PRE-CONGRESS COURSE 2

Treating the man with evidence based medicine

SIG Andrology
Munich - Germany, 29 June 2014
Treating the man with evidence based medicine

Munich, Germany
29 June 2014

Organised by
The ESHRE Special Interest Group Andrology
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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
Training tomorrow’s research Andrologists to embrace 21st century investigative techniques: the promise of the Reprotrain network

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

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

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 Initial Training 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:

**2012**
- Identification of the conserved core sperm nuclear proteins and characterization of their role in fertility

**2013**
- Genomics and epigenetics of male infertility and characterization of the function of novel genes and proteins

**2014**
- Genetic and proteomic characterization and selection for early embryo development in mice
- Male germ cell reprogramming by bone marrow and bone cell modulation during spermatogenesis in the mouse
- Sperm spermatozoa epigenetic characterization and epigenetic potential
- Sperm, in control and fraction of sperm protein in chrompelis
- Investigating the relationship between spermatozoa donors and fertility
- Stepping of gene expression associated to the epigenetic profile of spermatozoa in rats and humans
- Characterizing the role of DNA methylation in spermatozoa, identifying new markers for male infertility and research on spermatozoa, spermatozoa proteins and proteome profiles and male infertility through novel compounds regulation
- High-resolution CIC, chromatin remodelers are involved in spermatogenesis and functional role of the genes involved
- Y chromosome-linked CTS and their biological consequences
- Development of DDA-IP, a method to identify gene function
- Implementing drug discovery program for epigenetic modulation and biomarker development to identify existing entities to therapy in human cancer patients
- Clinical evaluation of cancer-based expression in spermatogenesis, diagnostic for prostate cancer and male fertility in population

**2014**
- Sperm Phenotypic assessment, Proteomics, Transcriptomics, Epigenetics

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*Sperm Phenotypic assessment, Proteomics, Transcriptomics, Epigenetics*

It was held from the 3rd to 7th of June 2012 in Barcelona.
Setting up the basis for subsequent collaborative EU funded projects

- Reprotrain training network

- Methodological approaches to study the sperm cell proteome
Two-dimensional electrophoresis

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Sperm cells

DTT treatment

Protein purification

Acidic polyacrylamide gel electrophoreses


Protein identified as Cytochrome c oxidase subunit VIb

MALDI-TOF principle

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Sperm cell nuclear proteome

Sperm cell \rightarrow Nuclei \rightarrow Protein extraction \rightarrow Peptides \rightarrow LC-MS/MS

408 proteins identified


Sperm tail proteome

Heads Tails

LC-MS/MS

1049 proteins identified

Siva et al., 2010
Martínez-Heredia et al., 2006
Baker et al., 2007
Others

How many proteins in the mature normal human sperm cell?

Compilation of published proteomes: 6198 proteins

Predicted to exist from the pathway analysis: ~1800 proteins
Estimation of the complete proteome: ~8000 proteins

Protein abundance increased or decreased in patients?

Patients / Controls

- Wash seminal fluid (50% Percoll)
- Extract with 0.5 M HCl
- Purify proteins
- Acidic polyacrylamide gel electrophoresis

Motility

Asthenozoospermia

2D-DIGE

- Extract proteins
- Label with Cy5 (RED)
- Label with Cy3 (GREEN)
- Run 2D GE
- MS → Protein identification

1717 proteins identified

In the “No pregnancy” group as compared to the “pregnancy” group:
-35 increased
-31 decreased
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.

• 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, metabolism 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.

Summary

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


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)

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 (Spinobulbar, PAI etc) < 5%
- Unknown others > 50%

All other infertility / subfertility ~90%

- Abnormal semen profiles ~ 40%
- Apparently normal semen profiles ~ 60%

Environmental impact?

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)

Spermatogenesis as a proxy of the testis?

Spermatogenic Developmental Programme

64 Days in Humans
Control of gene expression in spermatogenesis

Testis

Stages of Spermatogenesis

- Pre-meiotic spermatogonia
- Pre-meiotic spermatocytes
- Meiotic spermatocytes
- Post-meiotic spermatids
- Spermatozoa

<table>
<thead>
<tr>
<th>Matched Gene</th>
<th>Transcriptional Protein</th>
<th>Enzyme</th>
<th>Other</th>
<th>Protein Synthesis</th>
<th>RNA Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matched Gene</td>
<td>Pre-meiotic spermatogonia</td>
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<td>Spermatozoa</td>
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<td>Post-meiotic spermatids</td>
<td>Spermatozoa</td>
</tr>
</tbody>
</table>

Diagnostic potential of existing sperm function applications

- Embryo quality
  - p < 0.004
  - r = -0.421

- Embryo quality
  - p < 0.05
  - r = -0.223

Lower sperm DF: better embryo quality

Tonnau et al., Hum. Rep., 2002
DNA Fragmentation
Lower sperm DF: more pregnancies

Simon et al. Fert Steril, 2012

DNA fragmentation by AO assay

Morphology?
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.

Antinori et al., RBM Online, 2008

Clinical pregnancy rate (%)  
ICSI  58/219 (26.5%)  
IMSI  89/227 (39.2%)

Implantation rate (%)  
ICSI  59/521 (11.3%)  
IMSI  97/560 (17.3%)

Proteomics

Proteomics

Sperm Proteomics

1. Sperm cell
2. Protein extraction (Semen seminal fluid, density gradient)
3. Proteins

MS (MALDI-TOF)

de Mata et al. 2013.
Transcriptomics

Proteomics

Complex repertoire of sperm RNAs (>3000)

- mRNA
- sncRNA
- IncRNA
- antisense transcripts
- etc....
Origins of male infertility

Miller & Ostermeier, 2006

1 - 4 highly motile spermatozoa

1' - 4' poorly motile spermatozoa

5 granulosa cell cDNA

6 water blank

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

eNOS and nNOS levels

Lambard et al, 2004

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

Bonache S et al., 2012
Expression ratios of target genes according to the IUI PR.

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

IUI significant differences

Grp1(L)

Grp2 (M)

Grp3 (H)

IUI insignificant differences

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

Bonache S et al., 2012

Early macroarray profiling of sperm and testis RNA.
Sperm RNA profiling corresponds to phenotypes

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

Assessment of sperm using mRNA microarray technology
Interspecies comparison and characterisation of sperm RNA

Total RNA approach: four 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 RNA to maximise RNA information.

First look: Bull

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

First look: Sheep

Protamines and Transition Protein 2

Sperm

Testis

TNF2
PRM1
PRM2
PRM3
Assessment of sperm using NGS technology (many RNAs are fragmented)

RNA
DNA

Nucleosomes including modified histones

Nucleoprotamine

Acrosomal cap

Nuclear envelope

Sperm RNA: the zygote and beyond
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.
References


With thanks to:-

Martin Brinkworth, University of Bradford.

Stephen Kravetz et al, Wayne State University Detroit.

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

Also with thanks to:-

reprotrain MRC Medical Research Council.
Molecular messages in the ejaculate remain an underestimated resource for understanding male fertility

Sophie ROUSSEAU, MD, PhD
Research Director at INSERM

Saeed Khoshbin's team
INSERM U923
Grenoble University, FRANCE

ESIRE Munich 29th June 2014
Pre-congress course 2

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

Background knowledge
The epigenome is a regulated "genome signaling" 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
- Smc6 is a master regulator of male genome programming and post-meiotic compaction
- So far, yet unknown, histone post-translational modifications (PTMs) are also involved
- The testis-specific histone variant H3B has an essential role in decondensing nucleosomes, which can be compensated by highly specific targeted histone modifications (e.g., the absence of H3B)
- First models to understand the molecular mechanisms driving male genome programming

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

Epigenetic landscape

Tissue-specific gene expression pattern

Environment
Growth factors, hormones, diet, metabolites...
Development
Aging, stress...

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

Cell identity relies on a defined and specific gene expression profile.
**Epigenome** = regulated genome signposting system

[Diagram showing DNA, histone octamer, linker histone, gene repression, and gene expression with Brm domains]

[Diagram showing DNA, histone octamer, linker histone, gene repression, and gene expression with Brm domains]
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

Spermatogonia

Meiosis

Yeast in vegetative growth
Brdt: a "swissKnife" in male genome programming

Histone PTM

Histone K acetylation – guided action: Brdt

Epigenetic programming of the post-meiotic genome by histone PTM...

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

Functional studies of new histone PTMs

Collab. Yiqi Meng, Zhao University of Chicago

Tse et al. Cell 2011
Brdt et al. in press and work in progress
Epigenetic guidance by histone PTMs

Male genome

POST-MEIOTIC SPERM CELLS MATURATION
One of the most dramatic chromatin remodelling

Before histone removal:

Histone hyperacetylation wave

Histone variants

The role of histones in nucleosome disassembly

Do specific histones become part of the dissociating nucleosomes?

Strategy:

Haploid cells

Ac H4

=> Capture histones in the course of replacement
**Discovery of new testis histones variants**

H2AL2: late expressing H2A variant

![Diagram of H2AL2 and TP2 proteins]

**H2AL2** is enriched in sub-nucleosomal particles, which are highly sensitive to MNase.

**TH2B** is present in nucleosomal and sub-nucleosomal particles.

**Hypothesis**

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

![Diagram illustrating histone replacement wave]

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**TH2B is expressed in meiotic and post-meiotic cells**

<table>
<thead>
<tr>
<th>Mitosis</th>
<th>Meiosis</th>
<th>Spermiogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2N</td>
<td>4N</td>
<td>4N</td>
</tr>
</tbody>
</table>

- TH2B
- TH2B
- TH2B
- Acid

**TH2B knock-in mouse models**

- TH2B-less mouse model (NCO)
- TH2B+mouse model (NCO)

**TH2B-tag mice**

- TH2B-tag mice ↔ normal nucleosome assembly
Lack of TH2B => compensation

TH2B"  TH2B"

Histone PTMs?

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

The secret of histone disappearance

First molecular models
Implications for male fertility

Infertile mouse models with abnormal male genome compaction
⇒ Brd and H2A12 mutants...

Sperm epigenetic defects are associated with male infertility
⇒ New causes of infertility? (ex. BRD7/K6Q)
⇒ Risks and/or low success of ICSI?
⇒ Develop tests for sperm epigenome assessment

Implications for epigenetic inheritance

THE SINS OF THE FATHER

Non-exhaustive list of references for further reading (4)


Non-exhaustive list of references for further reading (3)


Non-exhaustive list of references for further reading (2)


Non-exhaustive list of references for further reading (1)


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
Prevalence data for reproductive disorders in newborn or young adult males

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prevalence</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptorchidism</td>
<td>6-9%</td>
<td>Prospective EU studies</td>
</tr>
<tr>
<td>Hypospadias</td>
<td>0.4-0.9%</td>
<td>Prospective EU studies</td>
</tr>
<tr>
<td>Low sperm counts</td>
<td>16-20%</td>
<td>Prospective EU studies</td>
</tr>
<tr>
<td>Testis germ cell cancer</td>
<td>0.45%</td>
<td>Registry data (reliable)</td>
</tr>
<tr>
<td>Low adult Testosterone</td>
<td>&gt;10%</td>
<td>Cross-sectional studies</td>
</tr>
</tbody>
</table>

(Compensated Leydig cell failure)

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
- Testis GC cancer
- Low sperm counts
- Low-normal T levels
Sometimes inspiration comes from unexpected sources

As it turns out, the answer is literally staring him in the face!

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 (e.g. 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)


Testosterone production
reproductive test differentiation

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

The ‘masculinisation programming window’ (MPW)

DBP
Treatment windows

ITT
Late window (LW)
Full window (FW)

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

AGD is positively related to sperm count
in adult men

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

From: Eisenberg et al (2011) PlosOne e18973
AGD in normal boys and boys with cryptorchidism at <2 years of age

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

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

Female mean AGD runs roughly along the lowest percentile

Age in months

AGD (mm)

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

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

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

Female mean AGD runs roughly along the lowest percentile

Age in months

The commonest reproductive disorders of the developing and young adult male

“Testicular dysgenesis syndrome”

- Cryptorchidism

- Hypospadias

- Testis GC cancer

- Low sperm counts

- Low-normal/compensated T levels

- Subnormal T production or action

- Fetal Period

- Birth

- Perinatal Period

- Puberty

- Adulthood
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


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

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

Evan Solomonoff, Ryan Leeman, Verola Bannhart, Beth Bourgeois, Jeff H. Anderson, and Jon E. Stamp.


Abstract: We have previously shown that fetal sex steroid production is strongly correlated with birth weight, and that fetal sex steroid production is also tightly linked to adult body weight and composition. In this study, we have examined the relationship between birth weight and adult testosterone levels, independent of adult body weight. Our results show that birth weight is positively associated with adult testosterone levels, even after controlling for adult body weight. This suggests that fetal sex steroid production may have a lasting effect on adult testosterone levels, and may be a factor in the development of obesity and other metabolic disorders.

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

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

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

Evan R. Kilcoyne, Ben R. Tsui, Vicco Chapegas, Beth Bourgeois, Jeff H. Anderson, Verola Bannhart, and Beth Bourgeois.


Supporting information available online at www.pnas.org.

Abstract: We have previously shown that fetal sex steroid production is strongly correlated with birth weight, and that fetal sex steroid production is also tightly linked to adult body weight and composition. In this study, we have examined the relationship between birth weight and adult testosterone levels, independent of adult body weight. Our results show that birth weight is positively associated with adult testosterone levels, even after controlling for adult body weight. This suggests that fetal sex steroid production may have a lasting effect on adult testosterone levels, and may be a factor in the development of obesity and other metabolic disorders.

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

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

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

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

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

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)

Grafted

Week

0 1 2 3 4 5 6

HCG 20IU subcutaneous

DBP 500mg/kg/day

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

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

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

Host blood testosterone level

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%

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

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, Endokrinolog, Diabetolog
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
"Good news and bad news, Kevin. You tested negative for steroids, but positive for estrogen."

οἰστρός
oistrós / oestrus

Thorn
Passion

Target organs of testosterone and its metabolites
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 metabolism – studies in humans
Leder et al. 2003 J Clin Endocrinol Metab 88:204-210

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

All with GnRH agonist
Bone metabolism – studies in humans
Leder et al. 2003 J Clin Endocrinol Metab 88:204-210

Resorption

Time (weeks)
Group 1 vs 2: P = 0.03
Group 1 vs 3: P = 0.02
Group 2 vs 3: P = 0.13

Bone metabolism – studies in humans

Visit  Entry  Baseline  Final
Weeks 0 1 2 3 4 5 6 7 8
Leuprolide  Leuprolide
Letrozole
T + E Patch
A: No Patch
B: E Patch
C: T Patch
D: T + E Patch

Bone metabolism – studies in humans

Resorption  Formation

Urinary N0.1, % change
Group A  Group B  Group C  Group D  Group E  Group F  Group G  Group H

Urinary N0.1, % change
Group A  Group B  Group C  Group D  Group E  Group F  Group G  Group H
Bone density in 18 women 46,XY with CAIS after gonadectomy

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

FEMORAL NECK

No estrogen replacement

Androgen-Receptor-Mutations

CAIS

Okay... So maybe it wasn't such a good idea to buy your estrogen pills off the internet after all...
Fracture Risk / Femur
Men > 70 years


<table>
<thead>
<tr>
<th>Sex Hormone Groups*</th>
<th>Number of Men With Hip Fracture</th>
<th>Incidence Rate (per 1000 person-years)</th>
<th>Unadjusted Hazard Ratio (95% CI)</th>
<th>Adjusted Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;8.5 ng/mL)</td>
<td>11/115</td>
<td>1.6</td>
<td>2.0 (1.3, 3.1)</td>
<td>3.1 (1.4, 6.9)</td>
</tr>
<tr>
<td>Normal (8.5–10.0 ng/mL)</td>
<td>13/194</td>
<td>1.4</td>
<td>1.8 (1.3, 2.5)</td>
<td>1.8 (1.1, 3.0)</td>
</tr>
<tr>
<td>High (&gt;10.0 ng/mL)</td>
<td>15/155</td>
<td>3.3</td>
<td>(reference)</td>
<td>(reference)</td>
</tr>
<tr>
<td>Reference group</td>
<td>15/113</td>
<td>1.5</td>
<td>(reference)</td>
<td>(reference)</td>
</tr>
</tbody>
</table>

*Adjusted for age, body mass index, height and smoking status.

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

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

Treatment with various SERMS

Börjesson et al, J Bone Min Res 2012
Treatment of gynecomastia induced by GnRH-antagonist therapy for PCa: Tamoxifen

Viani et al Int J of Radiation 2012

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Odds Ratio</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boccardo 2005</td>
<td>20.9% [0.9, 44.0%]</td>
<td>0.06</td>
</tr>
<tr>
<td>Pernot 2005</td>
<td>3.0% [0.9, 10.1%]</td>
<td>0.04</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>3.4% [0.9, 12.4%]</td>
<td>0.04</td>
</tr>
<tr>
<td>Total events</td>
<td>100.0%</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Effect on gynecomastia by TMX

Clomiphene citrate for treatment of hypogonadism

Katz et al. BJU 2011

| Baseline | After treatment, mean (SD) | P
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total testosterone, ng/dl</td>
<td>192 (37)</td>
<td>485 (169)</td>
</tr>
<tr>
<td>Free testosterone, pg/ml</td>
<td>22 (16)</td>
<td>85 (23)</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>30 (13)</td>
<td>32 (16)</td>
</tr>
<tr>
<td>Oestradiol, pg/ml</td>
<td>26 (22)</td>
<td>38 (16)</td>
</tr>
<tr>
<td>LH, IU/L</td>
<td>2.8 (2.3)</td>
<td>6.8 (4.0)</td>
</tr>
<tr>
<td>FSH, IU/L</td>
<td>1.8 (1.3)</td>
<td>1.8 (1.8)</td>
</tr>
</tbody>
</table>

N=86
Clomiphene citrate for treatment of hypogonadism

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>%</th>
<th>Treatment</th>
<th>%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased libido</td>
<td>73</td>
<td>32</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lack of energy</td>
<td>66</td>
<td>40</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Decreased strength/endurance</td>
<td>28</td>
<td>21</td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>Lack of energy</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>Decreased life enjoyment</td>
<td>85</td>
<td>40</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sedentary</td>
<td>60</td>
<td>30</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Erections weaker</td>
<td>12</td>
<td>8</td>
<td></td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>Decreased sports performance</td>
<td>56</td>
<td>25</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sleep after dinner</td>
<td>34</td>
<td>28</td>
<td></td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>Decreased work performance</td>
<td>46</td>
<td>38</td>
<td></td>
<td></td>
<td>0.28</td>
</tr>
</tbody>
</table>

Katz et al. BJU 2011

Clomiphene citrate for treatment of hypogonadism

<table>
<thead>
<tr>
<th></th>
<th>Improvement in 10 symptoms</th>
<th>%</th>
<th>Improvement in 10 symptoms</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>One symptom</td>
<td>90</td>
<td></td>
<td>Two symptoms</td>
<td>75</td>
</tr>
<tr>
<td>Three symptoms</td>
<td>60</td>
<td></td>
<td>Four symptoms</td>
<td>30</td>
</tr>
<tr>
<td>Five symptoms</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P < 0.05 was considered to indicate statistical significance.

Katz et al. BJU 2011

Clomiphene citrate for treatment of hypogonadism

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T, ng/dL</td>
<td>238 ± 48</td>
<td>612 ± 113</td>
<td>540 ± 201</td>
<td>540 ± 227</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>LH, mIU/mL</td>
<td>20.8 ± 1.6</td>
<td>8.6 ± 3.2</td>
<td>7.2 ± 4.0</td>
<td>8.2 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DHEA-S, mg/dL</td>
<td>37 ± 16</td>
<td>46 ± 22</td>
<td>42 ± 13</td>
<td>50 ± 30</td>
<td>0.03</td>
</tr>
<tr>
<td>ADAM (s response)</td>
<td>7.3 ± 2</td>
<td>9.2 ± 3.2</td>
<td>5.8 ± 2.5</td>
<td>5.8 ± 0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean BMI, kg/m²</td>
<td>32.2 ± 8</td>
<td>31.8 ± 9</td>
<td>29.8 ± 11</td>
<td>28.8 ± 4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

N=46

Moscovic et al BJU 2012
Clomiphene citrate for treatment of hypogonadism

Subfertility: enhancement of positive outcome

Use of aromatase inhibitors for treatment male infertility is under discussion and seems to have rare side effects
Progression of intima-media-thickness in 195 men aged 73 to 94 years in a 4-year period


Testosterone and Estradiol in Obesity

160 obese Men


Wang, Jackson, Jones, Matsumoto, Swerdloff, Zitzmann, Cunningham Diabetes Care 2011
Increase of LH and Testosterone in obese men receiving an aromatase inhibitor


"Lord knows I've battled my demons, Gordy, but you might want to chill on the recreational estrogen."
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

NVdG 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (Interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male age (yrs)</td>
<td>34.6 (22-63.6)</td>
</tr>
<tr>
<td>Duration of infertility (yrs)</td>
<td>2.9 (0-17.6)</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>85%</td>
</tr>
</tbody>
</table>

Sperm parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Concentration (10^6/ml)</td>
<td>5.0</td>
<td>11.4</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>TMSC</td>
<td>2.2</td>
<td>8.2</td>
</tr>
</tbody>
</table>
**Karyotype**

<table>
<thead>
<tr>
<th>Number</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype</td>
<td>1185</td>
</tr>
<tr>
<td>Abnormal karyotype</td>
<td>38 (3.1%)</td>
</tr>
<tr>
<td>Gonosomal</td>
<td>19</td>
</tr>
<tr>
<td>Autosomal</td>
<td>19</td>
</tr>
<tr>
<td>Translocation</td>
<td>12</td>
</tr>
<tr>
<td>Inversion</td>
<td>7</td>
</tr>
</tbody>
</table>

**Abnormality type**

<table>
<thead>
<tr>
<th>Abnormality type</th>
<th>Abnormality</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonosomal</td>
<td>47 XY</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>46 XY</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>46 XY</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>45,XY</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>45,XY,del(Yq11.2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>45,XY,del(Yq11.23)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY,del(Yq11.23)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY</td>
<td>1</td>
</tr>
</tbody>
</table>

**Abnormality type**

<table>
<thead>
<tr>
<th>Abnormality type</th>
<th>Abnormality</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Translocation</td>
<td>46,XY(11q44)(q11.2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY(12p13.3)(q12)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY(13p11.23)(q13)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY(13q10)(q22)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY(14q24)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY(15q21)(q24)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY,der(13)(q10)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>45,XY,del(14)(q10)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>45,XY,del(14)(q10)(q10)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>45,XY,del(15)(q10)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>45,XY,del(15)(p13)(q13.1)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XY,del(16)(q12)</td>
<td>1</td>
</tr>
</tbody>
</table>
Karyotype

<table>
<thead>
<tr>
<th>Abnormality type</th>
<th>Abnormality</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inversion</td>
<td>46, XY, inv (1)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46, XY, inv (2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46, XY, inv (2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46, XY, inv (2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46, XY, inv (1)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46, XY, inv (1)</td>
<td>1</td>
</tr>
</tbody>
</table>

Association with sperm parameters

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Normal</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>karyotype</td>
<td>karyotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=38)</td>
<td>(n=1185)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMSC (10^6)</td>
<td>0.2</td>
<td>2.2</td>
<td>0.98</td>
</tr>
<tr>
<td>Concentration (10^6/ml)</td>
<td>0.2</td>
<td>5.0</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Association with sperm parameters

<table>
<thead>
<tr>
<th>Sperm concentration (10^6/ml)</th>
<th>Prevalence abnormal karyotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.2</td>
</tr>
<tr>
<td>0-1</td>
<td>3.1</td>
</tr>
<tr>
<td>1-5</td>
<td>1.2</td>
</tr>
<tr>
<td>5-10</td>
<td>1.4</td>
</tr>
<tr>
<td>10-20</td>
<td>3.1</td>
</tr>
<tr>
<td>&gt;20</td>
<td>2.3</td>
</tr>
</tbody>
</table>
### Prevalence abnormal karyotype

<table>
<thead>
<tr>
<th></th>
<th>Abnormal karyotype (n=38)</th>
<th>Normal karyotype (n=1185)</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>15.2</td>
<td>84.8</td>
<td>7.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-azoospermia</td>
<td>2.3</td>
<td>97.7</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

### Association with patient characteristics

#### Azoospermic men

<table>
<thead>
<tr>
<th></th>
<th>Abnormal karyotype (n=12)</th>
<th>Normal karyotype (n=67)</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated gonadotrophins</td>
<td>82%</td>
<td>52%</td>
<td>4.20</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Abnormal karyotype (n=12)</th>
<th>Normal karyotype (n=67)</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive andrologic history</td>
<td>50%</td>
<td>78%</td>
<td>0.28</td>
<td>0.047</td>
</tr>
</tbody>
</table>

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---
Association with patient characteristics
Non-azoospermic men

<table>
<thead>
<tr>
<th></th>
<th>Abnormal karyotype (n=26)</th>
<th>Normal karyotype (n=1118)</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated gonadotrophins</td>
<td>42%</td>
<td>32%</td>
<td>1.50</td>
<td>0.49</td>
</tr>
<tr>
<td>Positive andrologic history</td>
<td>31%</td>
<td>50%</td>
<td>0.46</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Prevalence of chromosomal abnormalities

- Azoospermia (79) 15.2%
- Non-azoospermia (1144) 2.3%
- Gonadotrophins normal 6.7%
- Positive history 10.0%
- Gonadotrophins elevated 23.1%
- Negative history 30.0%

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

<table>
<thead>
<tr>
<th>Population risk</th>
<th>Increased risk of miscarriages and/or children with CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of men</td>
<td>24 14</td>
</tr>
<tr>
<td>Live born</td>
<td>64% 45%</td>
</tr>
<tr>
<td>normal child</td>
<td></td>
</tr>
<tr>
<td>Abnormal child</td>
<td>7% 5%</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>14% 45%</td>
</tr>
</tbody>
</table>

Number needed to screen

- Number of persons that need to be screened to prevent one adverse event
- Method to evaluate screening strategies
- Calculation based on absolute risk reduction:
  \[ NNS = \frac{1}{\text{absolute risk reduction}} \]

NNS - Example
HIV screening in pregnancy

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

<table>
<thead>
<tr>
<th>Number needed to screen</th>
<th>NNS for miscarriage</th>
<th>NNS for child with CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermic</td>
<td>80 - 88</td>
<td>790 - 3951</td>
</tr>
<tr>
<td>Non-azoospermic</td>
<td>315 - 347</td>
<td>2543 - 12723</td>
</tr>
</tbody>
</table>
Conclusion

Prevalence of chromosomal abnormalities in infertile men

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>15.2%</td>
</tr>
<tr>
<td>Non-azoospermia</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

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


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

Overview

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

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)
Having something to offer........

9. IDIOPATHIC MALE INFERTILITY

9.1 Introduction
No demonstrable cause of infertility is found in at least 40% of infertile men [5].

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 [5]. Androgens, HCG/HMG, bromocriptine, alpha-blockers, systemic corticosteroids and magnesium supplementation are not effective in the treatment of GAT syndrome. Follicle-stimulating hormone (FSH) might be beneficial in a select group of patients (Cochrane analysis shows)

A meta-analysis of FSH treatment in idiopathic male infertility showed a statistically significant increase in live birth rate (pooled OR = 4.86, 95% CI: 1.00-12.31, P = 0.0006, 20 studies) when compared with men taking the control treatment. No studies have reported harmful side effects from FSH treatment. The evidence suggests that FSH supplementation in subfertile men may improve the outcome of in vitro fertilization cycles. Further head-to-head comparisons are necessary to identify the superiority of one FSH treatment over another [6].

Recommendation
Medical treatment of male infertility is recommended only for cases of hypogonadotropic hypogonadism.

A

Anti-oxidants

“… because there might be excessive ROS in subfertile men”
Summary from the SR

<table>
<thead>
<tr>
<th>CPR</th>
<th>SCSA</th>
<th>COMET</th>
<th>TUNEL</th>
<th>COMBINED</th>
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<tbody>
<tr>
<td>IVF</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>ICSI</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>IVF + ICSI</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TOTAL</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

LBR

<table>
<thead>
<tr>
<th>SCSA</th>
<th>COMET</th>
<th>TUNEL</th>
<th>COMBINED</th>
</tr>
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<tr>
<td>IVF + ICSI</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>NS</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

Conclusions from the SR

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!

ROS & Risk of Miscarriage

- 20 men with iRPL and 20 control
Sperm DNA damage affects miscarriage

Anti-oxidants

“...should be an effective therapeutic modality”

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

What is the state of evidence

Two systematic reviews, 2010 & 2011
Summary of Results

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

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

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
“You said it may help if we took them, I want to do something and aren’t
Anti-oxidants
HARMLESS?”

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
Why not prescribe antioxidants to all subfertile men anyway?

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

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

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.
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

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

Acknowledgements

Mr Tarek El Toukhy – Guy’s & St Thomas’, London
Professor Arri Coomarasamy – Birmingham Women’s
Preserving fertility before puberty
What should the clinician know?

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

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
Outline of the presentation

- why prepubertal fertility preservation?
- what are the fertility preservation options?
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- take home messages

Spermatogonial stem cell loss

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

Delivrance et al. 2005
Sperm cryopreservation in male infertility due to genetic disorder
Cella Krause and Gianni Forti
Andrology Unit, Department of Clinical Pathophysiology

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
Cates 2012

Wilstrom and Dunkel
Horm. Res. 2006
MAJOR ADVANCEMENTS IN THE TREATMENT OF CHILDHOOD CANCER HAVE PAVED THE WAY FOR INCREASING NUMBERS OF INDIVIDUALS TO BECOME SURVIVORS OF CANCER. HOWEVER, MANY WILL EXPERIENCE NUMEROUS LONG-TERM EFFECTS FROM THE DISEASE AND TREATMENT THAT MAY OCCUR MONTHS TO YEARS FOLLOWING CESSION OF THERAPY.

Outline of the presentation

• why prepubertal fertility preservation?
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• towards clinical application
• take home messages
Spermatogenesis following male germ-cell transplantation

Ralph L. Brinster* and James W. Zinderlands

Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

Contributed by Ralph L. Brinster, August 21, 1994

Outline of the presentation

• why prepubertal fertility preservation?
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• take home messages
Regeneration of spermatogenesis by grafting testicular tissue or injection of testicular cells into the testes of sterile mice: a comparative study

Denis Van Saele, M.D., Elwin Geuens, Ph.D., Guy De Moor, and Wolfgang Vanneste, M.D., Ph.D.
Center for Reproductive Medicine and Research Laboratories for Reproductive Medicine, Ghent University Hospital and Medical School, Ghent, Belgium; Brussels Free University, Brussels, Belgium
Outline of the presentation

• why prepubertal fertility preservation?
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• take home messages

A future, on ICE

An experimental approach promises to change the future for boys diagnosed today with cancer, allowing them to genetically father children of their own instead of facing a life of infertility. But will the science be ready when the children grow up, or are researchers placing their bets on another successful scientific feat, a hope that might not pay off? Roger McNamee reports on the cutting-edge science—and controversy—surrounding the freezing of prepubertal tissue.
Testicle transplant makes sperm

By Chris soldier
Wesley House staff in Medical

This transplant operation is expected to work in a way that will help women to have children, as doctors have the success of putting testicle tissue in the womb.

The new technique preserves the "germ cells" which make sperm, which are frozen and then transplanted back into the man whose testes have been damaged or removed.

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

The Greek scientist behind the advance has already managed to give those born only within the testicle of a rat, and says that making use of tissue instead of sperm will be a much better idea for widespread use.

Who would you rather see live?

researchamen.org
Spermatogonial stem cell transplantation between syngeneic mice

no differences in DNA methylation pattern of

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

Pgfl (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.

Goossens et al. Hum Reprod 2009

Accepable Strategy?
the expert's viewpoint
Parental desire and acceptability of spermatogonial stem cell cryopreservation in boys with cancer

H. van den Berg1,2, S. Repping3 and F. van der Veen2

Department of Paediatric Oncology, Antoni van Leeuwenhoek Hospital and 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/infusionation

<table>
<thead>
<tr>
<th>Opinion at diagnosis</th>
<th>Present opinion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
</tr>
<tr>
<td>Consent for biopsy, n (%)</td>
<td>94 (62%)</td>
</tr>
<tr>
<td>Consent for infusionation, n (%)</td>
<td>55 (35%)</td>
</tr>
</tbody>
</table>

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

**CHEMOTHERAPY**

Unpredictable outcomes:

- Jeff W.,
  - Age 13 @ dx.
  - ALL/CGG #123 B
  - Treatment
    - Cytoxan 23.4g/m2
    - 1800 cGy cranial xrt
    - Semen analysis
      - Conc=0
      - TMS=0

- David R.,
  - Age 13 @ dx.
  - ALL/CGG #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)

<table>
<thead>
<tr>
<th>Malignant diseases</th>
<th>Non-malignant diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>0</td>
</tr>
<tr>
<td>B-cell lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>Hermatocytosis</td>
<td>2</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>1</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>1</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
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</tr>
<tr>
<td>Medulloblastoma</td>
<td>1</td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>1</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
</tr>
</tbody>
</table>
Can pubertal boys with Klinefelter syndrome benefit from spermatogonial stem cell banking?

D. Van Saet, I. Giro, J. De Schepper, M. Vunana, and E. Gassens

Outline of the presentation

• why prepubertal fertility preservation?
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• 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.
<table>
<thead>
<tr>
<th>Event Description</th>
<th>Location</th>
<th>Dates</th>
<th>Website</th>
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<tbody>
<tr>
<td>Endoscopy in reproductive medicine</td>
<td>Leuven, Belgium</td>
<td>15-17 October 2014</td>
<td><a href="http://www.eshre.eu/endoscopyoct">www.eshre.eu/endoscopyoct</a></td>
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<tr>
<td>From gametes to blastocysts – a continuous dialogue</td>
<td>Dundee, United Kingdom</td>
<td>7-8 November 2014</td>
<td><a href="http://www.eshre.eu/dundee">www.eshre.eu/dundee</a></td>
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<tr>
<td>Epigenetics in reproduction</td>
<td>Lisbon, Portugal</td>
<td>26-27 September 2014</td>
<td><a href="http://www.eshre.eu/lisbon">www.eshre.eu/lisbon</a></td>
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<tr>
<td>Controversies in endometriosis and adenomyosis</td>
<td>Liège, Belgium</td>
<td>4-6 December 2014</td>
<td><a href="http://www.eshre.eu/liege">www.eshre.eu/liege</a></td>
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<tr>
<td>Bringing evidence based early pregnancy care to your clinic</td>
<td>Copenhagen, Denmark</td>
<td>11-12 December 2014</td>
<td><a href="http://www.eshre.eu/copenhagen">www.eshre.eu/copenhagen</a></td>
</tr>
</tbody>
</table>

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