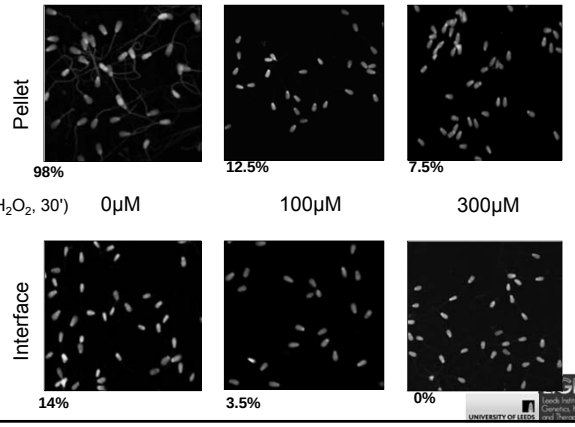
Tomsu et al, Hum Rep, 2002

DNA Fragmentation
Lower sperm DF: more pregnancies



Simon et al, Fert Steril, 2012

DNA fragmentation by AO assay



Morphology?

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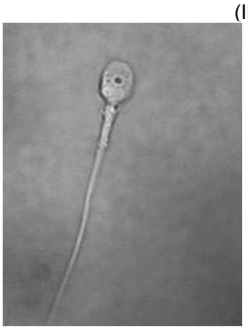
Intracytoplasmic morphologically selected sperm injection (IMSI)



- Semen sample processed.
- Sperm imaged using Nomarski interference contrast microscopy.
- Images captured by HD camera and displayed on HD monitor.
- >6000 x magnification (compared with typical 600 x)
- Each sperm examined by two embryologists.

	ICSI	IMSI
Clinical pregnancy rate (%)	58/219 (26.5%)	89/227 (39.2%)
Implantation rate (%)	59/521 (11.3%)	97/560 (17.3%)

Antinori *et al.*, RBM Online, 2008

Proteomics

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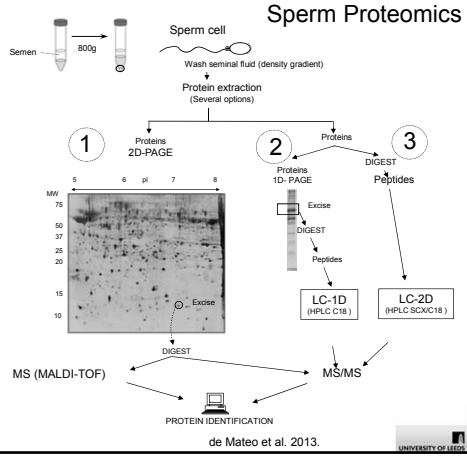
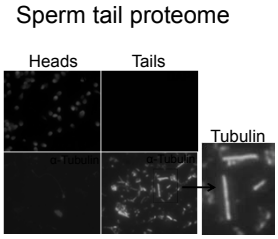
Sperm Proteomics



de Mateo et al. 2013.

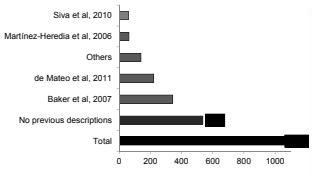
Sperm tail proteome

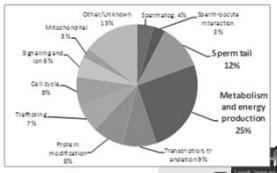


Dr. Alexandra Amaral

LC-MS/MS

1049 proteins identified





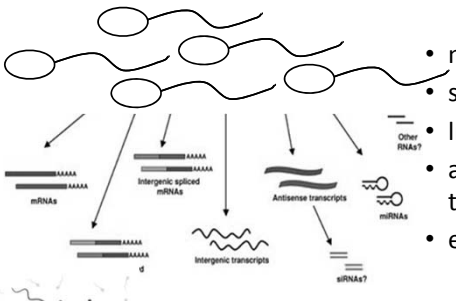
Amaral et al. 2013

Transcriptomics

⇕

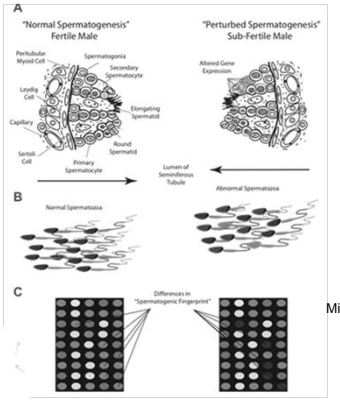
Proteomics

Complex repertoire of sperm RNAs (>3000)



- mRNA
- sncRNA
- lncRNA
- antisense transcripts
- etc....

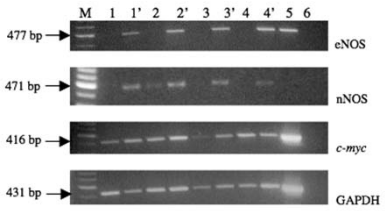
Origins of male infertility



Miller & Ostermeier, 2006

Good versus poor forward progressive motility in human spermatozoal sub-populations

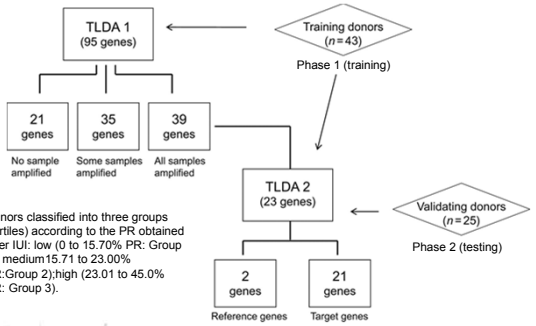
eNOS and nNOS levels



1 - 4	highly motile spermatozoa
1' – 4'	poorly motile spermatozoa
5	granulosa cell cDNA
6	water blank

Lambard et al, 2004

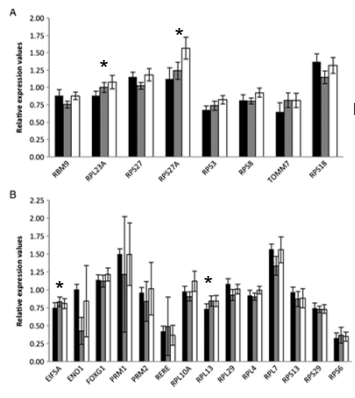
TaqMan low density array (TLDA) workflow for assessing sperm RNA expression profile in relation to (IUI) pregnancy rate



Donors classified into three groups (tertiles) according to the PR obtained after IUI: low (0 to 15.70% PR: Group 1); medium15.71 to 23.00% PR:Group 2);high (23.01 to 45.0% PR: Group 3).

Bonache S et al., 2012

Expression ratios of target genes according to the IUI PR.



IUI significant differences

Grp1(L)

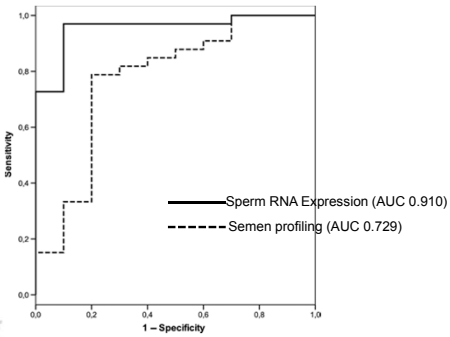
Grp2 (M)

Grp3 (H)

IUI insignificant differences

Bonache S et al. 2012 ($p < 0.05$, Kruskal–Wallis test)

ROC curves for predictive classification of donors (sperm RNA expression superior to semen profiling)



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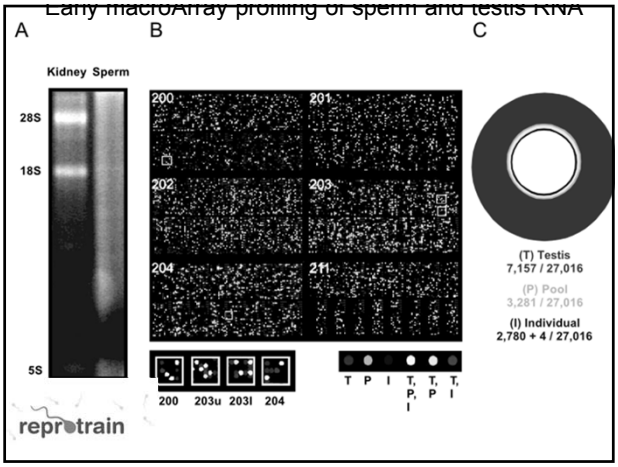
Bonache S et al, 2012

Early macroArray profiling of sperm and testis RNA



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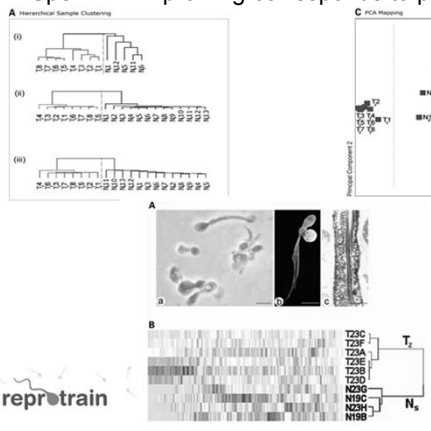
Sperm RNA profiling corresponds to phenotypes



Platts et al, 2007

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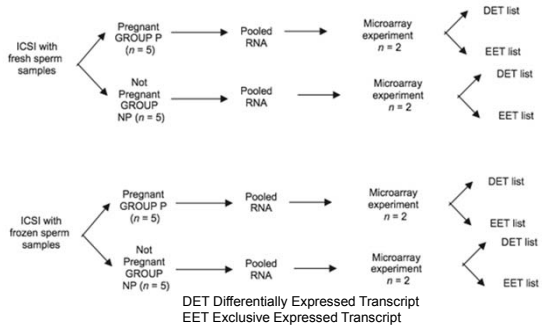
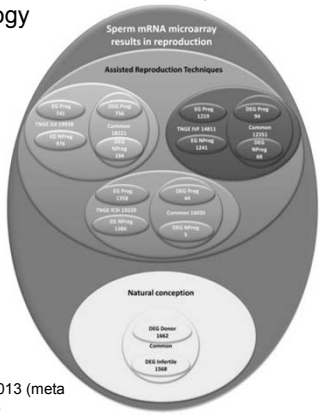
Assessment of sperm using mRNA microarray (Affymetrix U133 plus 2) platform



DET Differentially Expressed Transcript
EET Exclusive Expressed Transcript

García-Herrero ,et al, 2009

Assessment of sperm using mRNA microarray technology



IVF
IUI
ICSI
NC

Garrido et al, 2013 (meta study)

Interspecies comparison and characterisation of sperm RNA

→ Total RNA approach: four species




→ development of optimized RNA extraction of spermatozoa from each species
- → issue: femtograms of RNA in each spermatozoon.
- → Issue: Difficulty of RNA extraction from sperm varies with each species (human easier than bull, for example).
- → Issue: How to get rid of the rRNA (>80% of total RNA)?

→ next-generation RNA sequencing based on total RNA, less rRNA to maximise RNA information.

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First look: Bull

Sperm:

counts	symbol
157078	RN7SL1
3024	CABS1
2302	PRM1
1977	TUBA3E
1584	C25H16orf82
1448	HMGB4
1348	SPEM1
1291	TBC1D5
1244	KIF17
1236	GOLGA4
1134	SF3B1
1124	ODF2
1061	BRCA1
1011	LPIN1
898	CHMP5
893	TCP11
887	OAZ3
847	BAZ2B
841	ANKS3
806	HDAC11
803	ADAMTS6
752	ZCCHC6

counts	symbol
745	BCL2L11
738	OSBP2
733	PRKCD
729	HEATR8
722	CCDC181
690	CRIP2
679	PLXNB2
672	LOC524676
639	SUGP1
623	TTC7A
608	LTN1
600	RAD21
594	QSER1
582	SSRP1
569	APOPT1
563	ADAM32
558	ZMIZ2
557	MGC134473
545	RANGAP1
545	HSPB9
507	BRWD1
505	REEP6

RN7SL1:
Full name: signal recognition particle (SRP)
Gene type: ncRNA

CABS1
Full name: calcium-binding protein, spermatid-specific 1
Gene type: protein coding

PRM1:
Full name: protamine 1
Gene type: protein coding

SPEM1:
Full name: spermatid maturation 1
Gene type: protein coding

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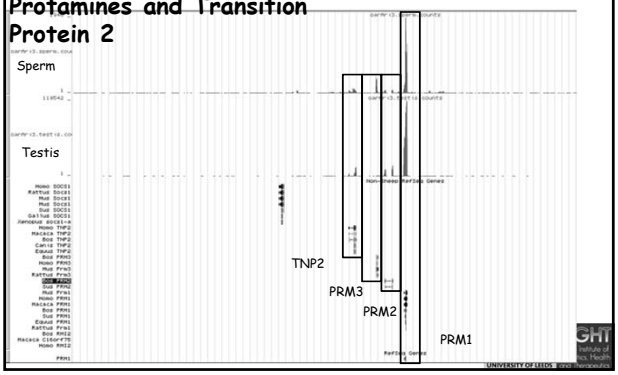
First look: Sheep

Protamines and Transition Protein 2



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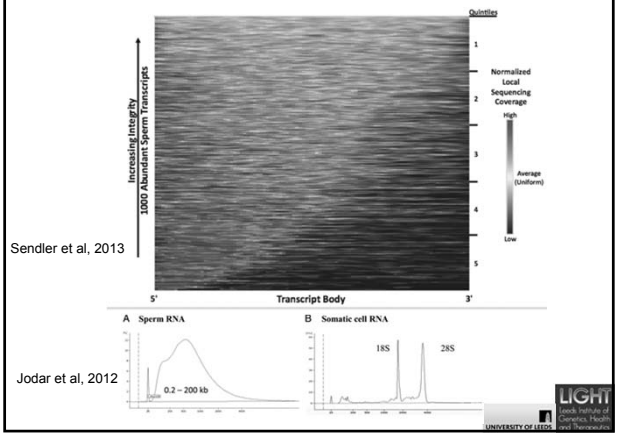
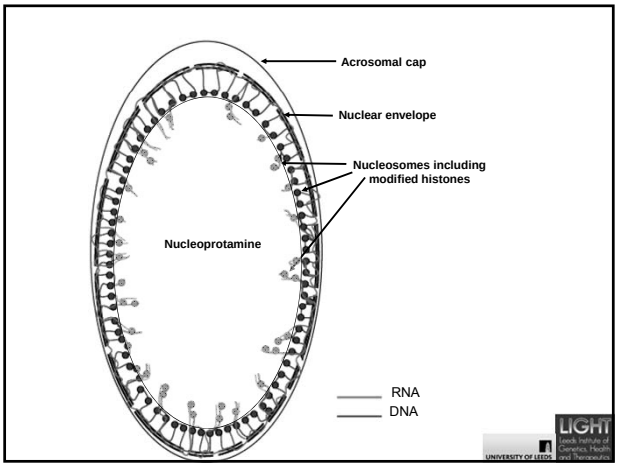
GHT Institute of Genetics, Health and Therapeutics

Assessment of sperm using NGS technology (many RNAs are fragmented)

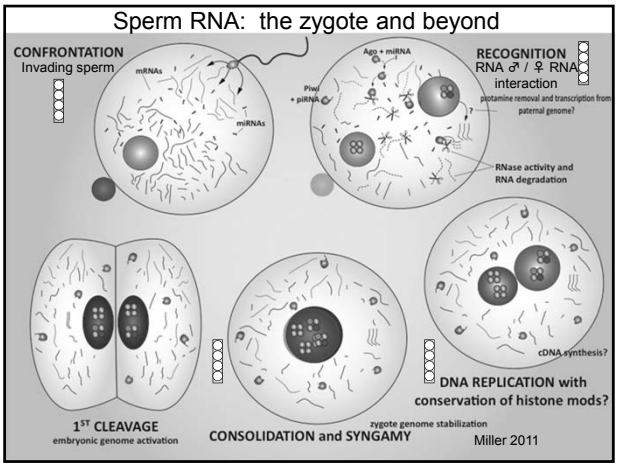


Sendler et al, 2013

Jodar et al, 2012



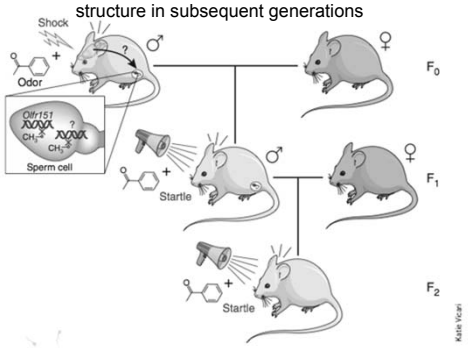
Sperm RNA: the zygote and beyond



Miller 2011

EPIGENETICS

Parental olfactory experience influences behavior and neural structure in subsequent generations



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Dias and Ressler, Nat NeuroSci 2013; commentary by Moshe Szyf

The Case of the Midwife Toad, 1926

If a person acquires a limp during their lifetime, can that limp be passed on to their children? Or if a person acquires a scar, will that scar be hereditary? Modern scientific theory denies this is possible, but a theory called Lamarckianism held that not only was it possible, but it was the means by which evolutionary change occurred.

During the 1920s, Austrian scientist Paul Kammerer designed an experiment involving a species called the Midwife Toad to prove that Lamarckian inheritance was possible.

Links and References

- Koestler, Arthur. (1971). *The Case of the Midwife Toad*. Random House.

Categories: Science, Biology, Scientific Fraud, 1914-1949

was forced to mate in water, it would eventually acquire the same bumps that naturally water-mating toads possessed — and that the toad's offspring would inherit these bumps via Lamarckian inheritance.

Kammerer filled a fishtank full of water, placed some Midwife Toads in it, and then waited as generations of toads were born and died. Finally he announced success. A generation of Midwife Toads had been born with black scaly marks on their hindlimbs. This appeared to prove that Lamarckian inheritance was possible.

Conclusions

- It is >50 years since sperm RNA (transcription) was first reported (Bhargava, 1957)
- The presence of the RNA transcription was considered surprising in view of the dormancy of the sperm nucleus and originally dismissed as an artefact (Markewitz et al, 1967).
- Residual RNA reported in human and rat sperm nuclei (Pessot et al, 1985).
- RNA reported in sperm and pollen of all species studied to date.
- Sperm RNA is complex but mainly comprises degraded rRNAs
- Sperm RNA has excellent diagnostic potential in assessing male fertility but tests should probably target 5' ends of mRNA.
- Sperm RNA has excellent prospects for assessing male fertility more accurately than WHO criteria.
- NGS is poised to transform sperm RNA based diagnostics.
- NGS will help illuminate functional aspects of sperm RNA

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Sendler E, Johnson GD, Mao S, Goodrich RJ, Diamond MP, Hauser R, Krawetz SA: Stability, delivery and functions of human sperm RNAs at fertilization. *Nucleic acids research* 2013, 41(7): 4104-4117.

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With thanks to:-

Martin Brinkworth,
University of Bradford.

David Iles, Stefanie Nadj,
University of Leeds and
the clinical embryologists
at Seacroft Hospital,
Leeds

Stephen Krawetz et al,
Wayne State University
Detroit

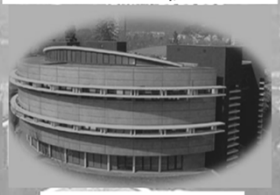
Also with thanks to:- reprotrain MRC Medical Research Council

Molecular messages in the ejaculate remain an underestimated resource for understanding male fertility

①

Sophie ROUSSEAUX, MD, PhD
Research Director at INSERM

Saadi Khochbin's team
INSERM U823
Grenoble University, FRANCE



ESHRE Munich 29th June 2014
Pre-congress course 2

Learning Objectives

③

Molecular messages in the ejaculate remain an underestimated resource for understanding male fertility

Background knowledge
The **epigenome** is a regulated "genome signposting" system
Spermatozoa's mission is to deliver the **male genome/epigenome messages** to the oocyte

The question
Molecular events driving male genome programming and compaction?

The findings
⇒ A genome-wide **histone acetylation wave** occurs in post-meiotic elongating spermatids before the replacement of histones by protamines
⇒ **Brdt** is a master regulator of male genome programming and post-meiotic compaction
⇒ Many, yet unknown, **histone post-translational modifications (PTM)** are also involved
⇒ The **testis-specific histone variant tH2B** has an essential role in destabilizing nucleosomes, which can be compensated by highly specific targeted histone modifications (in the absence of tH2B)
=> First models to understand the molecular mechanisms driving male genome programming

Implications
Abnormal genome programming and male infertility / ICSI failures
Spermatozoa can transmit "non-genetic" messages to the next generations

---

Background knowledge: the epigenome is a regulated "genome signposting" system

④

Epigenetic landscape

Tissue-specific gene expression pattern

Each cell type has a *specific identity*, which underlies its function.

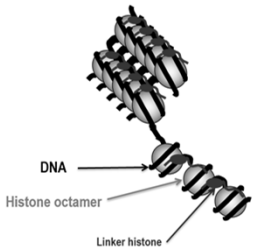
Environment

Growth factors, hormones, diet, metabolites…
Development
Aging, stress…

Cell identity relies on a defined and specific gene expression profile.

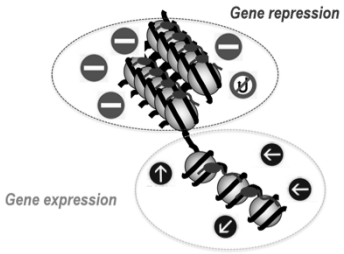
Background knowledge: the epigenome 5

Epigenome = regulated genome signposting system



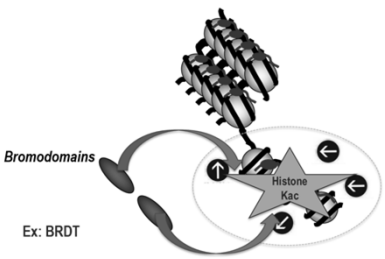
Background knowledge: the epigenome 6

Epigenome = regulated genome signposting system



Background knowledge: the epigenome 7

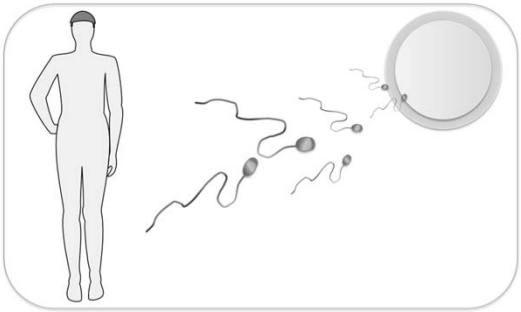
Epigenome = regulated genome signposting system



Sperm cells mission is to deliver a package = the male genome

DNA DELIVERY BOYS

Spermatozoa are the only cells that leave the organism

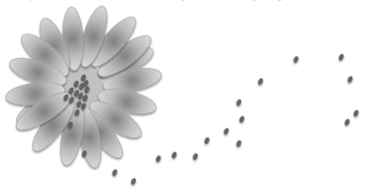


How to pack the genome for a safe trip?

How to pack the genome for a safe trip?

Long journey in a **harsh environment**

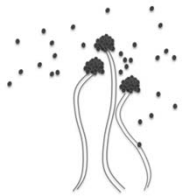
Very conserved phenomenon in the life cycle of many organisms



Ex. **pollen formation** in plants

Facing a harsh environment

Very conserved phenomenon in the life cycle of many organisms

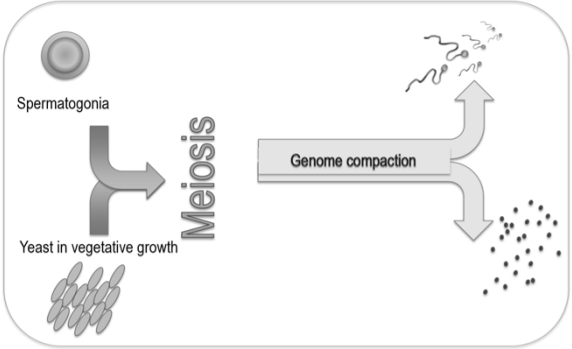


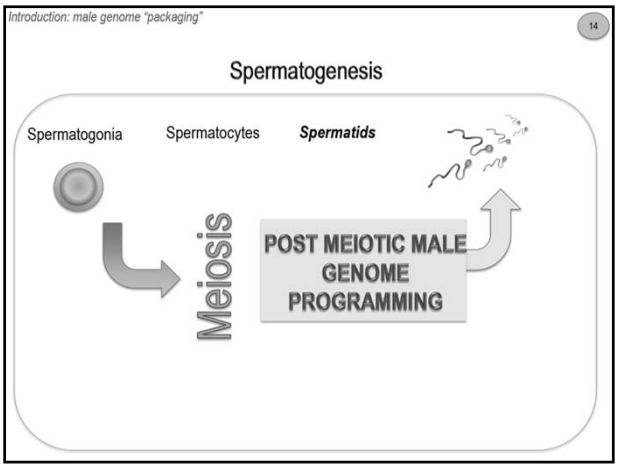
Sporulation

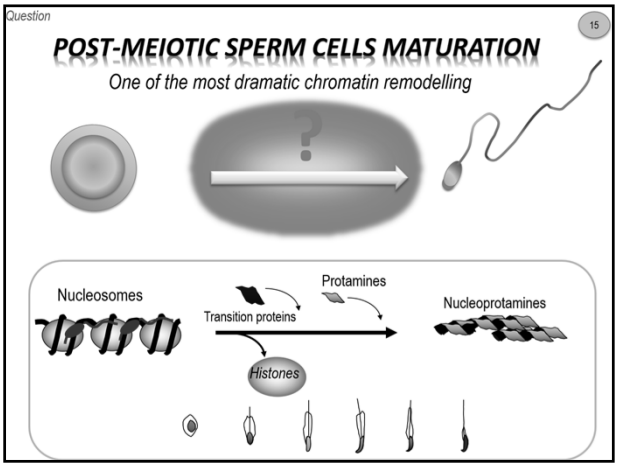
Genome compaction :

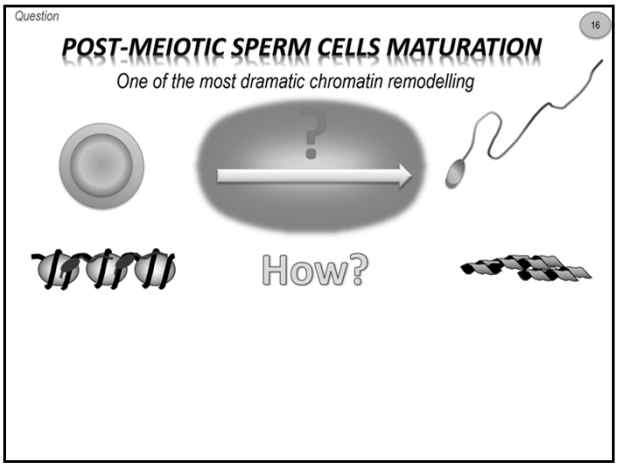
Generation of a tightly packed & almost inert genome

Spermatogenesis is an evolved sporulation




Introduction: male genome "packaging"
14
Spermatogenesis
Spermatogonia
Spermatocytes
Spermatids
Meiosis
POST MEIOTIC MALE GENOME PROGRAMMING


Question
15
POST-MEIOTIC SPERM CELLS MATURATION
One of the most dramatic chromatin remodelling
?
Nucleosomes
Transition proteins
Protamines
Nucleoprotamines
Histones

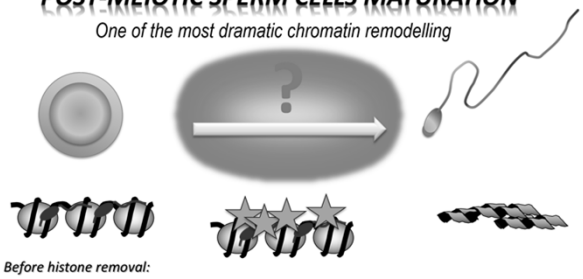

Question
16
POST-MEIOTIC SPERM CELLS MATURATION
One of the most dramatic chromatin remodelling
?
How?

Question

17

POST-MEIOTIC SPERM CELLS MATURATION

One of the most dramatic chromatin remodelling



Before histone removal:

Histone hyperacetylation wave

Histone variants

Question

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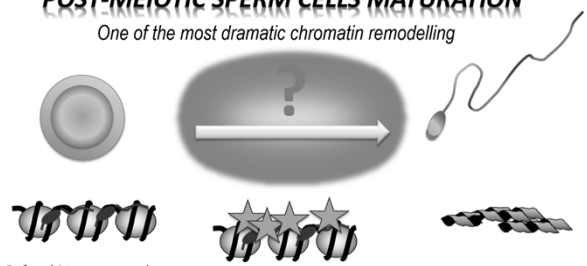
POST-MEIOTIC SPERM CELLS MATURATION

One of the most dramatic chromatin remodelling



Before histone removal:

Histone hyperacetylation wave

Histone variants

Histone hyperacetylation wave

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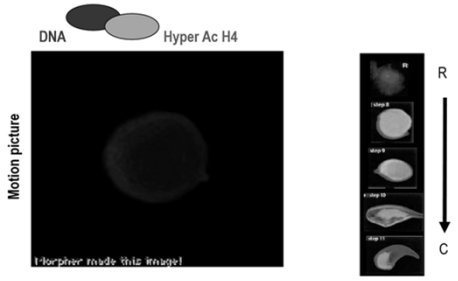
Histone replacement is preceded by a genome-wide histone hyperacetylation wave

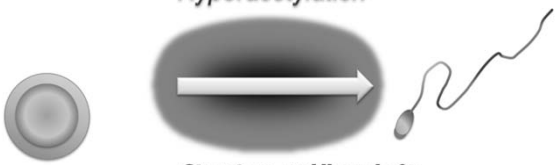


Hyperacetylation wave = signal for histone eviction?

Histone hyperacetylation wave

20

Hyperacetylation



Structure and ligand of a histone acetyltransferase bromodomain

Christophe Dhalluin*, Justin E. Carlson*, Lei Zeng, Cheng He, Aneel K. Aggarwal & Ming-Ming Zhou

NATURE | VOL 399 | 3 JUNE 1999 |

Bromodomain
Testis

Brdt
BET member

First molecular characterizations

Pivot-Pajot et al. , Mol. Cell. Biol. 2003

Brdt

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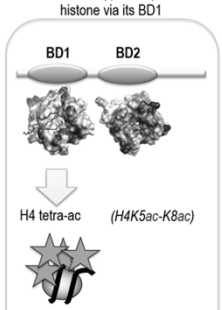
Structural and functional investigation of Brdt's bromodomains

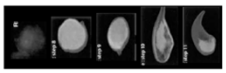
Brdt binds hyperacetylated histone via its BD1



Morinière et al. Nature 2009

Collaboration : Christoph Muller and Carlo Petosa (EMBL)

Hyperacetylation wave in elongating spermatids



=> Hypothesis: during spermatogenesis Brdt should act in elongating spermatids (hyperacetytion wave)

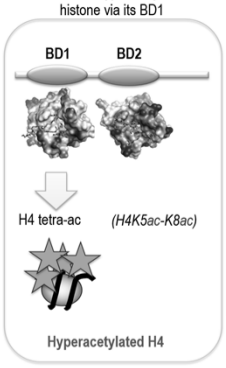
Jonathan Gaucher

Brdt

22

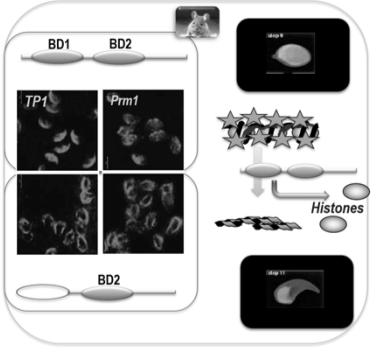
Brdt directly links chromatin hyperacetylation to histone removal

Brdt binds hyperacetylated histone via its BD1



Morinière et al. Nature 2009

In germ cells from Brdt BD1 del mice histones are not removed



Gaucher et al. EMBO J 2012

Brdt

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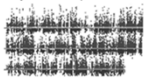
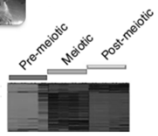
Brdt's functions in male germ cells

Mapping genome wide acetylation-dependent Brdt binding



Transcriptomic analysis of Brdt regulated genes
- *Brdt KO*
- *Brdt Δ BD1*



Brdt

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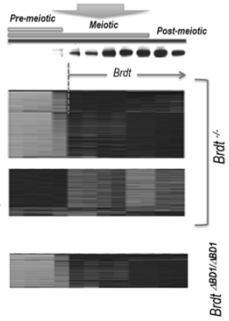
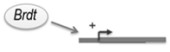
Brdt is a specific transcriptional regulator

Transcriptome : KO – ΔBD1 / WT

Repressed *Activated*



Brdt activation operates a gene expression shift



Brdt activates meiotic & post-meiotic genes
Brdt primes post-meiotic genes

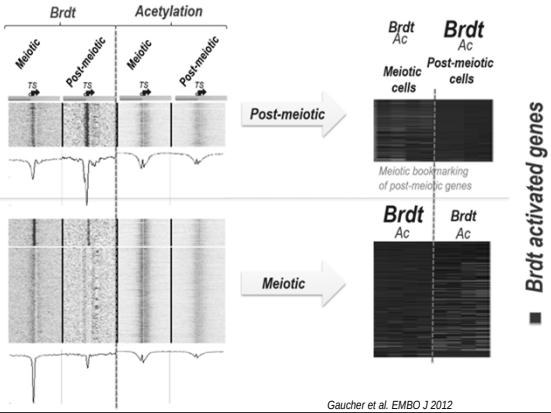
Brdt-represses pre-meiotic genes

Brdt 1st bromodomain is an important player

Brdt

26

ChIP-seq data => Brdt binds on acetylated TSS



Gaucher et al. EMBO J 2012

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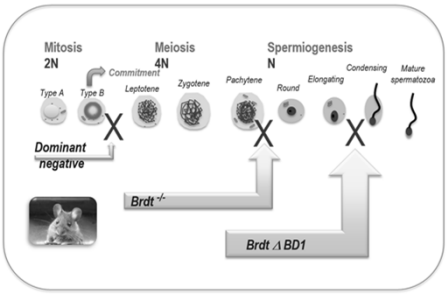
Brdt : a “swissKnife” in male genome programming



Gaucher et al. EMBO J 2012

29

Histone K acetylation – guided action : Brdt

*Epigenetic programming of the post-meiotic genome by **histone PTM…***

30

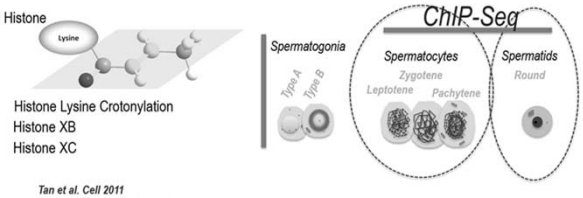
Cell

Resource

Identification of 67 Histone Marks and Histone Lysine Crotonylation as a New Type of Histone Modification

Collab.
Yingming Zhao
University of Chicago

Functional studies of new histone PTMs



Tan et al. Cell 2011
Dai et al. in press and work in progress

Histone PTM

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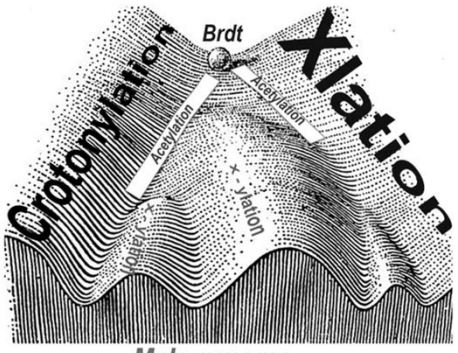
Epigenetic guidance by histone PTMs



Question

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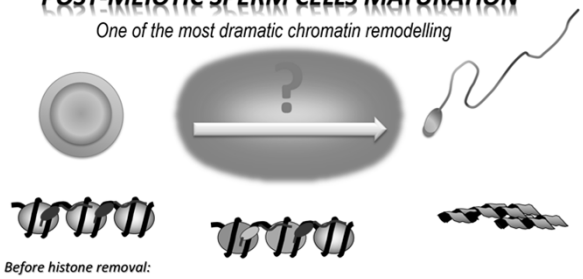
POST-MEIOTIC SPERM CELLS MATURATION

One of the most dramatic chromatin remodelling



Before histone removal:

Histone hyperacetylation wave

Histone variants

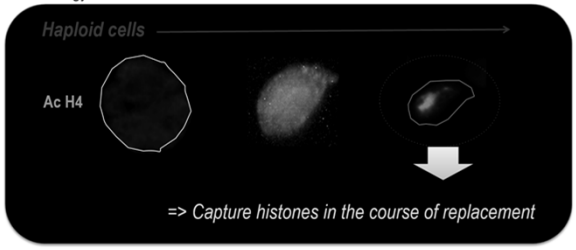
Histone variants

37

The role of histones in nucleosome disassembly

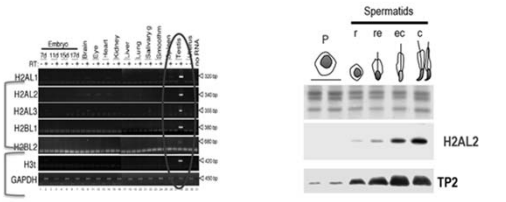
Do specific histones become part of the dissociating nucleosomes?

Strategy :



39

Discovery of new testis histones variants

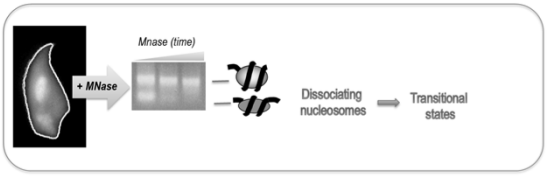


H2AL2= late expressing H2A variant

Govin et al. J. Cell Biol. 2007

40 41

***H2AL2* is enriched « sub-nucleosomal » particles, which are highly sensitive to MNAse**



***TH2B* is present in nucleosomal and « sub-nucleosomal » particles**

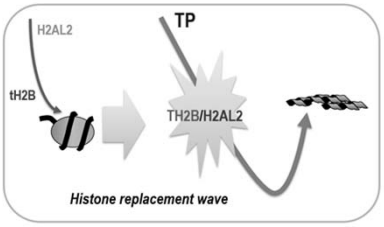
Working model:

Govin et al. J. Cell Biol. 2007

42

Hypothesis

***TH2B* could favour the assembly of variant nucleosomes to induce instability and facilitate histone removal**



Histone variants

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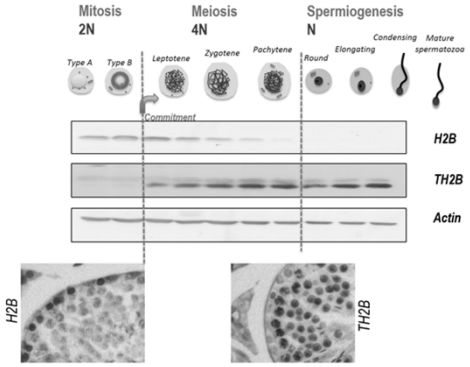
TH2B is expressed in meiotic and post-meiotic cells



Histone variants

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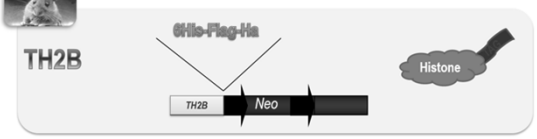
TH2B knock-in mouse models



- TH2B-less mouse model *(+ NEO)*
- TH2B^{tag} mouse model *(Δ NEO)*

Emilie Montellier

Histone variants

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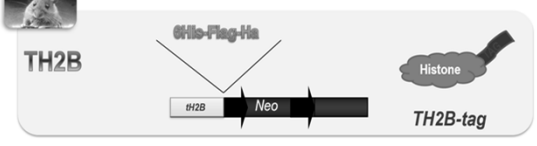
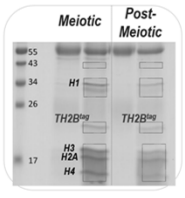
TH2B knock-in mouse models



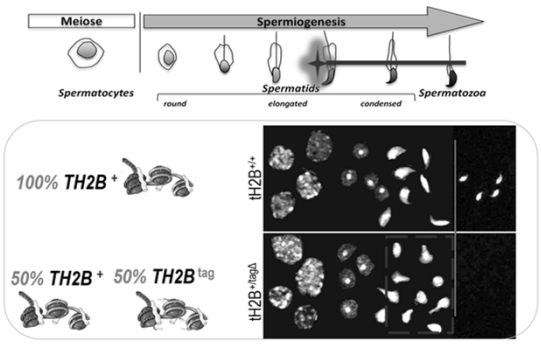
TH2B-tag mice
=> normal nucleosome assembly



Histone variants

46

TH2B-tag mice: defects only in elongating spermatids



Montellier et al. Genes & Dev 2013

Histone variants

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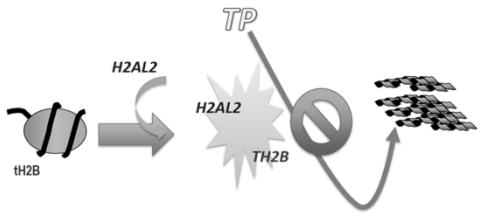
TH2B-tag mice: defective histone replacement



=> *TH2B-tag* has a "dominant negative" effect: prevents the dissociation of the subnucleosomal variants and the incorporation of TPs

Histone variants

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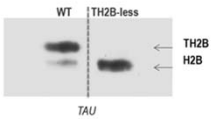
TH2B-less mouse model

Rescue

Spermatogenesis : normal



Compensation by H2B

Histone variants

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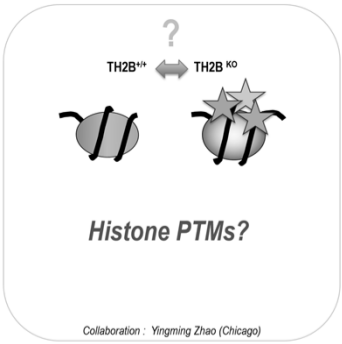
Lack of TH2B => compensation



Histone variants

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Lack of TH2B: a highly specific compensation mechanism is activated in the absence of TH2B

TH2B = H2B + histone PTMs

TH2B

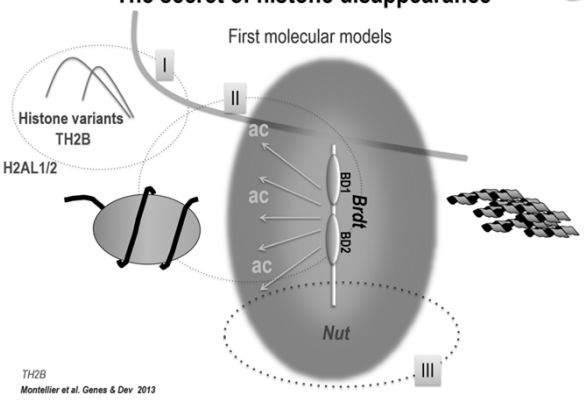
Histone modifying machineries

A major role of TH2B would be to set appropriate nucleosome stability parameters required for histone replacement

Histone variants

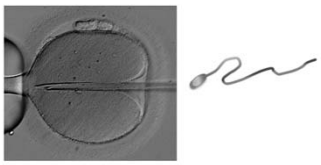
52

The secret of histone disappearance



Implications for male fertility

53



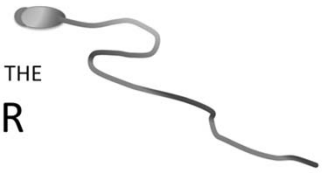
Infertile mouse models ***with abnormal male genome compaction***
⇒ *Brdt and Nut mutants*
⇒ *TH2B and H2AL2 mutants…*

Sperm epigenome defects ***are associated with male infertility***
⇒ *New causes of infertility? (ex. BRDT K62Q)*
⇒ *Risks and/or low success of ICSI ?*
⇒ *Develop tests for sperm epigenome assessment*

Implications for epigenetic inheritance

54

THE SINS OF THE
FATHER



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Dias, B. G. & Ressler, K. J. Nature Neurosci. 17, 89–96 (2014).
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Steroidogenesis in the fetal testis and its susceptibility to disruption The latest advances

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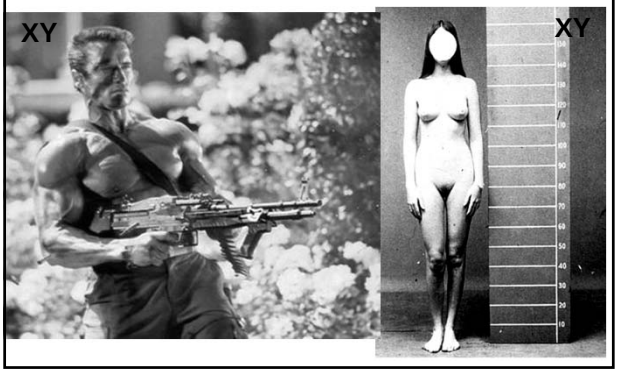
MRC Centre for Reproductive Health

Why should we be interested in fetal testis steroidogenesis in humans?

1. Because it determines if you become a phenotypic male

2. Because growing evidence indicates that subtle deficiency in early gestation androgen exposure may underlie most (common) male reproductive disorders

Fetal masculinisation
Its all down to androgens, not the Y chromosome

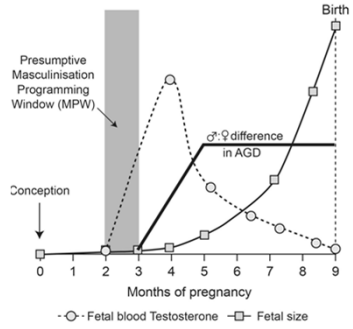


Prevalence data for reproductive disorders in newborn or young adult males

Parameter	Prevalence	Evidence
Cryptorchidism	6-9%	Prospective EU studies
Hypospadias	0.4-0.9%	Prospective EU studies
Low sperm counts	16-20%	Prospective EU studies
Testis germ cell cancer	0.45%	Registry data (reliable)
Low adult Testosterone (Compensated Leydig cell failure)	>10%	Cross-sectional studies

Environmental/lifestyle factors are clearly implicated in the high/increasing prevalence of these disorders. How this occurs is unknown

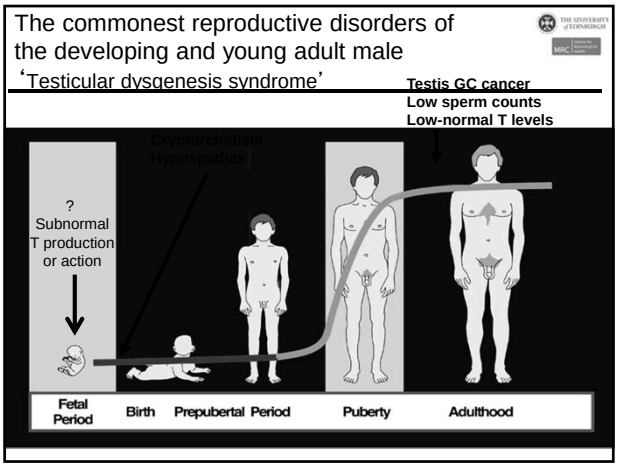
The masculinization programming window (MPW) in humans



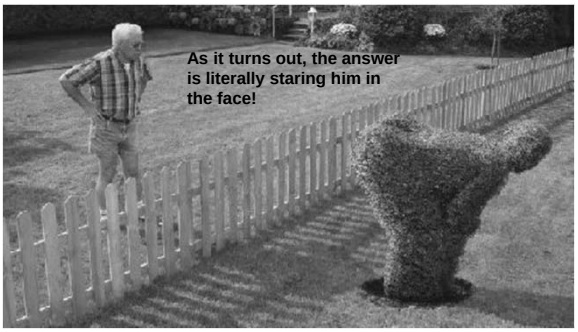
From Dean & Sharpe (2013) J Clin Endocrinol Metab 98: 2230-2238

The commonest reproductive disorders of the developing and young adult male

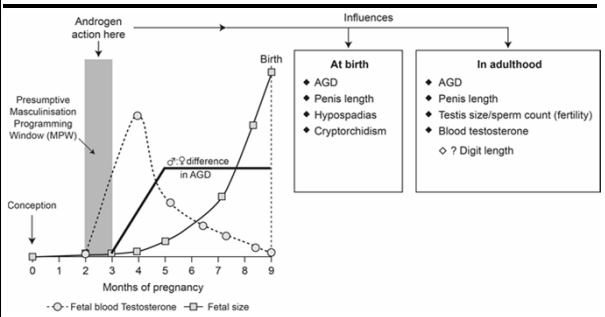
'Testicular dysgenesis syndrome'



Sometimes inspiration comes from unexpected sources



The masculinization programming window (MPW) in humans: reproductive influences



From Dean & Sharpe (2013) J Clin Endocrinol Metab 98: 2230-2238

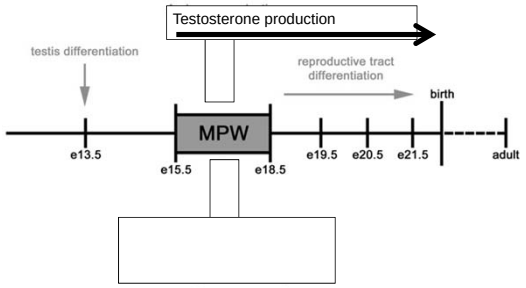
An animal model for human TDS?
Effects of fetal DBP exposure in the rat

- Gestational exposure (E13-E21) of the rat to high doses of certain phthalate esters (eg **dibutyl phthalate (DBP)**) results in:

Dose-dependent induction in male offspring of:

- Cryptorchidism
- Hypospadias
- Low testis weight/sperm production/subfertility
- Compensated adult Leydig cell failure (High LH/T)

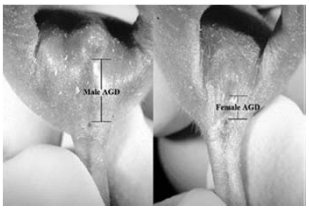
The 'masculinisation programming window' (MPW)

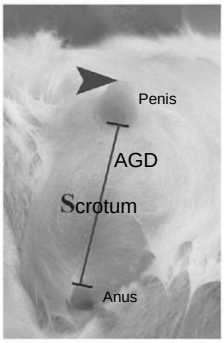


Adapted from M Welsh et al (2008) J Clin Invest 118: 1479-1490

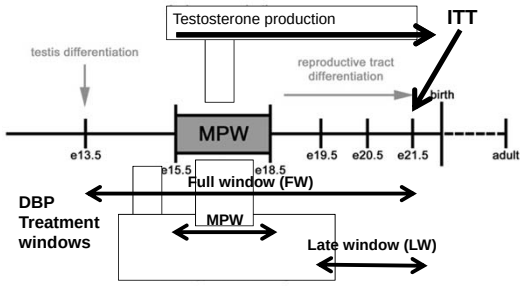
Anogenital distance (AGD)

~Twice as long in males as in females (rats and humans)



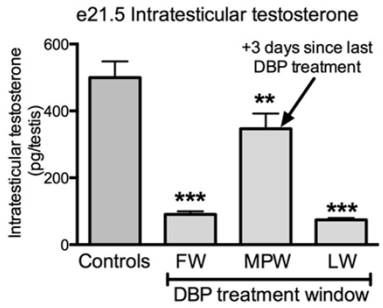


The 'masculinisation programming window' (MPW)



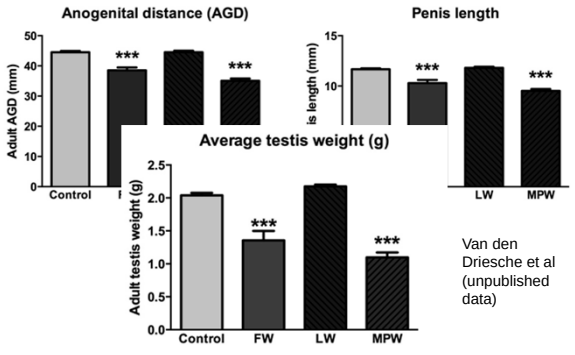
Adapted from M Welsh et al (2008) J Clin Invest 118: 1479-1490

Effect of different DBP treatment windows on intratesticular testosterone (ITT) at e21.5



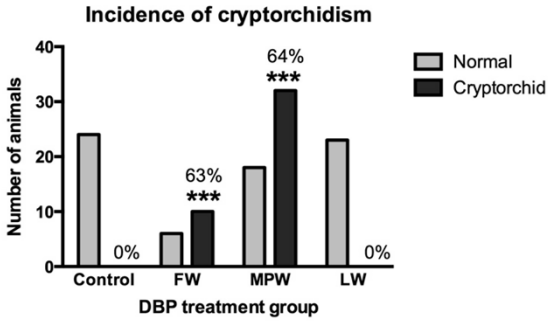
Adapted from Van den Driesche et al. (2012) PLoS One e30111

Effect of DBP-induced reduction in fetal testis testosterone in different time windows: Adult phenotype



Van den Driesche et al (unpublished data)

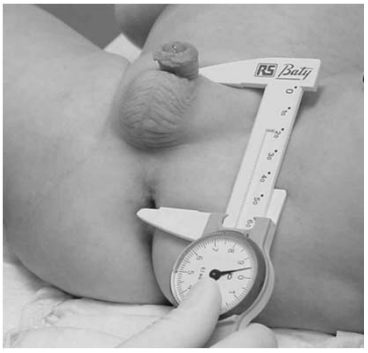
Effect of fetal DBP exposure window on phenotype of male rats in adulthood



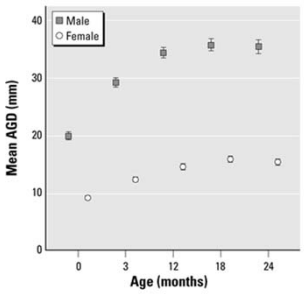
Van den Driesche et al (unpublished data)

Measuring anogenital distance in boys

A read-out of fetal androgen exposure?



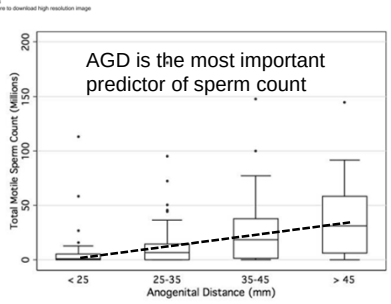
Male-female difference in AGD in human in the first two years from birth



Means ± 95% CI
N=137-285 per age

From: Thankamony et al 2009 Environ Health Perspect 117:1786-1790

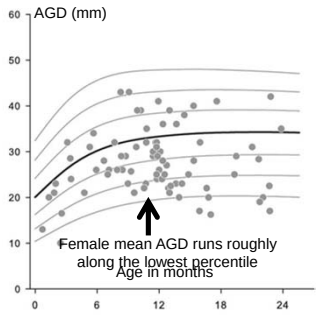
AGD is positively related to sperm count in adult men



AGD is the most important predictor of sperm count

From: Eisenberg et al (2011) PlosOne e18973

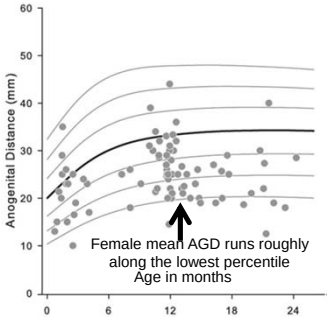
AGD in normal boys and boys with cryptorchidism at <2 years of age



P<0.0001 for mean AGD SDS in boys with cryptorchidism compared with normal population

From: Thankamony et al (2014) Environ Health Perspect - online

AGD in normal boys and in boys with hypospadias at age <2years

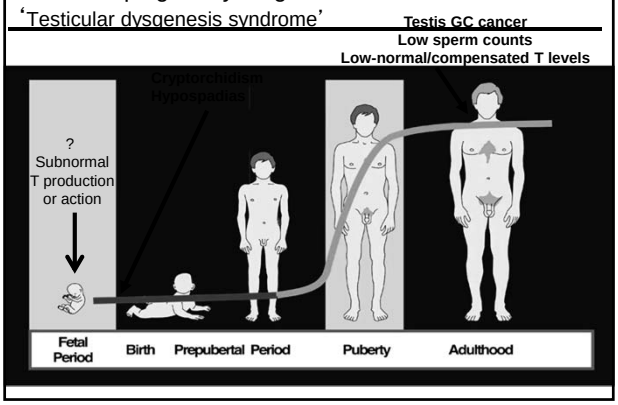


P=0.005 for mean AGD SDS in boys with hypospadias compared with normal population

From: Thankamony et al (2014) Environ Health Perspect - online

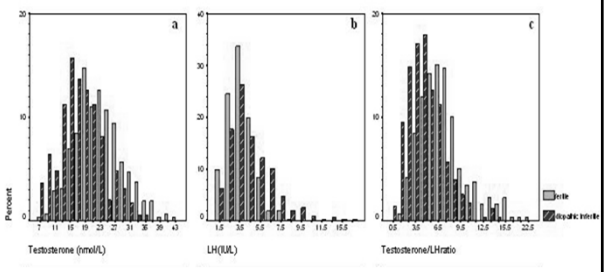
The commonest reproductive disorders of the developing and young adult male

'Testicular dysgenesis syndrome'



Altered testosterone/LH* ratios in adult men with infertility/low sperm counts

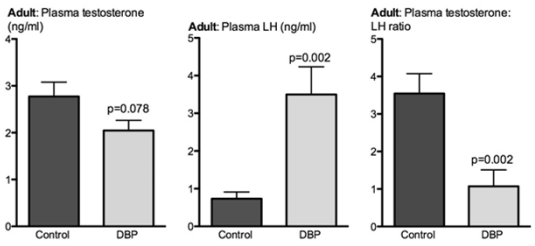
*lower testosterone/LH ratio = 'compensated Leydig cell failure'



Red columns = idiopathic infertility
Green columns = fertile men

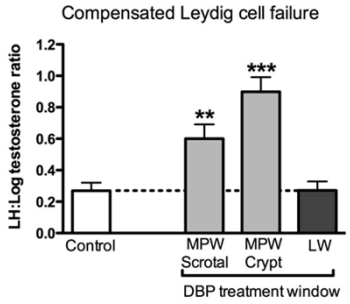
From: Andersson et al (2004) *JCEM*

Effect of in utero exposure of rats to DBP Induction of compensated adult Leydig cell failure



From K Kilcoyne et al (2014) Proc Natl Acad Sci USA

Compensated adult Leydig cell failure in rats originates in the masculinization programming window



How can adult Leydig cell function be altered by fetal events when adult Leydig cells do not appear until puberty?

van den Driesche et al – unpublished data

Birth weight is positively associated with adult testosterone levels (independent of adult bodyweight)

Birth Weight in Relation to Sex Steroid Status and Body Composition in Young Healthy Male Siblings

Griet Vanbillemont, Bruno Lapauw, Veerle Bogaert, Hélène De Naeyer, Dirk De Bacquer, Johannes Ruige, Jean-Marc Kaufman, and Youri E. C. Taes

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Context: Sex steroid concentrations have a strong genetic determination, but environmentall factors and body composition play an important role. From studies in children with intrauterine growth restriction, low birth weight has been associated with altered gonadotropin concentrations.

Objective: We aim to investigate sex steroid concentrations in healthy young brothers in relation to birth weight (normal gestational age), body composition, and parental steroid concentrations.

Design and Setting: We conducted a cross-sectional, population-based sibling pair study with inclusion of parental data.

Participants: A total of 677 men (25–45 yr old) were included in this study, with 296 independent pairs of brothers and 122 fathers.

Main Outcomes: We measured testosterone, estradiol, leptin, adiponectin, IGF-I (immunoassays), and free steroid hormones (calculated) in relation to birth weight and changes in body composition (dual-energy x-ray absorptiometry).

Results: Birth weight was associated with serum testosterone ($P = 0.0004$) and SHBG ($P = 0.0001$), independent from weight, age, or fat mass, whereas no association with (free) estradiol, LH, or FSH was found. Paternal testosterone ($P = 0.002$, estradiol $P = 0.04$), and indeed $P = 0.0004$) were associated with the respective sex steroid concentrations in the brothers. Weight increase (population rank) during life, was associated with lower testosterone (−15%; $P < 0.001$), independent from current weight and with higher free estradiol concentrations (+8%; $P = 0.002$), whereas weight decrease was associated with higher testosterone (+13%; $P < 0.001$).

Conclusion: Birth weight and paternal steroid concentrations are associated with testosterone concentrations, independent from adult weight. These findings support the concept of in utero programming across the range of birth weight. (*J Clin Endocrinol Metab* 95: 1587–1594, 2010)

PNAS

Fetal programming of adult Leydig cell function by androgenic effects on stem/progenitor cells

Karen R. Kilcoyne[a], Lee B. Smith[a], Nina Atanassova[b], Sheila Macpherson[a], Chris McKinnell[a], Sander van den Driesche[a], Matthew S. Jobling[a], Thomas J. G. Chambers[a], Karel De Gendt[c], Guido Verhoeven[c], Laura O'Hara[a], Sophie Platts[a], Luiz Renato de Franca[d], Nathália L. M. Lara[d], Richard A. Anderson[a], and Richard M. Sharpe[a,1]

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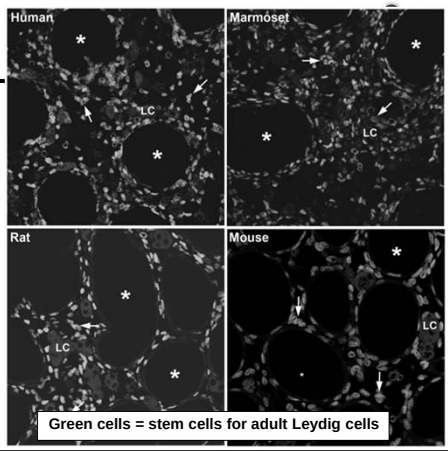
Edited by John J. Eppig, The Jackson Laboratory, Bar Harbor, ME, and approved March 25, 2014 (received for review November 7, 2013)

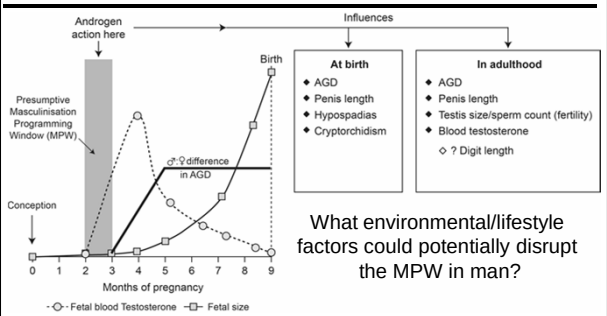
Red = fetal LC
Green = COUP-TFII
Blue = SMA
* = seminiferous cords



From K Kilcoyne et al (2014) Proc Natl Acad Sci USA

Green cells = stem cells for adult Leydig cells

The masculinization programming window (MPW) in humans



What environmental/lifestyle factors could potentially disrupt the MPW in man?

From Dean & Sharpe (2013) J Clin Endocrinol Metab 98: 2230-2238

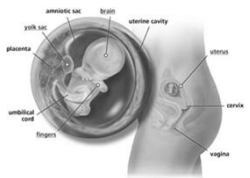
The three test 'endocrine disruptors'

- Dibutyl phthalate (500mg/kg/day)
- Diethylstilboestrol (potent oestrogen)
- Paracetamol (Acetaminophen)

In rat studies all of the above have been shown to reduce fetal intratesticular testosterone levels in vivo: DES by >90%, DBP by 50-80%, Paracetamol by 10-20%

Rodent-human differences in regulation of fetal testis steroidogenesis

Rats & Mice



8-12 weeks' gestation

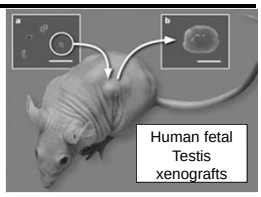
Fetal testis steroidogenesis is LHR-independent

LHR = LH Receptor

Fetal testis steroidogenesis Is CG(LHR)-dependent

Rodent-human differences in regulation of fetal testis steroidogenesis

Rats & Mice

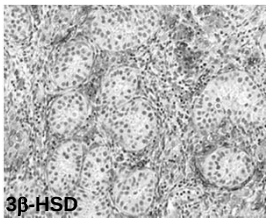


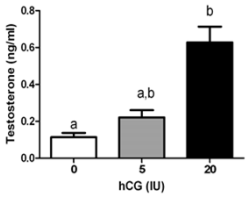
Fetal testis steroidogenesis is LHR-independent

LHR = LH Receptor

Fetal testis steroidogenesis Is CG(LHR)-dependent

Human fetal testis xenografts
Testosterone production is hCG-dependent

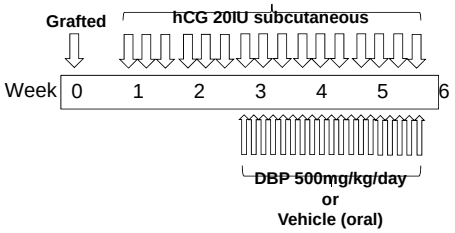




Host mice are injected every 3 days with hCG

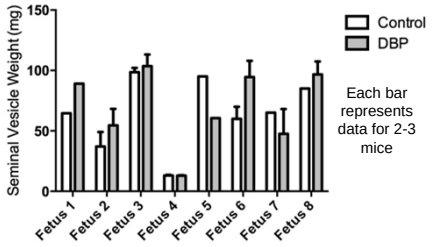
From: Mitchell et al. Human Reproduction. 2010

Human fetal testis xenografts
Exposure protocol – DBP (+hCG)



Exposure of human fetal testis xenografts to 500mg/kg/day DBP has no steroidogenic effects

Xenografts recovered + 6 weeks; hCG treatment from 1-6 weeks



Data show Means ± SEM for N=8 fetuses (14-20 weeks' gestation)
Statistical analysis was by 2-factor ANOVA

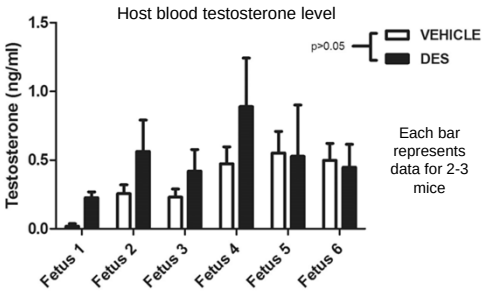
Adapted from: Mitchell et al 2012 J Clin Endocrinol Metab 97: E341-E348

The three test 'endocrine disruptors'

- Dibutyl phthalate (500mg/kg/day)
- Diethylstilboestrol (potent oestrogen)
- Paracetamol (Acetaminophen)

In rat studies all of the above have been shown to reduce fetal intratesticular testosterone levels in vivo: DES by >90%, DBP by 50-80%, Paracetamol by 10-20%

Lack of effect of DES on fetal human testis T production after xenografting into castrate nude mice



From Mitchell et al (2013) PLoS One 8: e61726

The three test 'endocrine disruptors'

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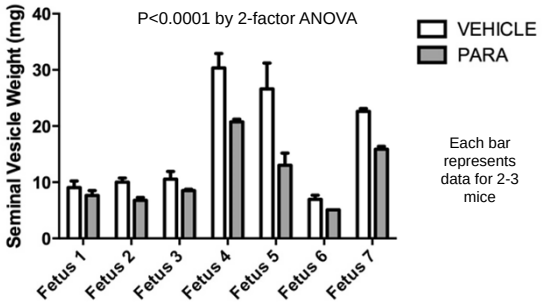
Effect of xenograft (host) exposure to paracetamol on fetal human testis T production

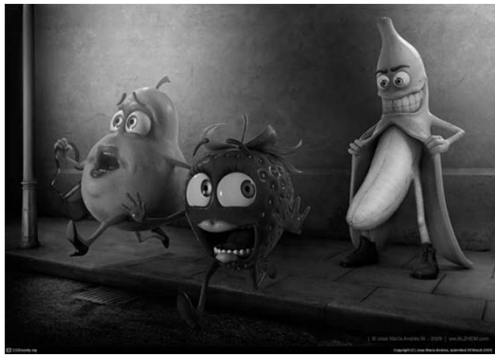


Rod Mitchell – unpublished data

Conclusions

- Testosterone (T) production by the fetal testis during the MPW* is critical for normal development and later function of the testis
- Deficiencies in (T) production during the MPW can alter adult T levels (which may have wider health implications)
- The rodent and human fetal testis are different in their regulation, and in their response to some, but not all, 'endocrine disruptors'

Thank you for your attention



Antiestrogens for treatment of male infertility or hypogonadism

Prof. Dr. Michael Zitzmann
Andrologist, Endokrinologist, Diabetologist
Sexual Medicine (FECSM)

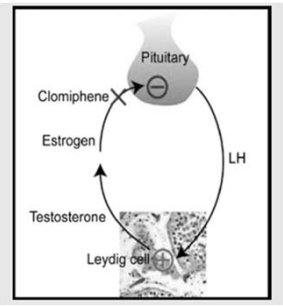
Clinical Andrology /
Centre for Reproductive Medicine and Andrology,
University Clinics Muenster
Germany

WHO Collaborating Centre for Research in Human Reproduction
Training Centre of the European Academy of Andrology

Disclosures

I have nothing to disclose in the context of this lecture

Treatment of hypogonadism and/or infertility with clomiphen citrate or tamoxifen



Kim et al, Fertil Steril 2013 epub

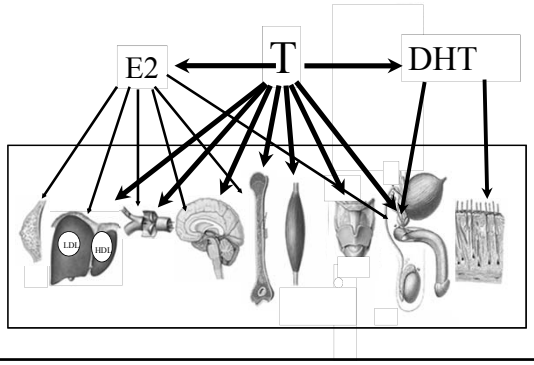
οἶστρος

oístrŏs / *oestrus*

Thorn

Passion

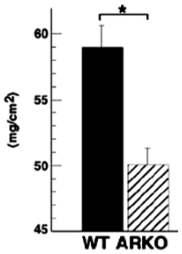
Target organs of testosterone and its metabolites



Bones

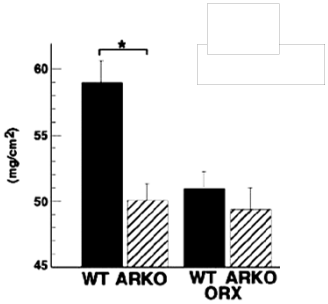
Bone density of WT and ARKO mice

Kawano et al. 2003 PNAS 100:9416



Bone density of WT and ARKO mice

Kawano et al. 2003 PNAS 100:9416



Bone density of WT and ARKO mice

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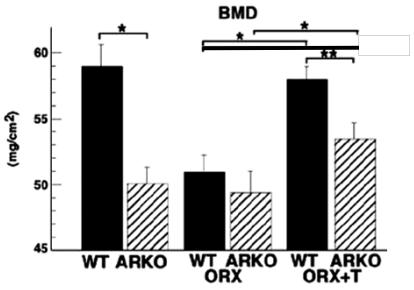

Bone density of WT and ARKO mice

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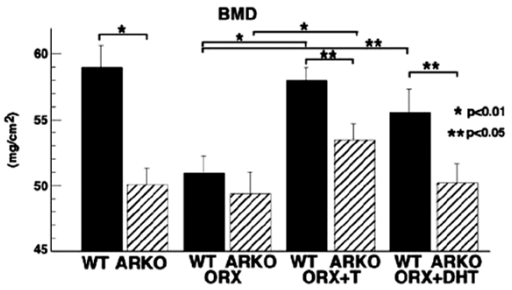
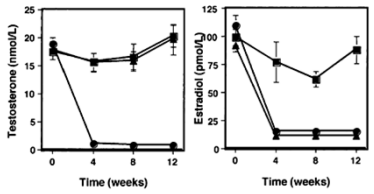


Bone metabolism – studies in humans

Leder et al. 2003 J Clin Endocrinol Metab 88:204-210



Group 1 vs 2: P < 0.001
Group 1 vs 3: P < 0.001
Group 2 vs 3: P = 0.721

Group 1 vs 2: P < 0.001
Group 1 vs 3: P = 0.649
Group 2 vs 3: P < 0.001

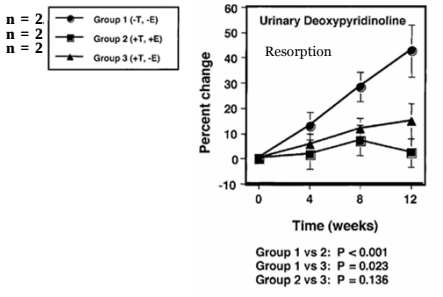
No further treatment, n = 25
Non-scrotal patch 5mg / d n = 22
Patch 5mg /d + aromatase n = 23 inhibitor

Group 1 (-T,-E)
Group 2 (+T, +E)
Group 3 (+T, -E)

All with GnRH agonist

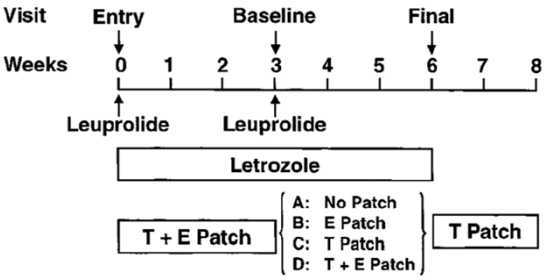
Bone metabolism – studies in humans

Leder et al. 2003 J Clin Endocrinol Metab 88:204-210



Bone metabolism – studies in humans

Falahati-Nini et al. 2000 J Clin Invest 106:1553–1560



Bone metabolism – studies in humans

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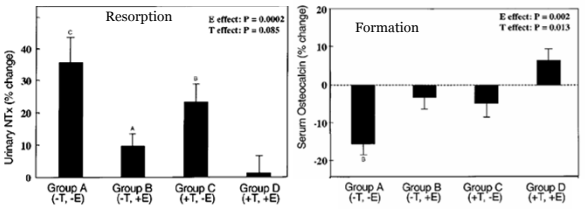
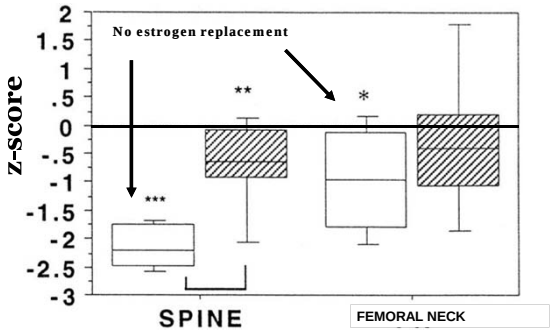


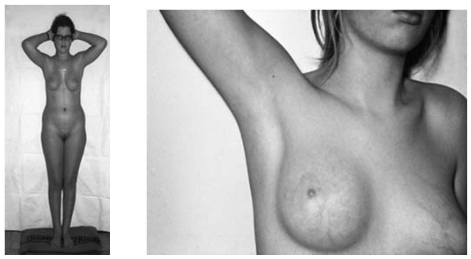
Bone density in 18 women 46,XY with CAIS after gonadectomy

Marcus et al. 2000 J Clin Endocrinol Metab. 85:1032-7



Androgen-Receptor-Mutations

CAIS



Fracture Risk / Femur
Men > 70 years

Amin et al. Am J Med 2006 119:426

Table 3 Incidence of Hip Fracture in Framingham Men by Sex Hormone Status

Sex Hormone Groups*	Number of Men With Hip Fracture/ Total Men Per Group†	Incidence Rate (per 1000 person-years)	Unadjusted Hazard Ratio (95% CI)	Adjusted Hazard Ratio‡ (95% CI)
Estradiol groups				
Low (2.0–18.1 pg/mL)	13/120	11.0	2.8 (1.3, 6.1)	3.1 (1.4, 6.9)
Middle (18.2–34.2 pg/mL)	13/358	3.4	0.9 (0.4, 1.9)	0.9 (0.4, 2.0)
High (≥34.3 pg/mL)	13/315	3.9	(referent)	(referent)
Testosterone groups				
Low (<3.85 ng/mL)	13/173	8.0	2.2 (1.0, 4.7)	1.8 (0.8, 3.8)
Middle (3.85–5.29 ng/mL)	12/281	4.1	1.1 (0.5, 2.4)	0.8 (0.4, 1.8)
High (≥5.30 ng/mL)	14/338	3.7	(referent)	(referent)

*Multiply by ~3.7 for conversion of estradiol to pmol/L and by ~3.5 for conversion of testosterone to nmol/L.
†Total number of men for estradiol groups is 793; total number of men for testosterone groups is 792.
‡Adjusted for age, body mass index, height and smoking status.

AR CAG repeats, estrogens and bone density

Zitzmann et al. 2001 Clin Endodrinol 55:649-657

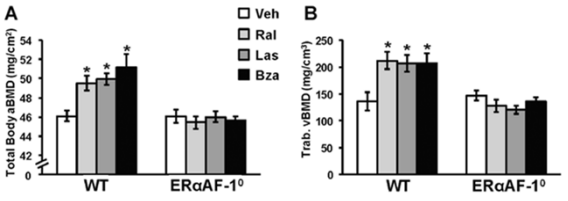
Predictor	Stand. Coeff. ß	t	Sign.	overall R^2 (%)
CAG repeats	-0.299	-3.29	p = 0.001	21.4 %
Estradiol	0.271	2.96	p = 0.004	p < 0.001
log age	-0.212	-2.31	p = 0.004	

n = 110, age 20 – 50 years

Orchiectomized male mice, with or without ER-alpha AF-1 dysfunction

Treatment with various SERMS

Raloxifene (Ral), Lasofoxifene (Las), Bazedoxifene (Bza) or vehicle



Börjesson et al, J Bone Min Res 2012 epub

Treatment of gynecomastia induced by GnRH-antagonist therapy for PCa: Tamoxifen

Viani et al Int J of Radiation 2012

Table 2 Incidence of gynecomastia, breast pain, and complications comparing radiotherapy (RT) and tamoxifen (TMX)

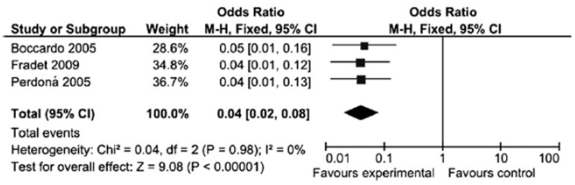
Comparation	Prophylatic	Observation	EER (%)	CER (%)	ARR (%)	NNT
Incidence of gynecomastia						
RT vs. observation	46/140	99/159	32.8	62.2	29.4	3.4
TMX vs. observation	13/121	113/151	10.7	74.8	64.1	1.56
Incidence of all degrees of breast pain						
RT vs. observation	69/140	110/159	49.2	69.1	19.9	5
TMX vs. observation	9/121	83/151	7.4	55	47.6	2.1
Comparation	Prophylatic	Observation	EER (%)	CER (%)	ARI (%)	NNH
Incidence of complications*						
RT vs. observation	45/155	48/175	29	27.4	1.6	62.5
TMX vs. observation	47/121	44/151	38.8	29	9.8	10

Abbreviations: ARR = absolute risk reduction; ARI = absolute risk increase; CER = control event rate; EER = experimental event rate; NNH = number need to harm; NNT = number needed to treat.

* RT complications include skin reaction, erythema, pruritus, and hyperpigmentation. TMX complications include hot flushes, dizziness, constipation, asthenia, and cardiologic or neurologic effects.

Treatment of gynecomastia induced by GnRH-antagonist therapy for PCa

Viani et al Int J of Radiation 2012



Effect on gynecomastia by TMX

Clomiphene citrate for treatment of hypogonadism

	Baseline, mean (SD)	After treatment, mean (SD)	P
Total testosterone, ng/dL	192 (87)	485 (165)	<0.01
Free testosterone, pg/mL	22 (16)	95 (35)	<0.01
SHBG, nM/L	30 (12)	32 (15)	0.72
Oestradiol, pg/mL	26 (22)	39 (18)	<0.05
LH, IU/mL	2.6 (2.2)	6.8 (2.8)	<0.01
FSH, IU/mL	1.9 (1.7)	7.6 (1.9)	<0.01

TABLE 1
Effects of CC on serum hormone profiles

P < 0.05 was considered to indicate statistical significance.

N=86

Katz et al. BJU 2011

Clomiphene citrate for treatment of hypogonadism

	Baseline, %	After treatment, %	P
Decreased libido	72	32	<0.01
Lack of energy	65	40	<0.01
Decreased strength/endurance	28	21	0.18
Lost height	4	5	0.45
Decreased life enjoyment	85	40	<0.001
Sad/grumpy	60	30	<0.01
Erections weaker	12	8	0.29
Decreased sports performance	55	25	<0.001
Sleep after dinner	34	28	0.17
Decreased work performance	45	38	0.28

TABLE 2 Alterations in individual symptoms with CC treatment based on the ADAM questionnaire

P < 0.05 was considered to indicate statistical significance.

Katz et al. BJU 2011

Clomiphene citrate for treatment of hypogonadism

TABLE 3 Symptom improvement based on the ADAM questionnaire

Improvement in at least:	%
One symptom	90
Two symptoms	75
Three symptoms	60
Four symptoms	30
Five symptoms	10

Katz et al. BJU 2011

Clomiphene citrate for treatment of hypogonadism

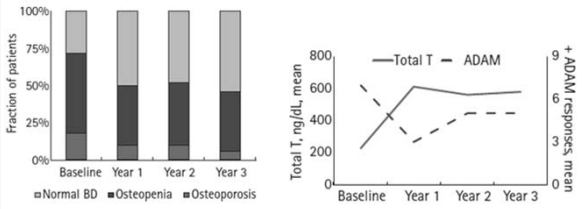
TABLE 1 Baseline and follow-up hormone, symptom and BMI data for patients (data are means ± SD)

	Baseline	Year 1	Year 2	Year 3	*P* value
Total T, ng/dL	228 ± 48	612 ± 212	562 ± 201	582 ± 227	<0.001
LH, IU/mL	2.0 ± 1.6	8.6 ± 3.2	7.2 ± 4.0	8.2 ± 1.9	<0.001
Oestradiol, pg/mL	37 ± 16	48 + 22	42 ± 13	50 ± 30	0.02
ADAM (+ responses)	7 ± 2	3 ± 2	5 ± 2.5	5 ± 3	0.01
Mean BMI, kg/m^2	32 ± 8	31 ± 9	29 ± 11	28 ± 4	<0.05

N=46

Moscovic et al BJU 2012

Clomiphene citrate for treatment of hypogonadism



Moscovic et al BJU 2012

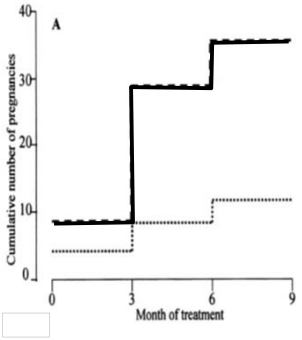
Subfertility: enhancement of positive outcome

Using Tamoxifen 20 mg/d and Andriol 120 mg/d

vs.

Placebo

Each group n= 106

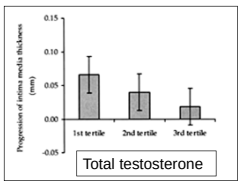


Adamopoulos et al. 2003

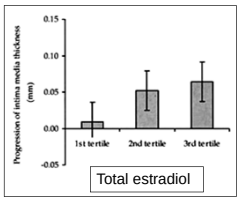
Use of aromatase inhibitors for treatment male infertility is under discussion and seems to have rare side effects

Schlegel Fertil Steril 2012 epub

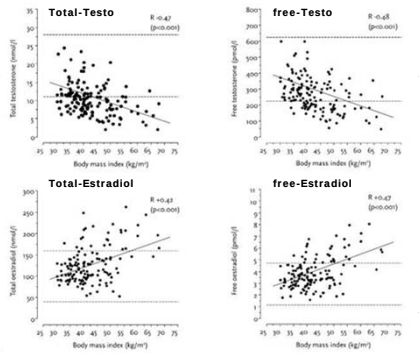
Progression of intima-media-thickness in 195 men aged 73 to 94 years in a 4 – year period



Muller M et al. Circulation 2004 109:2074

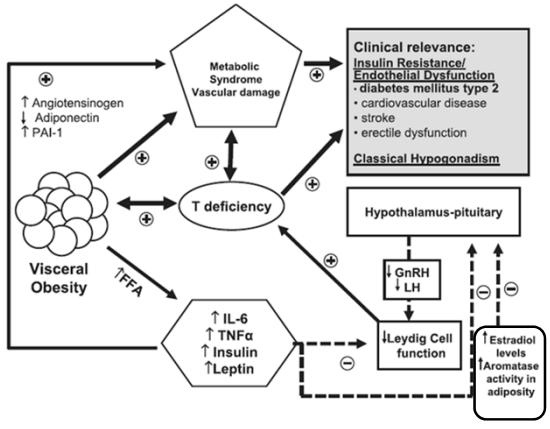


Testosterone and Estradiol in Obesity



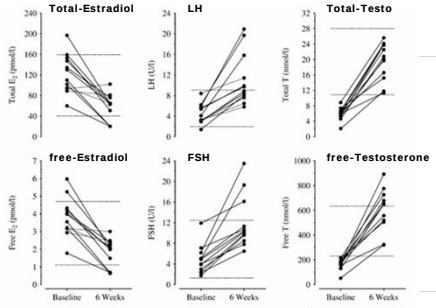
160 obese Men

Hofstra et al. 2009 Neth J Med 66: 103



Wang, Jackson, Jones, Matsumoto, Swerdloff, Zitzmann, Cunningham Diabetes Care 2011

Increase of LH and Testosterone in obese men receiving an aromatase inhibitor



Loves et al. Eur J Endocrinol 158: 741-747 (2008)

Genetic tests

how does male karyotyping impact on ART outcomes?

Elsbeth C. Dul, MD
Department of Obstetrics and Gynaecology
University Medical Center Groningen
The Netherlands

Conflict of interest

Our department received research grants from MSD, Ferring Pharmaceuticals, and Merck, the Netherlands.

Learning Objectives

- Prevalence of chromosomal abnormalities in different subgroups of infertile men
- Relation between chromosomal abnormalities and adverse pregnancy outcomes
- Strategy based on NNS to prevent one adverse pregnancy outcome

Introduction

Prevalence of chromosomal abnormalities
- 3-19% in infertile men
- 1% in newborns

Association with sperm parameters?

Introduction of ICSI: international guidelines

Karyotyping is costly and time-consuming

Dul et al, 2010; Nielsen et al, 1991

Content of Dutch guideline

Recommendation for karyotyping

- Male partners of ICSI couples, irrespective of sperm quality
- Azoospermic men

NVOG Guideline, 1999

Research questions

- Prevalence of chromosomal abnormalities
- Association with sperm parameters or other patient characteristics
- Consequences for the offspring

- Who should be screened for chromosomal abnormalities before ICSI treatment?

Materials & methods

Cohort 1223 men eligible for ICSI

1994-2007 UMCG

Retrospective data collection

- Sperm analyses
- Hormonal analyses
- Medical and reproductive history
- Karyotype
- Pregnancy outcome

Baseline characteristics

Male age (yrs)	34.6 (22-63.6)
Duration of infertility (yrs)	2.9 (0-17.6)
Primary infertility	85%

Sperm parameters

Parameter	Median	Interquartile range
Volume (ml)	3.7	2.4
Concentration (10^6/ml)	5.0	11.4
Motility (%)	18	23
TMSC	2.2	8.2

Karyotype

	Number
Normal karyotype	1185
Abnormal karyotype	38 (3.1%)
Gonosomal	19
Autosomal	19
Translocation	*12*
Inversion	*7*

Karyotype

Abnormality type	Abnormality	Frequency
Gonosomal	47, XXY	5
	47, XYY	3
	Mos 47,XXY/46,XY	2
	Mos 47,XYY/46,XY	1
	Mos 46,XY/45,X	3
	Mos 45,X/46, X, idic (Y)(q11.2)	1
	Mos 45,X/46, X, der(Y), ish r (Y)(cp923.1-, SRY+, DYZ4+, DYZ3+)	1
	46,XX.ish(X)(SRY+)	1
	46, X, der(Y), del (Y)(q11.223) inv dup (Y)(p11.2pter)	1
	46, X, t(Y;18;20)	1

Karyotype

Abnormality type	Abnormality	Frequency
Translocation	46, XY, t (1;14)(q44;q11.2)	1
	46, XY, t (2;9)(q37.3;q12)	1
	46, XY, t (3;11)(p21.3;q13)	1
	46, XY, t (3;16)(q12;q23)	1
	46, XY, t (4;5)(q32;q14)	1
	46, XY, t (15;21)(q24;q22.3)	1
	45, XY, der (13;14)(q10;q10)	2
	45, XY, dic (13;14)(p11.2;p11.2)	1
	45, XY, der (14;21)(q10;q10)	1
	45, XY, der (15;21)(q10;q10)	1
Translocation and inversion	45, XY, inv (5)(p13.1;q13.1), der (13;14)(q10;q10)	1

Karyotype

Abnormality type	Abnormality	Frequency
Inversion	46, XY, inv (1)(p13;q21)	1
	46, XY, inv (2)(p11.2;q13)	1
	46, XY, inv (2)(p21;q14.2)	1
	46, XY, inv (3)(q12;q23)	1
	46, XY, inv (10)(p11;q21.2)	1
	46, XY, inv (11)(q21;q23.3)	2

Association with sperm parameters

	Abnormal karyotype (n=38)	Normal karyotype (n=1185)	OR	p
TMSC (10^6)	0.2	2.2	0.98	0.35
Concentration (10^6/ml)	0.2	5.0	0.98	0.15

Association with sperm parameters

Sperm concentration (10^6/ml)	Prevalence abnormal karyotype (%)
0	15.2
0-1	3.1
1-5	1.2
5-10	1.4
10-20	3.1
>20	2.3

Prevalence abnormal karyotype

	Abnormal karyotype (n=38)	Normal karyotype (n=1185)	OR	p
Azoospermia	15.2	84.8	7.7	<0.001
Non-azoospermia	2.3	97.7	1.0	

Association with patient characteristics Azoospermic men

	Abnormal karyotype (n=12)	Normal karyotype (n=67)	OR	p
Elevated gonadotrophins	82%	52%	4.20	0.08

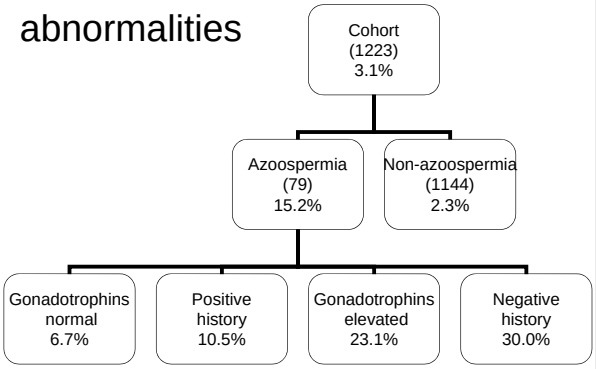
Association with patient characteristics Azoospermic men

	Abnormal karyotype (n=12)	Normal karyotype (n=67)	OR	p
Elevated gonadotrophins	82%	52%	4.20	0.08
Positive andrologic history	50%	78%	0.28	0.047

Association with patient characteristics Non-azoospermic men

	Abnormal karyotype (n=26)	Normal karyotype (n=1118)	OR	p
Elevated gonadotrophins	42%	32%	1.50	0.49
Positive andrologic history	31%	50%	0.46	0.07

Prevalence of chromosomal abnormalities



Classification of chromosomal abnormalities

- Risk of miscarriages and/or children with congenital anomalies increased
- Risk of miscarriages and children with congenital anomalies equal to population risk

Pregnancy outcome

	Population risk	Increased risk of miscarriages and/or children with CA
Number of men	24	14
Live born normal child	64%	45%
Abnormal child	7%	5%
Miscarriage	14%	45%

Number needed to screen

- Number of persons that need to be screened to prevent one adverse event
- Method to evaluate screening stategies
- Calculation based on absolute risk reduction:

  NNS = 1 / absolute risk reduction

NNS - Example HIV screening in pregnancy

	Prevalence 0.15%	Prevalence 5%
Women screened	10 000	10 000
HIV positive women	15	500
Rate of transmission in absence of interventions	14-25%	14-25%
Infected children prevented by screen and treat	0.8-2.9	27-95
NNS	3500-12 170	105-365

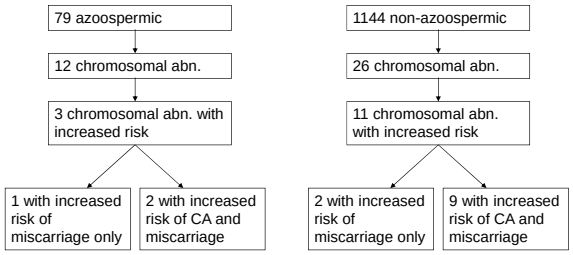
NNS in infertile men

For azoospermic and non-azoospermic men

Risk of miscarriage and child with congenital anomalies based on incidence in cohort

Absolute risk reduction based on comparison with population risk

Number needed to screen



Number needed to screen

	NNS for miscarriage	NNS for child with CA
Azoospermic	80 - 88	790 - 3951
Non-azoospermic	315 - 347	2543 - 12723

Conclusion

Prevalence of chromosomal abnormalities in infertile men	
Azoospermia	15.2%
Non-azoospermia	2.3%

Conclusion

	NNS for miscarriage	NNS for child with CA
Azoospermia	80 - 88	790 - 3951
Non-azoospermia	315 - 347	2543 - 12723

Recommendations

Karyotype all azoospermic men

Karyotype non-azoospermic infertile men in case of:

- Recurrent miscarriage
- Positive family history

Future research

Cost-effectiveness studies

- Costs of screening
- Costs of adverse pregnancy outcomes
- Impact of prenatal diagnosis and preimplantation diagnosis
- Societal willingness to pay

Genetic tests

how does male karyotyping impact on ART outcomes?

Karyotyping *all* infertile men will have little influence on ART outcome due to

- Low prevalence of chromosomal abnormalities (3.1%)
- Low risk for adverse pregnancy outcome

Karyotyping selected subgroups can benefit ART outcome due to

- High prevalence of chromosomal abnormalities (15.2% in azoospermic men)
- Low numbers needed to screen for adverse pregnancy outcomes

References

Nielsen J and Wohlert M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Hum Genet* 1991:**87**: 81-83.

Dul EC, van Ravenswaaij-Arts CMA, Groen H, van Echten-Arends J and Land JA. Who should be screened for chromosomal abnormalities before ICSI treatment? *Hum Reprod* 2010;**25**:2673-2677.

NVOG (Dutch Society of Obstetrics and Gynaecology). Guideline: Assessment and treatment for male subfertility, 1999. NVOG-richtlijn 17:1-5. Available on (in Dutch): http://nvog-documenten.nl/uploaded/docs/17_onder_behan_manne sub.pdf

Acknowledgements

Research group and co-authors:

Jolande Land	Dept Obst & Gyn
Conny van Ravenswaaij-Arts	Dept Genetics
Jannie van Echten-Arends	Dept Obst & Gyn
Henk Groen	Dept Epidemiology
Trijnie Dijkhuizen	Dept Genetics

Dietary Supplements
Are they any help?

Jackson C Kirkman-Brown
Birmingham Women's NHS Foundation Trust

We Will

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Overview

- Why consider supplements
- Rationale for using antioxidants
- Evidence in relation to value of antioxidants
- Risks
- Conclusions

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Why supplement?

Desire to offer 'something' to men in ART

Likelihood of 'improving' idiopathic male parameters / ART outcome (**majority**)

Known problem that might be assisted (minority)

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Having something to offer……..

European Association of Urology

Guidelines on **Male Infertility**

9. IDIOPATHIC MALE INFERTILITY

9.1 Introduction

No demonstrable cause of infertility is found in at least 44% of infertile men (1).

9.2 Empirical treatments

A wide variety of empirical drug treatments of idiopathic male infertility have been used; however, there is little scientific evidence for an empirical approach (2). Androgens, hCG/HMG, bromocriptine, alpha-blockers, systemic corticosteroids and magnesium supplementation are not effective in the treatment of OAT syndrome. Follicle-stimulating hormone (3) might be beneficial in a selection of patients (3). A Cochrane analysis showed that men taking oral antioxidants had an associated significant increase in live birth rate (pooled OR = 4.85; 95% CI: 1.92-12.24; P = 0.0008; I(2) = 0%) when compared with men taking the control treatment. No studies have reported harmful side effects from antioxidant therapy. The evidence suggests that antioxidant supplementation in subfertile men may improve the outcomes of live birth and pregnancy rate for subfertile couples undergoing assisted reproduction technique (ART) cycles. Further head-to-head comparisons are necessary to identify the superiority of one antioxidant over another (4).

Recommendation	GR
Medical treatment of male infertility is recommended only for cases of hypogonadotrophic hypogonadism.	A

Anti-oxidants

"… because there *might* be excessive ROS in subfertile men"

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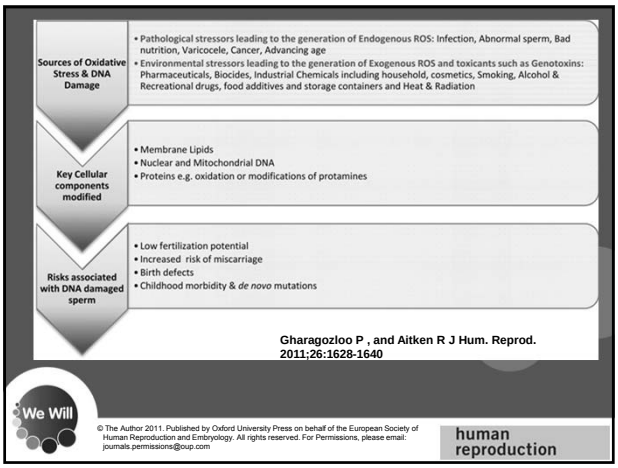
Guy's and St Thomas' NHS Foundation Trust

Birmingham Women's NHS Foundation Trust

Assisted Conception Unit



Gharagozloo P , and Aitken R J Hum. Reprod. 2011;26:1628-1640

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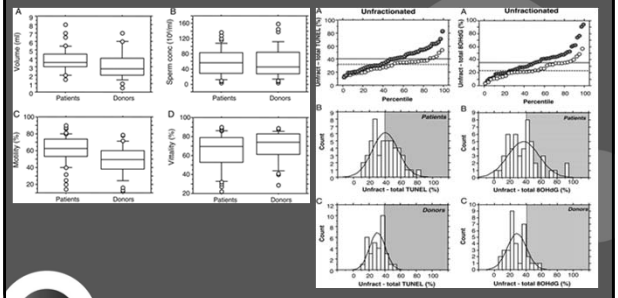
human reproduction

Conventional criteria of semen quality in the patient and donor populations



Aitken R J et al. Hum. Reprod. 2010;25:2415-2426

human reproduction

Antioxidant 'power' of semen

Physiology Section

The Total Antioxidant Power of Semen and Its Correlation with the Fertility Potential of Human Male Subjects

PRANJALI PRABHAKARRAO PAHUNE, AJAY RAJESHWAR CHOUDHARI, PARIKSHIT ASHOK MULEY

Group	Sperm concentration (million/ml)	Sperm Motility (%)	Sperm Morphology (%)	Total Antioxidant Capacity (µmol/L)
Normozoospermics (n=80)	96.25 ± 30.66	56.87 ± 8.66	38.65 ± 5.56	1980.82 ± 160.58
Asthenoteratozoospermics (n=25)	41.4 ± 12.64	37.88 ± 6.10	24.46 ± 4.39	1777.88 ± 239.87*
Oligoasthenoteratozoospermics (n=26)	14.92 ± 4.67	19.46 ± 4.84	17.26 ± 1.04	1389.72 ± 242.11*
Azoospermics (n=19)	0	0	0	1702.67 ± 485.95*

[Table/Fig-1]: Different Physical Parameters and TAC levels of semen in study group.
*Values significantly different compared to control P< 0.05

Journal of Clinical and Diagnostic Research. 2013 June Vol-7(6): 991-995

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Effects of sperm DNA damage on ART outcome

Osman et al., Human Reproduction 2013, Vol 28 (S1), i9: O-021.

25 Studies
3360 couples

SCSA	TUNEL	COMET
14 (1621 couples)	8 (1233 couples)	3 (506 couples)

IVF	5	6	2
ICSI	8	4	2
IVF + ICSI	6	1	1

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Summary from the SR

CPR	SCSA	COMET	TUNEL	COMBINED
IVF	S	S	S	S
ICSI	**NS**	**NS**	**S**	**S**
IVF + ICSI	NS	NS	NS	NS
TOTAL	S	S	S	S

LBR	SCSA	COMET	TUNEL	COMBINED
IVF	NS	S	S	S
ICSI	NS	NS	NS	NS
IVF + ICSI	NS	NS	-	NS
TOTAL	NS	S	S	S

Osman et al., Human Reproduction 2013, Vol 28 (S1), i9: O-021
Thanks to Tarek El Toukhy

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Conclusions from the SR

Osman et al., Human Reproduction 2013, Vol 28 (S1), i9: O-021
Thanks to Tarek El Toukhy

1- Sperm DNA damage appears to influence IVF and possibly ICSI outcome by 17-21%

2- Need large prospective studies

- LBR as primary outcome
- Agreed test threshold level
- Standardized inclusion and exclusion criteria

3- Need to study interventions *e.g. supplements!*

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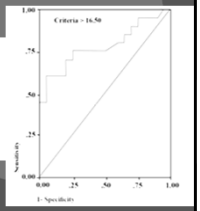
ROS & Risk of Miscarriage

Idiopathic Recurrent Pregnancy Loss: Role of Paternal Factors; A Pilot Study

Syed Nazar Imam [1], Monis Bilal Shamsi [1], Kishlay Kumar [1], Dipika Deka [2], Rima Dada [1*]

- 20 men with iRPL and 20 control

Category	DFI	ROS (RLU/min/20 million sperm)	TAC (*mM* trolox equivalent)
Cases (20)	25.36 (8.5,44.7)* [&]	47427.00 [&]	2.98 ± 1.2 [¶ &]
Controls (20)	12.70 (7.7,25.8)*	13644.57	6.95 ± 1.01 [¶]



J Reprod Infertil, Vol 12, No 4, Oct/ Dec 2011

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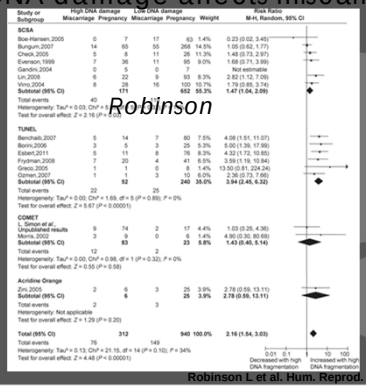
Sperm DNA damage affects miscarriage



Robinson

Robinson L et al. Hum. Reprod. 2012;27:2908-2917

human reproduction

ROS do seem to be a problem as is sperm DNA damage

Anti-oxidants

"…should be an effective therapeutic modality"

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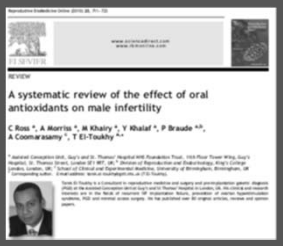
What is the state of evidence

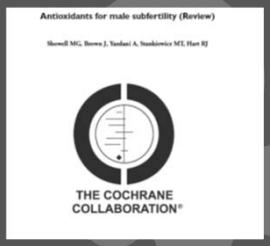
Two systematic reviews, 2010 & 2011



REVIEW

A systematic review of the effect of oral antioxidants on male infertility

C Ross *, A Morriss *, M Khairy *, Y Khalaf *, P Braude *,b, A Coomarasamy *, T El-Toukhy *,*



Antioxidants for male subfertility (Review)

Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ

THE COCHRANE COLLABORATION®

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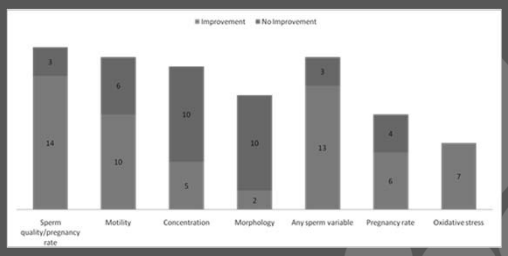
Summary of Results



- Moderate motility ↑ at 3, 6 and 9 m
- Limited concentration ↑ at 9 months

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MANY study design issues

- Variable methodology
- Extensive clinical heterogeneity & often no female partner data
- Different treatment regimens
  - Combinations & 'Reductive Stress' (Gharagozloo & Aitken, 2011)
- Different measures (e.g. DNA damage)
- Often no ongoing, live birth or miscarriage rates

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Conclusions from the SRs

- Oral antioxidant supplementation *may* improve pregnancy rate in male subfertility
- DO NOT expect WHO 2010 improvements
- Impossible from current literature to provide evidence-based recommendations
- Well-designed RCTs are needed

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"You said it may help if we took them, I want to do something and aren't

Anti-oxidants

HARMLESS?"

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Which one?!

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Which one?!

- They vary between 0mg and way above tested levels
- Have differing combinations / levels
- Difficult to assess any robustly on current evidence
- Properly organized, independent trials of specific formulations needed

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Why not prescribe antioxidants to all subfertile men anyway?

- Lack of effectiveness
- Waste valuable ♀ reproductive time
- Waste of resources
- Potential for harm

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Potential for harm

- Wrong (high) doses might have opposite effects
- Selenium and vitamin E cancer prevention trial (SELECT) indicated that for certain populations, supplement increased prostate cancer risk & severity
- beta-carotene is strongly counter-indicated as a lung cancer risk for smokers

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Other warning statements!

- Long term intake of 20mg Vitamin B6 may lead to mild tingling and numbness.
- Long term intake of 5mg of manganese may lead to muscle pain and fatigue.
- Long term intake of 30mg zinc may lead to anaemia.

Men will tend to not read these ….. Often thinking taking two is even better

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Conclusions

- There is good evidence that ROS / DNA damage detrimental to male fertility
- Shortage of well-conducted trials to demonstrate the effectiveness of antioxidant therapy – so requires a risk-balance
- Need to guide patient expectations
- Need to know which supplement and why
- High-quality trials are urgently needed

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Perhaps the advice of lifestyle change and healthy balanced diet is still the best, there are no quick solutions…

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Acknowledgements

Mr Tarek El Toukhy – Guy's & St Thomas', London

Professor Arri Coomarasamy – Birmingham Women's

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Preserving fertility before puberty
What should the clinician know ?

Herman Tournaye, M.D. Ph.D.
Centre for Reproductive Medicine Brussels

Universitair Ziekenhuis Brussel
Vrije Universiteit Brussel
Centrum voor Reproductieve Geneeskunde
bite biology of testis

The speaker's institution receives a research grants from Ferring to support research presented in this lecture

Outline of the presentation

- why prepubertal fertility preservation ?
- what are the fertility preservation options?
- present status of prepubertal fertility preservation
- towards clinical application
- take home messages

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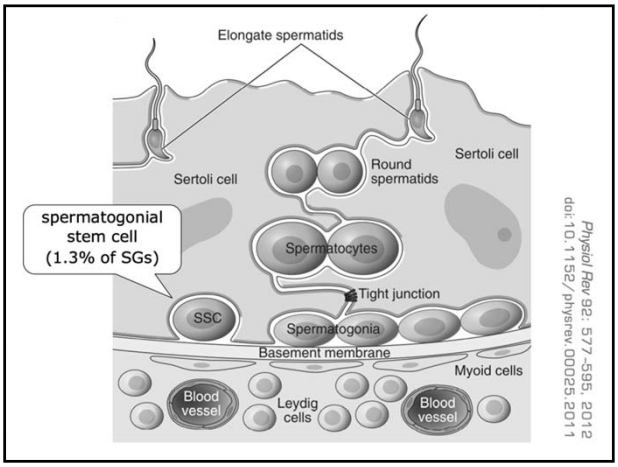
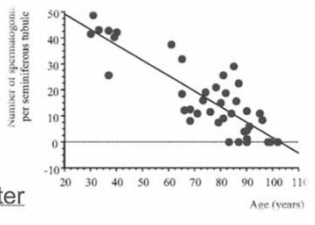


Physiol Rev 92: 577-595, 2012
doi:10.1152/physrev.00025.2011

Spermatogonial stem cell loss

- ageing
- genetic disorders
  - Yq deletions
  - 47, XXY Klinefelter
- gonadotoxic treatments

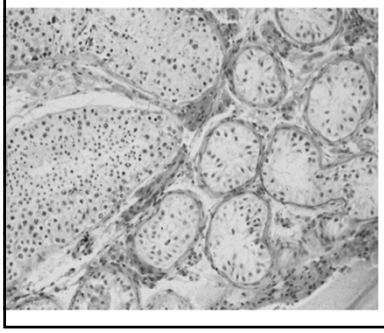


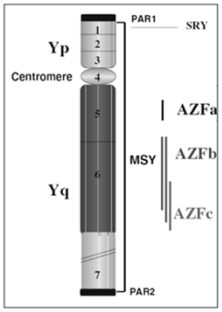
Dakouane et al. 2005

Cell and Tissue Banking (2006) 7:105–112

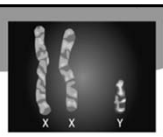
Sperm cryopreservation in male infertility due to genetic disorders

Csilla Krausz* and Gianni Forti
Andrology Unit, Department of Clinical Physiopathology





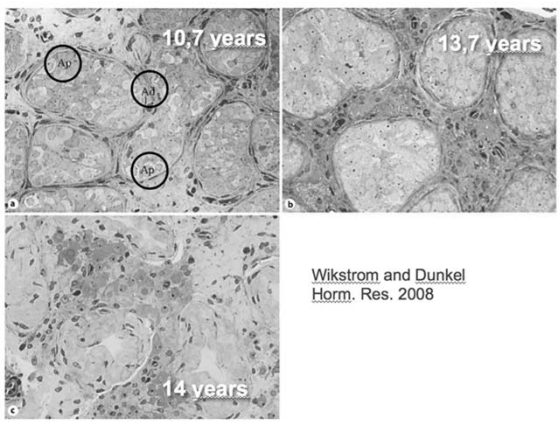
Testicular stem cell depletion



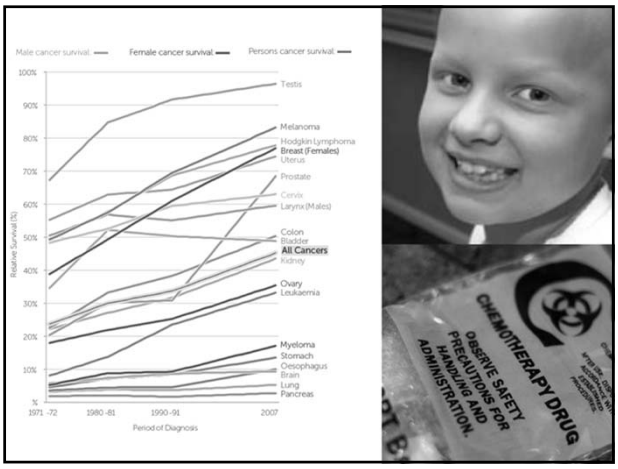
- reduced number of stem cells?
- 'minipuberty' with 'seminiferous activity'
- XXY spermatogonial stem cells go into apoptosis
- XXY Sertoli cells: dysfunctional niche?
- depletion resulting in azoospermia

Wikstrom et al., 2004
Oates 2012

Universitair Ziekenhuis Brussel
Vrije Universiteit Brussel
Centrum voor Reproductieve Geneeskunde



Wikstrom and Dunkel Horm. Res. 2008



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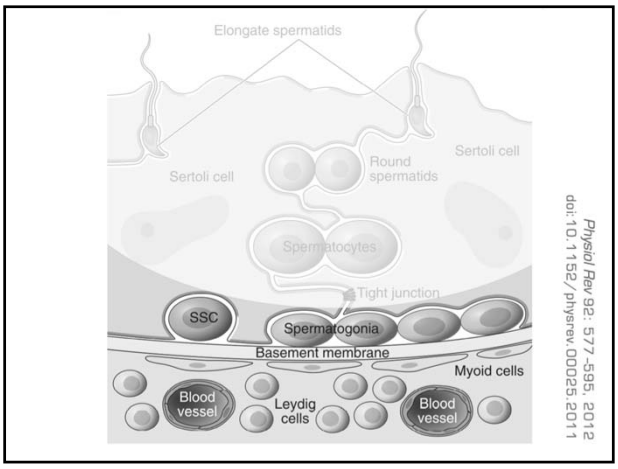
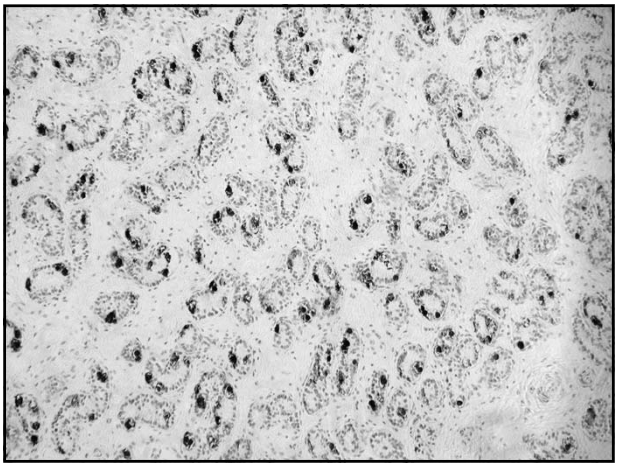
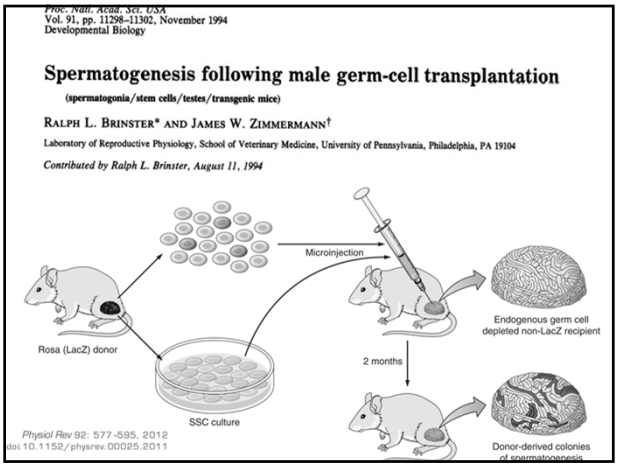

Elongate spermatids
Sertoli cell
Round spermatids
Sertoli cell
Spermatocytes
Tight junction
SSC
Spermatogonia
Basement membrane
Myoid cells
Blood vessel
Leydig cells
Blood vessel
Physiol Rev 92: 577-595, 2012
doi:10.1152/physrev.00025.2011




Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11298–11302, November 1994
Developmental Biology
Spermatogenesis following male germ-cell transplantation
(spermatogonia/stem cells/testes/transgenic mice)
RALPH L. BRINSTER* AND JAMES W. ZIMMERMANN†
Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104
Contributed by Ralph L. Brinster, August 11, 1994
Microinjection
Rosa (LacZ) donor
SSC culture
2 months
Endogenous germ cell depleted non-LacZ recipient
Donor-derived colonies of spermatogenesis
Physiol Rev 92: 577-595, 2012
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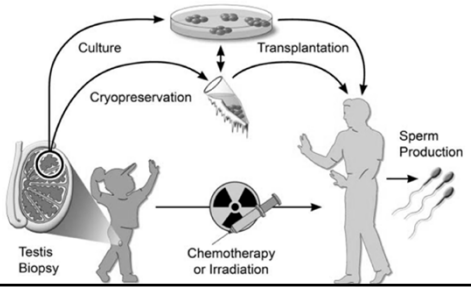

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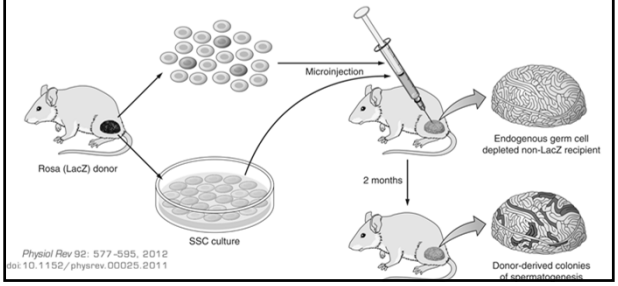
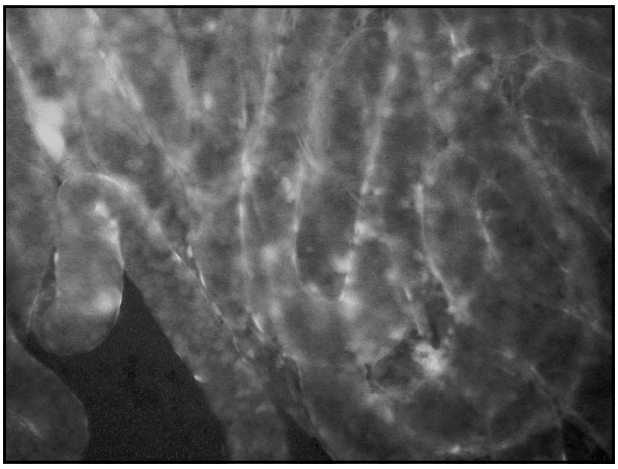
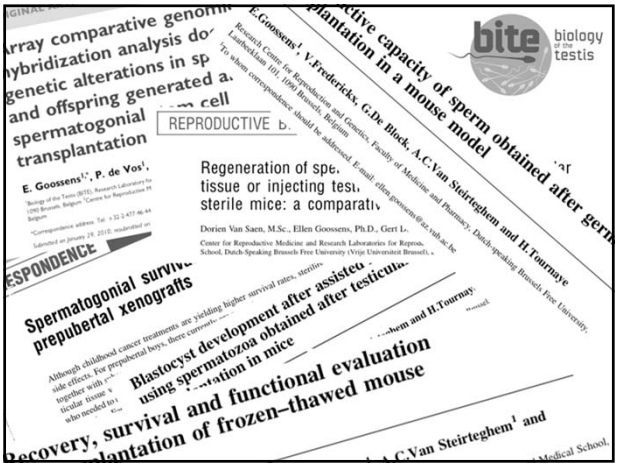
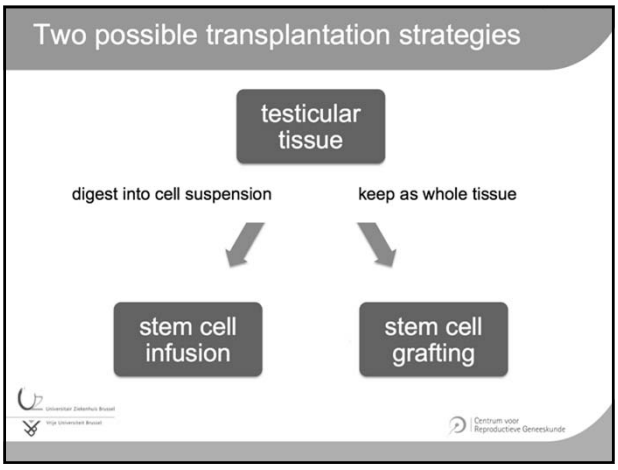
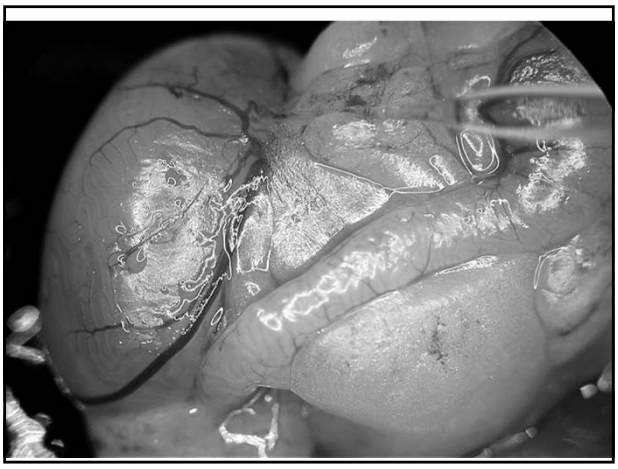


Physiol Rev 92: 577-595, 2012
doi:10.1152/physrev.00025.2011




bite
biology of the testis
Spermatogonial
prepubertal xenografts
Blastocyst development after assisted
using spermatozoa obtained after testicula
Recovery, survival and functional evaluation
plantation of frozen–thawed mouse
plantation in a mouse model
REPRODUCTIVE
Regeneration of spe
tissue or injecting tes
sterile mice: a comparati
E. Goossens
P. de Vos
Dorien Van Saen, M.Sc., Ellen Goossens, Ph.D., Gert L.
Center for Reproductive Medicine and Research Laboratories for Repros
School, Dutch-Speaking Brussels Free University (Vrije Universiteit Brussel),
E.Goossens, V.Fredericks, G.De Block, A.C.Van Steirteghem and H.Tournaye
Research Center for Reproduction and Genetics, Faculty of Medicine and Pharmacy, Dutch-speaking Brussels Free University,
To whom correspondence should be addressed. E-mail: ellen.goossens@az.vub.ac.be
A.C.Van Steirteghem and
Medical School,


Two possible transplantation strategies
testicular tissue
digest into cell suspension
keep as whole tissue
stem cell infusion
stem cell grafting
Universitair Ziekenhuis Brussel
Vrije Universiteit Brussel
Centrum voor Reproductieve Geneeskunde



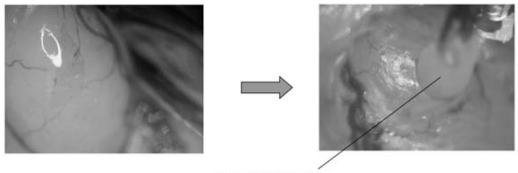
REPRODUCTIVE BIOLOGY

bite biology testis

Regeneration of spermatogenesis by grafting testicular tissue or injection of testicular cells into the testes of sterile mice: a comparative study

Dorien Van Saen, M.Sc., Ellen Goossens, Ph.D., Gert De Block, and Herman Tournaye, M.D., Ph.D.

Center for Reproductive Medicine and Research Laboratories for Reproductive Medicine, University Hospital and Medical School, Dutch-Speaking Brussels Free University (Vrije Universiteit Brussel), Brussels, Belgium



Prepubertal tissue

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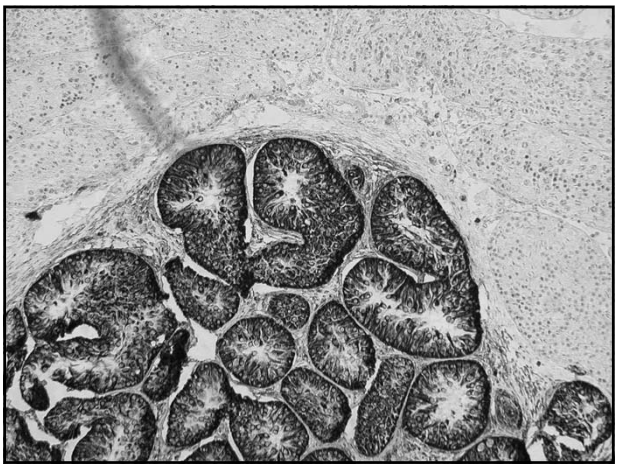
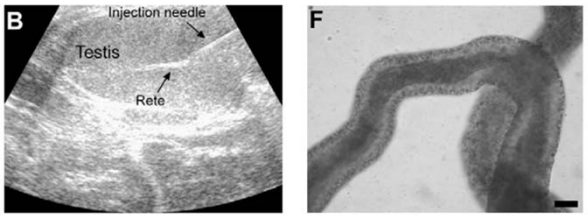
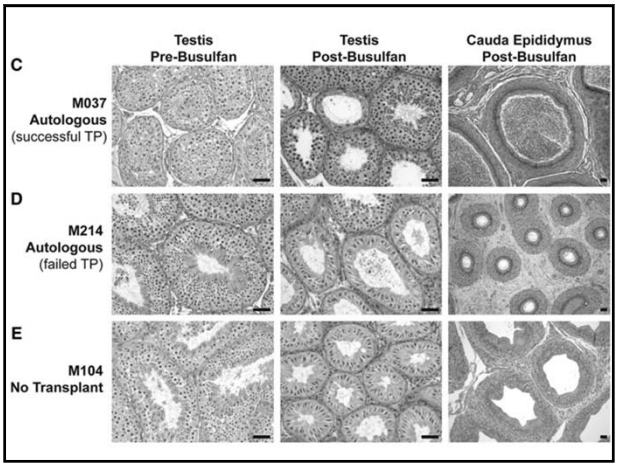


Cell Stem Cell
Clinical Progress

Spermatogonial Stem Cell Transplantation into Rhesus Testes Regenerates Spermatogenesis Producing Functional Sperm

Brian P. Hermann,[1,4,7,15] Meena Sukhwani,[7] Felicity Winkler,[7] Julia N. Pascarella,[7] Karen A. Peters,[7] Yi Sheng,[1,7] Hanna Valli,[6,7] Mario Rodriguez,[7] Mohamed Ezzelarab,[5] Gina Dargo,[13] Kim Peterson,[13] Keith Masterson,[8] Cathy Ramsey,[8] Thea Ward,[12] Maura Lienesch,[14] Angie Volk,[14] David K. Cooper,[5] Angus W. Thomson,[5] Joseph E. Kiss,[3,13] Maria Cecilia T. Penedo,[12] Gerald P. Schatten,[1,7] Shoukhrat Mitalipov,[6,9,10,11] and Kyle E. Orwig[1,2,4,7,*]





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Clinical Progress

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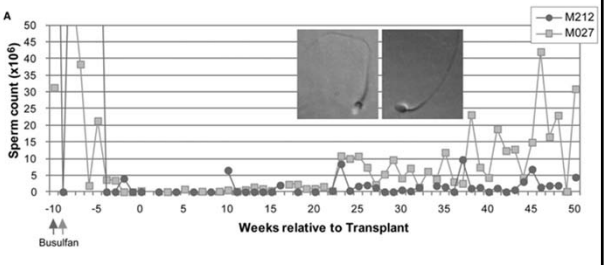

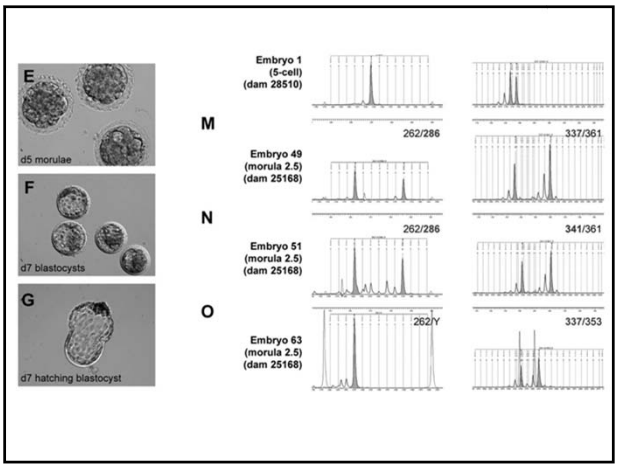

Outline of the presentation

- why prepubertal fertility preservation ?
- what are the fertility preservation options?
- present status of prepubertal fertility preservation
- towards clinical application
- take home messages

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Last Updated: Tuesday, 1 July, 2003, 09:03 GMT 10:03 UK

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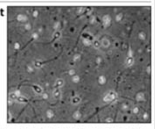
Testicle transplant makes sperm

By Martin Hutchinson
BBC News Online health staff in Madrid

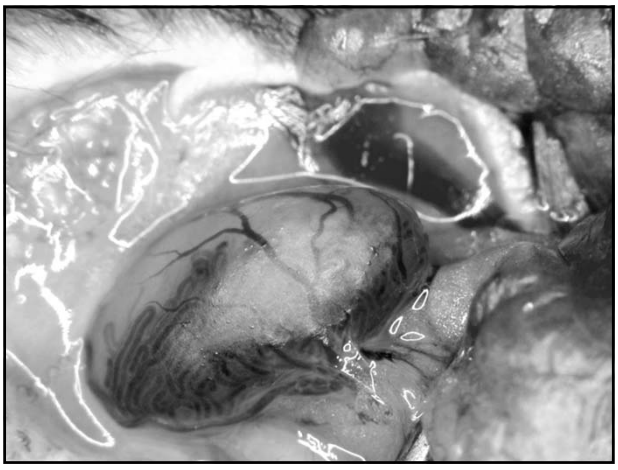


Men facing cancer treatment may not have to rely on a limited supply of frozen sperm to have children, as doctors hail the success of putting testicle tissue in storage instead.

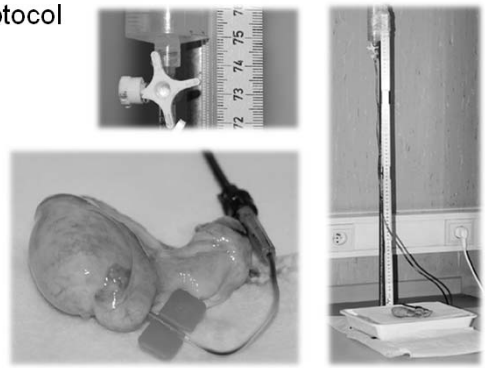
The new technique preserves the "germ cells" which make sperm, which are frozen and then transplanted back into the man when he is given the all-clear from the disease.

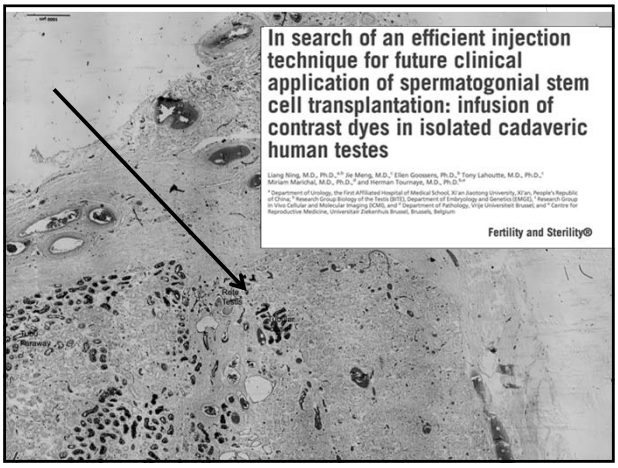
Remarkably, the frozen cells then "re-colonise" the testicle, and start producing enough sperm to allow fertility doctors to extract it from semen.

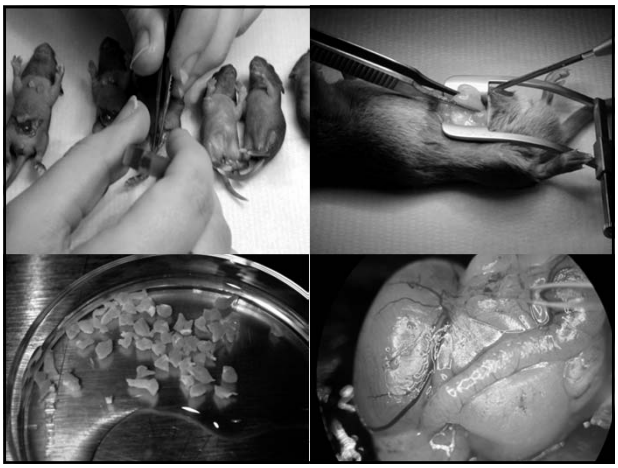
The Greek scientist behind the advance has already managed to grow these germ cells within the testicle of a rat, and says that storing testicle tissue instead of sperm will be a much better idea for would-be fathers.



Adapted transplantation protocol

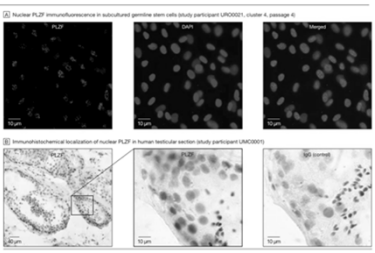






Propagation of Human Spermatogonial Stem Cells In Vitro

Hooman Sadri-Ardekani, MD
Sefika C. Mizrak, DVM, PhD
Saskia K. M. van Daalen, BSc
Cindy M. Korver, BSc
Hermien L. Roepers-Gajadien, BSc
Morteza Koruji, PhD
Suzanne Hovingh, MSc
Theo M. de Reijke, MD, PhD
Jean J.M.C.H. de la Rosette, MD, PhD
Fulco van der Veen, MD, PhD
Dirk G. de Rooij, PhD
Sjoerd Repping, PhD
Ans M. M. van Pelt, PhD



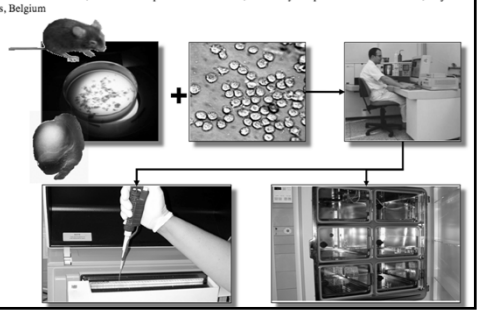
JAMA, November 18, 2009—Vol 302, No. 19

Human Reproduction Vol.22, No.3 pp. 733–742, 2007

The efficiency of magnetic-activated cell sorting and fluorescence-activated cell sorting in the decontamination of testicular cell suspensions in cancer patients

M.Geens[1,3], H.Van de Velde[2], G.De Block[1], E.Goossens[1], A.Van Steirteghem[2] and H.Tournaye[2]

[1]Research Centre for Reproduction and Genetics, [2]Centre for Reproductive Medicine, University Hospital and Medical School, Vrije Universiteit Brussel, Brussels, Belgium

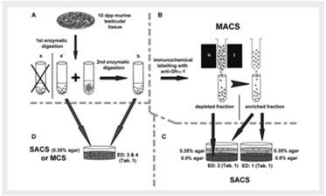


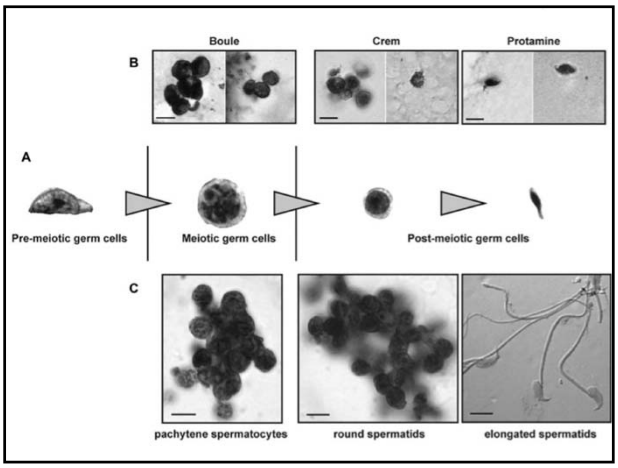
Molecular Human Reproduction, Vol.15, No.9 pp. 521–529, 2009
Advanced Access publication on June 27, 2009 doi:10.1093/molehr/gap052

MHR NEW RESEARCH HORIZON *Review*

New horizons for *in vitro* spermatogenesis? An update on novel three-dimensional culture systems as tools for meiotic and post-meiotic differentiation of testicular germ cells

Jan-Bernd Stukenborg[1,3], Stefan Schlatt[1,4], Manuela Simoni[1,4], Ching-Hei Yeung[1], Mahmoud Abu Elhija[2], Craig Marc Luetjens[1,5], Mahmoud Huleihel[2], and Joachim Wistuba[1]





Spermatogonial stem cell transplantation between syngeneic mice

no differences in DNA methylation pattern of

Igf2 (Insuline-like Growth Factor-2 (*Igf2*) = maternally methylated gene)

*Peg1 (*Paternally Expressed Gene-1)

alpha-Actin (not imprinted gene)

in spermatozoa obtained after SSCT

in liver, kidney and placental tissues of two subsequent generations of offspring obtained after SSCT.

bite biology of the testis

Goossens et al. Hum Reprod 2009

Acceptable strategy? the expert's viewpoint

A Strategy for Fertility Services for Survivors of Childhood Cancer

Author Multidisciplinary Working Group—British Fertility Society

Over the last 20 years there has been a very significant improvement in the outcome of treatments for children with cancer. Unfortunately, one of the side effects is either severe compromise or total destruction of fertility potential. For those of us practising in the reproductive endocrinology environment, this has become a very real problem. To that end, the British Fertility Society convened a multidisciplinary working group, which produced the above document under Ian Cooke's guidance. This is a tour de force. However, it is not really aimed at the general gynaecologist but at the organisations and units responsible for delivering such treatments and also as an aid to the Government in designing its strategies, nationally and regionally, for the provision of such services.

Fertilisation and Embryology Act must change to keep pace with the changing requirements of service provision. It also highlights the necessity of cancer networks involved in the management and coordination of care for these children to include fertility experts in their network.

The document has sections on counselling and sensibly suggests a combination approach, using those experienced in counselling children with childhood cancers and those experienced in counselling in the ART unit environment. Having said this, I am not convinced that the document deals adequately with how one copes with the teenager who requests storage of gametes but whose long-term survival chances are negligible. This is a

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Acceptable strategy? the parent's viewpoint

Parental desire and acceptability of spermatogonial stem cell cryopreservation in boys with cancer

H.van den Berg[1,3], S.Repping[2] and F.van der Veen[2]

Human Reproduction Vol.22, No.2 pp. 594–597, 2007

[1]Department of Paediatric Oncology, Emma Children Hospital and [2]Department of Obstetrics and Gynaecology, Centre for Reproductive Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

Table III. Number of parents giving consent for biopsy/hemicastration

	Opinion at diagnosis		Present opinion	
	Group A	Group B	Group A	Group B
n	94	108	94	108
Consent for biopsy, *n* (%)	58 (62%)	66 (61%)	70 (74%)	70 (65%)
Consent for hemicastration, *n* (%)	33 (35%)	33 (31%)	35 (37%)	42 (39%)

A, patient treated with protocols not considered to induce infertility; B, patient treated with protocols considered to induce infertility.

who should bank?

>80% risk for sterility after cytostatic treatment

- ✓ whole body irradiation
- ✓ conditioning for bone-marrow transplantation
- ✓ Hodgkin treated with alkylating agents
- ✓ metastatic Ewing's sarcoma
- ✓ metastatic soft-tissue sarcoma
- ✓ testicular radiotherapy

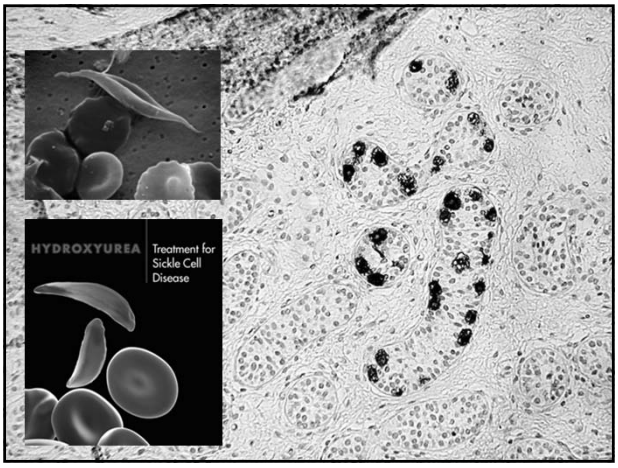
Wallace et al. Lancet Oncol. 2005

Example:

CHEMOTHERAPY

Unpredictable outcomes:

- Jeff W.
  - Age 13 @ dx.
  - ALL:CCG #123 B
  - Treatment
    - Cytoxan 23.4g/m2
    - 1800 cGy cranial xrt
  - Semen analysis
    - Conc=0
    - TMS=0
- David R.
  - Age 13 @ dx.
  - ALL:CCG #106 B
  - Treatment
    - Cytoxan 22.8g/m2
    - 1800 cGy cranial xrt
  - Semen analysis
    - Conc=216 million/ml
    - TMS=438 million



Prepubertal banking programme CRG Brussels
(as per January 1, 2014)

Malignant diseases		Non-malignant diseases	
Leukaemia	8	Drepanocytosis	14
B-cell lymphoma	2	Klinefelter syndrome	13
Hersentumor	2	Thalassemia	3
Testicular cancer	1	Granulomatous disease	2
Neuroblastoma	1	Ideopathic medullar aplasia	1
Osteosarcoma	1		
Rhabdomyosarcoma	1		
Medulloblastoma	1		
Anaplastic ependymoma	1		
Ewing sarcoma	1		
Nasopharynxcarcinoom	1		
Hodgkin lymphoma	1		
Total	21	Total	33

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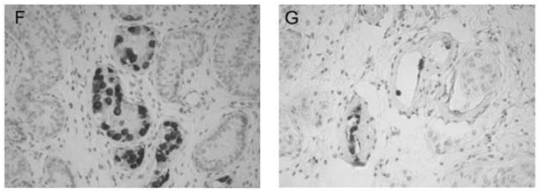
Human Reproduction, Vol.27, No.2 pp. 323–330, 2012
Advanced Access publication on December 12, 2011 doi:10.1093/humrep/der425

human reproduction ORIGINAL ARTICLE *Andrology*

Can pubertal boys with Klinefelter syndrome benefit from spermatogonial stem cell banking?

D. Van Saen [1,*], I. Gies [2], J. De Schepper [1,2], H. Tournaye [1,3], and E. Goossens [1]

[1]Research Group Biology of the Testis, Department of Embryology and Genetics, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium [2]Department of Pediatrics, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium [3]Centre for Reproductive Medicine, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium



www.brusselsoncofertility.be

Outline of the presentation

- why prepubertal fertility preservation ?
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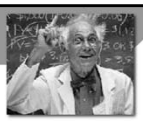
- present status of prepubertal fertility preservation
- towards clinical application
- take home messages

Take home messages



- spermatogonial stem cell transplantation works in mouse and rhesus monkey models
- in-vitro culture may be the key to success in the human
- although experimental, consider cryopreserving testicular tissue in prepubertal boys with a high risk profile

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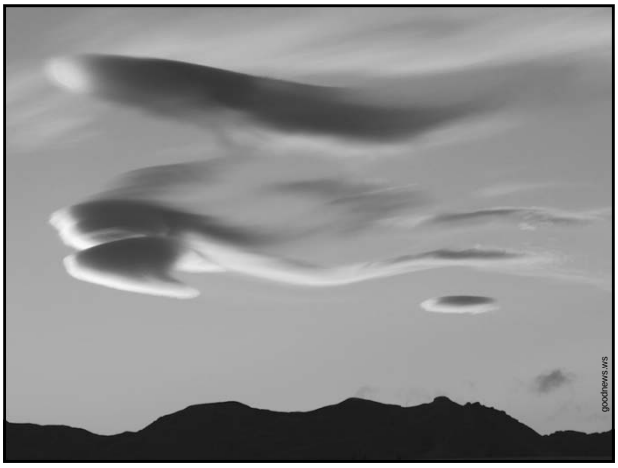

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// ESHRE CAMPUS EVENTS

ESHRE's 30th Annual Meeting

www.eshre2014.eu

Munich, Germany
29 June - 2 July 2014

Epigenetics in reproduction

www.eshre.eu/lisbon

Lisbon, Portugal
26-27 September 2014

Endoscopy in reproductive medicine

www.eshre.eu/endoscopyoct

Leuven, Belgium
15-17 October 2014

Making OHSS a complication of the past: State-of-the-art use of GnRH agonist triggering

www.eshre.eu/thessaloniki

Thessaloniki, Greece
31 October-1 November 2014

From gametes to blastocysts – a continuous dialogue

www.eshre.eu/dundee

Dundee, United Kingdom
7-8 November 2014

Controversies in endometriosis and adenomyosis

www.eshre.eu/liege

Liège, Belgium
4-6 December 2014

Bringing evidence based early pregnancy care to your clinic

www.eshre.eu/copenhagen

Copenhagen, Denmark
11-12 December 2014

An update on preimplantation genetic screening (PGS)

www.eshre.eu/rome

Rome, Italy
12-13 March 2014

For information and registration: www.eshre.eu/calendar
or contact us at info@eshre.eu

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