Lifestyle and male reproduction
Special Interest Group Andrology

3 July 2011
Stockholm, Sweden
Lifestyle and male reproduction

Stockholm, Sweden
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Organised by
Special Interest Group Andrology
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Course coordinators

Sheena Lewis (United Kingdom) and Lars Bjorndahl (Sweden)

Course description

This course will present the causal links between lifestyle choices, general male health, systemic disease and human reproductive health. The impact on male reproductive health will be addressed as follows:

i) prenatal influences, dietary habits during childhood and puberty,
ii) adolescents and adults: obesity, diabetes and other systemic disorders, sexually transmitted infections and cancer therapies
iii) alcohol, tobacco and recreational drug use on male reproductive health

Target audience

Clinicians, paramedical staff, embryologists and andrologists with an interest in the effects of lifestyle factors on human male reproduction
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.00 - 09.30</td>
<td><em>In utero influences on future male fertility: effects of environmental chemicals and life-style</em> – <strong>Olle Söder (Sweden)</strong></td>
</tr>
<tr>
<td>09.30 - 09.45</td>
<td>Discussion</td>
</tr>
<tr>
<td>09.45 - 10.15</td>
<td><em>Obesity and diabetes: disease and treatment effects on male fertility</em> – <strong>Stefan Arver (Sweden)</strong></td>
</tr>
<tr>
<td>10.15 - 10.30</td>
<td>Discussion</td>
</tr>
<tr>
<td>10.30 - 11.00</td>
<td>Coffee break</td>
</tr>
<tr>
<td>11.00 - 11.30</td>
<td><em>STIs</em> – <strong>Falk R. Ochsendorf (Germany)</strong></td>
</tr>
<tr>
<td>11.30 - 11.45</td>
<td>Discussion</td>
</tr>
<tr>
<td>11.45 - 12.15</td>
<td><em>Genetically determined susceptibility to iatrogenic therapies</em> – <strong>Yvonne Lundberg-Giwerman (Sweden)</strong></td>
</tr>
<tr>
<td>12.15 - 12.30</td>
<td>Discussion</td>
</tr>
<tr>
<td>12.30 - 13.30</td>
<td>Lunch</td>
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<tr>
<td>13.30 - 14.00</td>
<td><em>Cancer: impact of disease and therapy on male fertility</em> – <strong>Bernard Robaire (Canada)</strong></td>
</tr>
<tr>
<td>14.00 - 14.15</td>
<td>Discussion</td>
</tr>
<tr>
<td>14.15 - 14.45</td>
<td><em>Recreational drugs (smoking, alcohol and cannabis)</em> – <strong>Sheena Lewis (United Kingdom)</strong></td>
</tr>
<tr>
<td>14.45 - 15.00</td>
<td>Discussion</td>
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<td>15.00 - 15.30</td>
<td>Coffee break</td>
</tr>
<tr>
<td>15.30 - 16.00</td>
<td><em>Good sperm, good brain?</em> – <strong>Arand Pierce (United Kingdom)</strong></td>
</tr>
<tr>
<td>16.00 - 16.15</td>
<td>Discussion</td>
</tr>
<tr>
<td>16.15 - 16.45</td>
<td><em>Exercise: Fit sperm?</em> – <strong>Diana Vaamonde (Spain)</strong></td>
</tr>
<tr>
<td>16.45 - 17.00</td>
<td>Discussion</td>
</tr>
</tbody>
</table>
What is ESHRE?

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

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

Executive Committee 2009/2011

Chairman
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- Anna Veiga
- Joep Geraedts
- Jean François Guérin
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- Ursula Eichenlaub-Ritter
- Antonis Makrigiannakis
- Miroslav Stojkovic
- Anne-Maria Sukkar
- Carlos Plancha
- Françoise Shenfield
- Etienne Van den Abbeel
- Jolienklo Schoonenberg-Pomper
- Veljko Vlaisavljevic
- Søren Ziebe

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- Anna Veiga

Past Chairman
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Italia
- Spain
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- Turkey
- Germany
- Greece
- Serbia
- Finland
- Portugal
- United Kingdom
- Belgium
- Netherlands
- Slovenia
- Denmark
General Assembly of Members

Central Office

ESHRE Consortia

PGD Consortium

Executive Committee

Committee of Nat. Representatives

Sub-Committees

Finance Sub-Committee

Comm. Sub-Committee

Publ. Sub-Committee

SIG Sub-Committee

SIG Coordinators

Task Forces

ESHRE Organisation

Finance Sub-Committee

Comm. Sub-Committee

Publ. Sub-Committee

SIG Sub-Committee

SIG Coordinators

Task Forces

ESHRE Journals

Human Reproduction with impact factor 3.859

Human Reproduction Update with impact factor 7.042

Molecular Human Reproduction with impact factor 3.005

Campus Activities and Data Collection

Campus / Workshops

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

Data collection and monitoring

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

- Embryology Certification
- Guidelines
- Position papers
- News magazine “Focus on Reproduction”

ESHRE COMMUNITY

RSS feeds for news in reproductive medicine

Since launch 12/2009: **1,360 Fans**
Since launch 12/2009: **190 followers**
(journalists, scientific organisations, patient societies, governmental bodies)

Retweets to MHR

ESHRE Membership (1/3)

TOTAL MEMBERSHIP*: **5 659 members**

* as of July 2010
ESHRE Membership (2/3)

<table>
<thead>
<tr>
<th>Membership Type</th>
<th>1 yr</th>
<th>3 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary Member</td>
<td>€60</td>
<td>€180</td>
</tr>
<tr>
<td>Paramedical Member*</td>
<td>€30</td>
<td>€90</td>
</tr>
<tr>
<td>Student Member**</td>
<td>€30</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

*Paramedical membership applies to support personnel working in a routine environment such as nurses and lab technicians.

**Student membership applies to undergraduate, graduate and medical students, residents and post-doctoral research trainees.

ESHRE Membership – Benefits (3/3)

1) Reduced registration fees for all ESHRE activities:
   - Annual Meeting: Ordinary €480 (€720)
   - Workshops*: All members €150 (€250)

2) Reduced subscription fees to all ESHRE journals – e.g. for Human Reproduction €191 (€573)
3) ESHRE monthly e-newsletter
4) News Magazine “Focus on Reproduction” (3 issues p.a.)
5) Active participation in the Society’s policy-making

*Workshop fees may vary

Special Interest Groups (SIGs)

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

- Andrology
- Psychology & Counselling
- Early Pregnancy
- Reproductive Genetics
- Embryology
- Reproductive Surgery
- Endometriosis / Endometrium
- Stem Cells
- Ethics & Law
- Reproductive Endocrinology
- Safety & Quality in ART
Task Forces
A task force is a unit established to work on a single defined task / activity
• Fertility Preservation in Severe Diseases
• Developing Countries and Infertility
• Cross Border Reproductive Care
• Reproduction and Society
• Basic Reproductive Science
• Fertility and Viral Diseases
• Management of Infertility Units
• PGS
• EU Tissues and Cells Directive

ESHRE – Annual Meeting
• One of the most important events in reproductive science
• Steady increase in terms of attendance and of scientific recognition

Track record:
ESHRE 2010 – Rome: 9,204 participants
ESHRE 2009 – Amsterdam: 8,095 participants
ESHRE 2008 – Barcelona: 7,559 participants

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

ESHRE 2011, Stockholm, Sweden
When: 3 - 6 July 2011
Where: Stockholmsmässan, Mässvägen 1, Älvsjö, Sweden
www.stockholmsmassan.se
Chair of conference: Kersti Lundin

Hotel and Travel:
MCI - Stockholm Office
Phone: +46 (0)8 54651500
E-mail: eshre@mci-group.com
For updates visit www.eshre.eu
ESHRE 2011, Stockholm

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

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

MHR Symposium – The paternal genome
Sperm chromatin packaging – B. Robaire (CDN)
The human sperm epigenome – B. Cairns (USA)

ESHRE 2011, Stockholm: Debates

This house believes that obese women should not receive treatment until they have lost weight
• Yes: Mark Hamilton (UK)
• No: Guido de Wert (NL) - TBC

Paramedical invited session: Should we pay donors?
• Yes: Herman Tournaye (BE)
• No: Laura Witjens (UK)

Annual Meeting – Pre-Congress Courses

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

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

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

### Certificate of attendance

1. Please fill out the evaluation form during the campus.
2. After the campus you can retrieve your certificate of attendance at www.eshre.eu.
3. You need to enter the results of the evaluation form online.
4. Once the results are entered, you can print the certificate of attendance from the ESHRE website.
5. After the campus you will receive an email from ESHRE with the instructions.
6. You will have TWO WEEKS to print your certificate of attendance.
In utero influences on future male fertility: Effects of environmental chemicals and life-style

Olle Söder, MD, PhD
Professor of Pediatrics
Paediatric Endocrinologist
Paediatric Endocrinology Unit
Department of Women’s and Children’s Health
Karolinska Institutet
Stockholm, Sweden

Disclosure:
The speaker has received honorarium/grants for consultancy, educational assignments and research projects from the following pharmaceutical companies:

Novo Nordisk
Ferring
Ipsen

Learning Objectives:
• Basic aspects of male prenatal sexual differentiation
• Sensitive periods of male sex development
• Adverse trends in male reproductive functions
• Definition and concepts of endocrine disruptors (EDCs)
• Potential targets of EDCs affecting male reproduction
• Knowledge gaps of EDCs
Reproductive Life Cycle

- Conception
- Sex determination
- Sex differentiation
- Birth
- Growth
- Puberty
- Adolescence
- Fertility
- Ageing
- Death

Chronology of early steps in human sex differentiation

<table>
<thead>
<tr>
<th>Event (start)</th>
<th>Age (dpc)</th>
<th>CRL(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic sex (fertilisation)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PGC differentiation and migration</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>Formation of gonadal ridge</td>
<td>32</td>
<td>5</td>
</tr>
<tr>
<td>PGCs reach gonadal ridge</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Sex determination</td>
<td>♂ testis</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>♀ ovary</td>
<td>49</td>
</tr>
<tr>
<td>Leydig cells appear</td>
<td>55</td>
<td>30</td>
</tr>
<tr>
<td>Androgen, INSL3; estrogen</td>
<td>63</td>
<td>40</td>
</tr>
<tr>
<td>Testicular descent (1st phase)</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

Testis determination in human embryo

From Bendsen et al. Hum Reprod 2003
External genitalia

GW 7
GW 11
GW 14

Girl
Boy

Differentiation of external genitalia

Homologous structures:
- Glans penis = Clitoris
- Corpus penis = Labiae min.
- Scrotum = Labiae maj.

Due to androgen action:
- T/DHT + AR (5α-R)
- T to ♀ = Virilisation
- Too little T to ♂ = Incomplete masculinization

Temporal events in male and female prenatal sex differentiation

- Migration of primordial germ cells
- Leydig cell activity
- Sertoli cell activity
- Regression of Müllerian ducts
- Development of Wolffian ducts
- Start of folliculogenesis
- Differentiation of external genitalia
- Regression of external genitalia
Sertoli cells critical for testis development

Adult Sertoli cell number determines testicular size and volume of spermatogenesis (sperm output)

Leydig cells produce androgen

Leydig cells are active in fetal life, neonatally and from puberty throughout adult life
Hormonal status of the human fetus

♂ Androgen↑
♀ Estrogen~ (=♀)
♀ Estrogen~
♂ Androgen↓ (active protection)

Gender dimorphic differentiation of the brain important part of human sex differentiation:

- Prenatal
  - genetic
  - hormonal
- Postnatal
  - neonatal?
  - pubertal

Affects:
- psychosexual identity
- behavior

“Imprinting” of the brain

Pre- and postnatal development of the brain
Androgen-sensitive events in male prenatal sex differentiation

- Development of male external genitalia
- Testicular descent
- Development of male internal (Wolffian) structures
- Priming of male-type metabolism
- Differentiation of the CNS

Increased incidence of disorders affecting male reproductive function

Testicular cancer

Cryptorchidism

Declining sperm count

Hypospadias

Intersex
Decline in human sperm count

Increasing incidence of testicular cancer

Undescended Testicles (Cryptorchidism)

Most common malformation in boys
Androgen-dependent 2nd phase of descent mostly affected. Anti-androgens?
Poor data in old incidence studies

Danish-Finnish study shows great regional variability mirroring incidence of testicular cancer:
4x > birth incidence Denmark vs. Finland
4x increase in Denmark since 1950s

(Boisen et al., 2004)
Suggested common pathogenesis of disorders of male reproductive functions

Bay K et al., 2006

Early events!

Global perspective on synthetic chemicals

• >100,000 man-made chemicals
• 400 million tonnes yearly production
• 85% no safety information
• 300 synthetic chemicals in human blood (incl. in blood of EU Commissioners -WWF)
• Higher levels of “modern” chemicals in children in “three generations study” (WWF Detox)
• >200 chemicals in cord blood (www.ewg.org/reports/bodyburden2)
Estimated increase of chemicals production, world population, and GDP (1995-2020)

OECD 2001

Endocrine disrupting chemicals (EDCs)

EDCs are chemicals common in the environment such as pharmaceuticals and over-the-counter drugs, natural hormones, personal care products like soaps and cosmetics, industrial by-products, plastics and pesticides.

Kavlock et al., 1996: “an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes”

WHO, 2002: “an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations”

Putative endocrine disrupting chemicals

Endocrine disrupters

(endocrine disruptor, endocrine modulators, endocrine toxicants, hormonally active chemicals, hormone mimics)
Putative EDCs have structures akin to hormones, and include:

- Several pesticides and their breakdown products that are now banned, such as DDT
- PCBs, a persistent group of chemicals still found in electrical equipment that pollutes lake and stream sediments in many industrial regions
- Dioxins, a group of toxic chemical byproducts from paper production and incineration
- Compounds used in plastics such as phthalates and bisphenol A.

EDCs CONT'D

- Several naturally occurring substances such as phytoestrogens (e.g., soy isoflavones – genestein) and antioxidants (e.g., resveratrol)
- Fungicides used in fruit (e.g., vinclozolin)
- Brominated flame retardants

...and many more

Important chemicals of "modern life". Often small lipophilic molecules that pass cell membranes and are easily absorbed not only via food and water, but also through the skin or by inhalation.

Many EDCs structurally similar to steroid hormones

[Diagram showing structural similarities between steroids and pollutants]

Supomkitch V et al. 2005
Classification of Endocrine Disruptors

Hormone-modulating effects of EDCs:

1. estrogenic activity, e.g., phytoestrogens
2. anti-androgenic activity, e.g., pesticides
3. anti-estrogenic activity
4. androgenic activity
5. thyroid hormonal effects

Evidence base for endocrine disrupting actions

1. Chemicals found in environment
2. Exposure data (wild life, humans, human fetuses)
3. Epidemiology of disorders (genital malformations, sperm counts, etc.)
4. “Disease” related to exposure
   a. Wild life (fish, reptiles, birds, whales, turtles, etc.)
   b. Experimental animals (“proof of concept”)
   c. Humans disorders

Proof-of-principle studies in exp. animals
(primary cultures)

See, Svechnikov et al. 2010
Cytokine level in rat testis after single oral dose of di-pentyl-phthalate

Granholm et al. 1992

IL-1\(\alpha\) induces StAR expression in immature but not adult Leydig cells

See, Svechnikov et al., 2010

Late changes in timing of onset of puberty
Proposed causes of changes of pubertal timing

1. Nutritional (obesity/anorexia; cf., secular trend)
2. Psychosocial ("exposure to a sexualized society")
3. Environmental (EDCs)
4. Combinations of above
5. Wrong diagnosis ("breast" in obesity)

<table>
<thead>
<tr>
<th>Reported effect</th>
<th>Sex</th>
<th>EDC</th>
<th>Exposure</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>Earlier onset</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Menarche, pubarche</td>
<td>F</td>
<td>PBBs</td>
<td>Prenatal</td>
<td>Blanck et al. -00</td>
</tr>
<tr>
<td>Thelarche</td>
<td>F</td>
<td>Phthalates</td>
<td>Childhood</td>
<td>Colon et al. -00</td>
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<tr>
<td>Menarche</td>
<td>F</td>
<td>DDE</td>
<td>Prenatal</td>
<td>Vasiliu et al. -04</td>
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<tr>
<td>CPP</td>
<td>F</td>
<td>DDE</td>
<td>Pre-/postnatal?</td>
<td>Krstevska-K et al. -01</td>
</tr>
<tr>
<td>Later onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast stage</td>
<td>F</td>
<td>Dioxin</td>
<td>Childhood</td>
<td>Den Hond et al. -02</td>
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<tr>
<td>Pub. stage, test. vol.</td>
<td>M</td>
<td>PCBs</td>
<td>Childhood</td>
<td>Den Hond et al. -02</td>
</tr>
<tr>
<td>Genital stage</td>
<td>M</td>
<td>PCBs, PCDFs</td>
<td>Prenatal</td>
<td>Guo et al. -04</td>
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<tr>
<td>Pub. stage, menarche</td>
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<td>Lead</td>
<td>Childhood</td>
<td>Wu et al.</td>
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<tr>
<td></td>
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<td></td>
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<td>Sedman et al. -03</td>
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<tr>
<td>No association</td>
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<td></td>
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<tr>
<td>Pub. stage, F/M</td>
<td></td>
<td>PCBs</td>
<td>Prenatal, lactat.</td>
<td>Gladen et al. -00</td>
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<td>Menarche, pub. stage</td>
<td>F/M</td>
<td>DDE</td>
<td>Prenatal, lactat.</td>
<td>Gladen et al. -00</td>
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<td>M</td>
<td>PCBs</td>
<td>Prenatal</td>
<td>Non et al. -02</td>
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<td>F</td>
<td>Dioxin, PCBs</td>
<td>Childhood</td>
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<td>Prenatal</td>
<td>Vasiliu et al. -04</td>
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<tr>
<td>Menarche</td>
<td>F</td>
<td>Dioxin</td>
<td>Prepubertal</td>
<td>Warner et al. -04</td>
</tr>
</tbody>
</table>

Stem cells novel target for EDC actions?

Goldman-Johnson-DR et al. 2008
Androgen Receptor expressed in murine embryonic stem cells

Knowledge gaps
Still poor data (incl. low power) on:
• environmental contamination of chemicals
• human exposure
• fetal exposure
• reporting of congenital malformations
• neurocognitive disorders
• gender identity
• fertility, sperm counts
• effects of low doses/complex mixtures rather than single substances

Preventive strategies
Proactive attitude
Fill the knowledge gaps
Precautionary principle
Cited Papers and Recommended Reading


Obesity and Diabetes: disease and effects on male fertility

Stefan Arver MD, PhD
Assoc Professor, Director
Centre for Andrology and Sexual Medicine
Karolinska University Hospital and Karolinska Institutet
Stockholm, Sweden

Theme of the Lecture

• Male reproductive function depends on
  Spermatogenesis producing “fertile spermatozoa”
  Ejaculation of “healthy gamets”
  Erectile function sufficient for intercourse
  Sexual drive and sufficient frequency of gamet delivery
• Fertile female partner with similar interest

Diabetes and Obesity

• Hypothalamic-Pituitary-Gonadal axis
• Spermatogenesis
• Ejaculatory Function
• Sexual function
• Psycho-social impact of Obesity and Diabetes
• Co-morbidities and pharmacological
• Susceptibility to environmental factors
Solution?

IVF – ICSI

Insulin dependant diabetes mellitus: implications for male reproductive function

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (n = 29)</th>
<th>Diabetic (n = 27)</th>
<th>P-value</th>
<th>WHO normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.0 ± 2.0</td>
<td>32.7 ± 0.7</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.3 ± 0.1</td>
<td>8.2 ± 0.5</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Semen volume (ml)</td>
<td>4.0 ± 0.2</td>
<td>2.6 ± 0.3</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Sperm concentration (x10^6/ml)</td>
<td>750 (130–2400)</td>
<td>420 (74–1510)</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Motility (%)</td>
<td>47.3 ± 2.8</td>
<td>46.0 ± 4.2</td>
<td>0.79</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>11.7 ± 0.8</td>
<td>11.1 ± 0.6</td>
<td>0.56</td>
<td></td>
</tr>
</tbody>
</table>

- b Values expressed as mean ± SEM.
- c Values expressed as median [inter-quartile range].

A boxplot comparing mtDNA deletions in sperm from control (n = 29) and diabetic men (n = 27, Type 1 diabetes mellitus) (c) mtDNA-deletion number.

© The Author 2007. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org
Table 2. Assisted reproductive outcomes by treatment. Values are n (%)a.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Patients</th>
<th>Cycles</th>
<th>Eggs harvested</th>
<th>Eggs fertilized</th>
<th>Normally fertilized</th>
<th>Embryo transfers</th>
<th>Clinical pregnancies/cycle</th>
<th>Clinical pregnancy rate per cycle of ART (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF</td>
<td>6</td>
<td>12</td>
<td>66</td>
<td>45 (68)</td>
<td>35 (70)</td>
<td>7</td>
<td>31/138 (27.2)</td>
<td></td>
</tr>
<tr>
<td>ICSI</td>
<td>13</td>
<td>20</td>
<td>108</td>
<td>121 (62)</td>
<td>110 (90)</td>
<td>18</td>
<td>94/134 (70.1)</td>
<td></td>
</tr>
<tr>
<td>BET</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>30/131 (23.1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18c</td>
<td>39</td>
<td>264</td>
<td>266 (67)</td>
<td>263 (92)</td>
<td>37</td>
<td>240/895 (26.8)</td>
<td></td>
</tr>
</tbody>
</table>

Increased BMI decreased sperm count

N = 3281

Arver, Bjørndahl, , Ekström, , Kist, Lethiset, Tu, to be published 2011
Relationship Between Testosterone Levels, Insulin Sensitivity, and Mitochondrial Function in Men

Increasing Insulin Resistance Is Associated with a Decrease in Leydig Cell Testosterone Secretion in Men

Mechanism of Erection i.e. smooth muscle relaxation
Initial dilatation Neurogenic

Continued dilatation
Shear Stress, VEGF + NO

Diabetes & vascular & erectile function

Diabetic endothelial dysfunction was originally described in human corpus cavernosum from men with ED

Acute & chronic hyperglycemia increases oxidative stress and reactive oxygen species with further progress of endothelial dysfunction.
Phosphorylation of eNOS Regulates NOS activity

Glucosylation at site Ser-1177 the site for Phosphorylation

Glycosylation inhibits phosphorylation

Summary

- Obesity and Diabetes type 2 are closely linked
- Suppressed Gonadal function is common in obesity independent of age
- Diabetes cause increased oxidative stress and glycosylation of key proteins
- Endothelial dysfunction and neuropathy cause erectile and ejaculatory dysfunction (Dunsimur WD and Holmes SA Diabetes Medicine 1996;13;700-708)
Disclosures

• No relationship or other activities to disclose in relation to this presentation
• No potential conflict of interest

Learning objectives

• After the lecture the participant is able to
  – describe the different prevalences worldwide
  – Explain the difficulties to define the real impact of STI’s
  – substantiate why STI’s are relevant for infertility
  – list 3 pathogens relevant for male infertility
  – describe how to care for HIV-infected couples
STI and male infertility

• Are STI's relevant?

• Which are relevant?

• Which sequelae?
  What to do?

STI prevalence (2001)

<table>
<thead>
<tr>
<th></th>
<th>Population aged 25 – 40 y (10^6)</th>
<th>Prevalence (10^6)</th>
<th>Prevalence/1000</th>
<th>Annual Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Europe</td>
<td>203</td>
<td>4</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Sub Saharan Africa</td>
<td>269</td>
<td>32</td>
<td>119</td>
<td>69</td>
</tr>
<tr>
<td>South and South East Asia</td>
<td>955</td>
<td>48</td>
<td>50</td>
<td>151</td>
</tr>
</tbody>
</table>

After: Lunenfeld & van Steirteghem 2004

Relevance of STI's for infertility

• Primary infertility
  – Developing countries < 2 % - 4 % – 8 %

• Secondary infertility
  – Egypt, Bolivia, Peru 15 – 20%
  – Bangladesh, Haiti 20 – 25 %
  – Kambodscha, India, Indonesia > 25 %
  – 14/23 Sub-Saharan states > 25 %
  – Zimbabwe > 62 %

→ Causes:
  – STI, poor hygiene post partum

After: Lunenfeld & van Steirteghem 2004
STI and infertility in Nigeria

Case control study; 150 fertile, 150 infertile men

Okonofua et al. 2005

STI's in Germany

STI's in Germany

Page 41 of 145
Chlamydia—Rates by Sex, United States, 1990–2009

NOTE: As of January 2000, all 50 states and the District of Columbia had regulations that required chlamydia cases to be reported.


NOTE: As of 1997, all 10 U.S. Department of Health and Human Services (HHS) regions, which represent all 50 states, the District of Columbia, and outlying areas, reported chlamydia positivity data.

Gonorrhea—Rates, United States, 1941–2009

NOTE: Data for 1941–1960 are from the Centers for Disease Control and Prevention (CDC) and the National Center for Health Statistics (NCHS). Data for 1961–2009 are from the CDC and the NCHS.
Syphilis—Reported Cases by Stage of Infection, United States, 1941–2009

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Female → male</th>
<th>Male → female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spreading of disease</td>
<td>Male disease</td>
<td>Female disease</td>
</tr>
<tr>
<td>Changes of</td>
<td>Male sterility</td>
<td></td>
</tr>
<tr>
<td>- Spermatogonia</td>
<td></td>
<td>Female infertility</td>
</tr>
<tr>
<td>- Leydig cells</td>
<td>Hypoospermia, male infertility</td>
<td>Immunologic infertility, obstruction</td>
</tr>
<tr>
<td>Infiltration of leukocytes in reproductive tract</td>
<td>Epididymitis, obstruction, immunologic infertility</td>
<td>Immunologic infertility, obstruction</td>
</tr>
<tr>
<td>Incorporation of virus genome in genome of spermatogonia</td>
<td>Risk of transmission to next generation</td>
<td></td>
</tr>
</tbody>
</table>

Problems to define relevance

High → high predictive value of a positive test result
Low → low predictive value of a positive test result

Prevalence of infection in a population

Length of exposure
Virulence of agent
Dose of agent
Immunologic response
Host factors
Infection Urethra
Prostate/sem. vesicles
Epididymis
Testis
Spermatozoa

Functional disturbance?

Invasion <- -> contamination
Growth < - -> colonisation
Reaction = infection

acute recurrent chronic

Inferile men

- Restortion
  - Immunol. response

False negative
- Prevalence of infection
- Sensitivity/spec. of test
- Long ago
- Chronic recurrent
- Infection in epid/ testis not reachable

Test for pathogens

False positive
- Agent commensal
- High prevalence pop.
- Irrelevant (infection of urethra)
- Agent irrelevant (other cause)

STI’s and fertility

- Are STD’s relevant?
- Which are relevant?
- Which sequelae?
  What to do?
<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Etiologic agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Syphilis</td>
<td>Treponema pallidum</td>
</tr>
<tr>
<td></td>
<td>Gonorrhoea</td>
<td>Neisseria gonorrhoeae</td>
</tr>
<tr>
<td></td>
<td>Ulcus molle</td>
<td>Haemophilus ducreyi</td>
</tr>
<tr>
<td></td>
<td>Granuloma inguinale</td>
<td>C. trachomatis, M. genitalium, H. ducreyi, G. vaginalis</td>
</tr>
<tr>
<td></td>
<td>Lymphogranuloma venereum</td>
<td>Chlamydia trachomatis L1-L3</td>
</tr>
<tr>
<td></td>
<td>Not gonococcal urethritis / adnexitis</td>
<td>C. trachomatis D-K, M. genitalium spp., Ureaplasma urealyticum</td>
</tr>
<tr>
<td></td>
<td>Bacterial vaginosis</td>
<td>Gardnerella vaginalis u.a.</td>
</tr>
<tr>
<td>Viruses</td>
<td>Herpes genitalis</td>
<td>Herpes-simplex-Virus 1/2</td>
</tr>
<tr>
<td></td>
<td>Genital ulcers (in HIV-infection)</td>
<td>Zytomyelovirus</td>
</tr>
<tr>
<td></td>
<td>Herpesvirus vulgatus, Condylomata acuminate, Bowenoid papulosis</td>
<td>Human Papillomavirus</td>
</tr>
<tr>
<td></td>
<td>Mollusca cornea</td>
<td>Mollusca-cornea-Virus</td>
</tr>
<tr>
<td></td>
<td>Papilloma</td>
<td>Papilloma-B-Virus, Pap-Virus</td>
</tr>
<tr>
<td>Yeasts</td>
<td>Balanitis, Vulvovaginitis, colpitis, urethritis</td>
<td>Candida species</td>
</tr>
<tr>
<td>Protozoa</td>
<td>urethritis, vulvovaginitis</td>
<td>Trichomonas vaginalis</td>
</tr>
<tr>
<td>Ectoparasites</td>
<td>pediculosis pubis</td>
<td>Pediculus humanus</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Effect on male/female fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Syphilis</td>
<td>No Yes</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Ulcus molle</td>
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</tr>
<tr>
<td></td>
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<td></td>
<td>Papilloma</td>
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</tr>
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<td>Yeasts</td>
<td>Balanitis, Vulvovaginitis, colpitis, urethritis</td>
<td>Yes Yes</td>
</tr>
<tr>
<td>Protozoa</td>
<td>urethritis, vulvovaginitis</td>
<td>Unclear, probably negligible</td>
</tr>
<tr>
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<td>pediculosis pubis</td>
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<td>Papilloma</td>
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</tr>
<tr>
<td>Ectoparasites</td>
<td>pediculosis pubis</td>
<td>No</td>
</tr>
</tbody>
</table>

Page 45 of 145
Semen microbiology in fertile/infertile population

<table>
<thead>
<tr>
<th>Non-bacterial</th>
<th>Subfertile population (n=144)</th>
<th>Fertile population (n=143)</th>
<th>Difference significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ureaplasma urealyticum</td>
<td>7.6</td>
<td>12.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>1.4</td>
<td>2.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>Roth</td>
<td>5.4</td>
<td>5.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>C. Trachomatis</td>
<td>4.2</td>
<td>7.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total</td>
<td>16.6</td>
<td>27.2</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Bacterial

<table>
<thead>
<tr>
<th>Non-bacterial</th>
<th>Subfertile population (n=144)</th>
<th>Fertile population (n=143)</th>
<th>Difference significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>20.1</td>
<td>13.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>Positive</td>
<td>85.7</td>
<td>72</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Problems: C. trachomatis and male infertility

- Detection method
- Material investigated
- Detection: acute or prior infections
- Relevance of immunologic responses
- Relevance of co-infections
- Group sizes
STD and fertility

- Are STD’s relevant?
- Which are relevant?
- Which sequelae?
  What to do?

Relevant Diseases

- Urethritis (Gonorrhoe, Ureaplasmen)
- Adnexitis (C. trachomatis)
- Herpes genitalis
- Trichomonas vaginalis
- HIV-Infection
Gonorrhoe

- Urethritis no effect  
  Nuss et al. 1997
- [strictures +]  
  Favel et al. 1987
  Dohle 2003

Gonorrhea

- 2 yrs after unilateral epididymo-orchitis:  
  → only 21 % sufficient semen quality
- biopsy: bilateral testicular damage
- Tubal occlusion in women  
  → RR 2.4 – 2.7  
  with history/serologic signs of prior gonorrhea
- Increased preterm birth (OR 2.9)

N.N. 1995
Grodstein et al. 1993

Gonococcal and chlamydia-infections

Infection with C. trachomatis often occurs among people who have gonococcal infection (referred to as concurrent infection). (See Gonococcal Infections; Dual Therapy for Gonococcal and Chlamydial Infections). The following recommendations are:

CDC 2010

Co-infection with Chlamydia trachomatis is common in patients with gonorrhea. Treatment for gonorrhea should routinely be followed with effective treatment for chlamydia due to transmission or re-infection of co-infections.

IUSTI 2004
Gonococcal urethritis

- Cefixim 400 mg p.o. 97.4 %  
- Ceftriaxon 250 mg i.m. 99.1 %  

PLUS

- Azithromycin 1 g p.o.  
- Doxycyclin 100 mg 2x/d 7 d

Chlamydia trachomatis and male infertility

- Deleterious effects: plausible  
- Evidence: weak  


Men: evidence for deleterious effects

- 6/241 (2%) pos. DNA  
  decreased sperm count  
- 244 infertile couples, 20 % pos.  
  Chl.antibodies; OR 2.6 for infertility  
- Association with inflammation  
- Transport to the female tract  

Idahl et al. 2007
Chlamydia and disease

- 17,764 men enrolled, retrospective 2001-05
  - 913 (5.14%): reproductive tract outcome
  - CT-positive men,
    cumulative incidence Hazard ratio
  - orchitis/epididymitis 4.28 % 1.38 (1.13-1.7)
  - Prostatitis 1.41 %
  - Infertility 1.27 %
  - urethral stricture 0.13 %
  - any outcome 1.37 (1.16-1.61)
  - infertility 1.36 (0.93-2.00)

Trei et al. 2008

Chlamydia and obstruction

- Obstructive azoospermia
  - 14 cases, 22 controls
- No C. trachomatis DNA
  - in testis or epididymis

Sripeda et al. 2010

Chlamydia-serology and prognosis

<table>
<thead>
<tr>
<th>Pair</th>
<th>Male factor</th>
<th>DNA-demonstration</th>
<th>Tubal-factor</th>
<th>Chance of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG Man</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>IgA Man</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>IgG Woman</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IgA Woman</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CHSP 60 man</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Idahl et al. 2007

"...the chlamydial serology results ... were not indicative of reduced sperm function or subsequent fertilizing capacity." Eggert-Kruse et al. 2011
Chlamydia and female infertility

- Infertility, ectopic pregnancies and chronic pelvic pain, are important consequences of PID, and since sexually transmitted microorganisms are the cause of acute PID in the majority of cases, then PID represents the link between sexually transmitted diseases (STDs) and infertility.

N.N. 2002

Therapy chlamydia

- Azithromycin 1 g p.o., single dose
- Doxycyclin 100 mg 2x/d for 7 d

“Azithromycin and doxycycline are equally efficacious in achieving microbial cure and have similar tolerability. Further head-to-head trials comparing these antibiotics are unnecessary. Lau & Qureshi 2002

Relevant Diseases

- Urethritis (Gonorrhoe, Ureaplasmen)
- Adnexitis (Chlamydia)
- Herpes genitalis
- Trichomonas vaginalis
- HIV-Infection
Acute epididymitis

Patient M.R.

- 29-yrs.
- Request for fertility status

Patient M.R.: Spermatogram

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Spermconcentration (10^6/ml)</strong></td>
<td>21.8</td>
</tr>
<tr>
<td><strong>Total count</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Motility a + b</strong></td>
<td>4</td>
</tr>
<tr>
<td><strong>Motility c</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Motility d</strong></td>
<td>95</td>
</tr>
<tr>
<td><strong>Morphology (nl)</strong></td>
<td>14</td>
</tr>
<tr>
<td><strong>Leukozytes</strong></td>
<td>9.8</td>
</tr>
</tbody>
</table>
History and physical examination

• 12 months ago: epididymitis
• Delayed diagnosis: chlamydia infection
• Epidididymis both sides: thickened

Patient M.R.: diagnosis and treatment

• Diagnosis:
  status after bilateral epididymitis
• Therapy
  – Diclofenac, Doxycylin, Vitamin E and C,
    Prednisone: no effect
  – Recommendation: ICSI
• Follow-up:
  after 18 months unchanged

Relevant Diseases

• Urethritis (Gonorrhoe, Ureaplasmen)
• Adnexitis
• Herpes genitalis
• Trichomonas vaginalis
• HIV-Infection
Ureaplasma/Mycoplasma

- Problem: Effect?
  - In-vitro: time- and dose-dependent
    - Chromatin-Decondensation
    - DNA damage
    - abortion?

  Reichart et al. 2000

Relevant Diseases

- Urethritis (Gonorrhoe, Ureaplasmen)
- Adnexitis durch C. trachomatis
- Herpes genitalis
- Trichomonas vaginalis
- HIV-Infektion

Herpes simplex in men

- Demonstrated in spermatozoa
- Incidence 0 - 3 – 24 - 56 %
  - PCR  in-situ Hybridisierung
- Association to low sperm numbers and motility?
- Pregnancies after aciclovir?

  Kotronias et al. 1998
  Kapranos et al. 2003
  Krause et al. 2002
Viruses in ejaculate

- HPV in ejaculate
  - 100 men with unprotected intercourse
    - 10/100 infected
    - 25% of sperm heads infected
  - 100 men without intercourse
    - No infection
  - Motility
    - Infected: 37,7 +/- 16,8
    - Non-infected: 53,7 +/- 18,2
    - Controls: 53,7 +/- 19

Relevant Diseases

- Urethritis (Gonorrhoe, Ureaplasmen)
- Adnexitis durch C. trachomatis
- Herpes genitalis
- Trichomonas vaginalis
- HIV-Infektion
Trichomonas vaginalis

- In 25%: only ejaculate positive
- Infertile men: higher incidence ??
- Quality of ejaculate improved 1 month after therapy ??
- No negative effect on motility, sperm-mucus interaction
- Apparently no impact on female fertility

Soper 2004

Relevant Diseases

- Urethritis (Gonorrhoe, Ureaplasmen)
- Adnexitis durch C. trachomatis
- Herpes genitalis
- Trichomonas vaginalis
- HIV-Infektion

Relevance of STD: increase risk of HIV

CDC

The Role of STD Detection and Treatment in HIV Prevention

STD treatment reduces the spread of HIV

STD treatment reduces the spread of HIV

STD treatment reduces the spread of HIV

STD treatment reduces the spread of HIV

STD treatment reduces the spread of HIV

STD treatment reduces the spread of HIV
HIV infection prevalence 2009

HIV in the male genital tract

- HIV-1 proviral DNA in spermatogonia
- Virus in ejaculate
- Intermittent “Shedding”
  - free in seminal plasma
  - from prostate gland?
- Spermatozoen?
  - No infection of motile sperm
- Lymphocytes
  - Macrophages
  - Monocytes
  - Rete testis, Epididymis

HIV infection in a semen donor

<table>
<thead>
<tr>
<th>Semen variable</th>
<th>Before HIV infection (n=63)</th>
<th>After HIV infection (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volumne (ml)</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Concentration</td>
<td>74</td>
<td>110</td>
</tr>
<tr>
<td>% motility</td>
<td>49</td>
<td>41</td>
</tr>
<tr>
<td>% morphology</td>
<td>55</td>
<td>46</td>
</tr>
<tr>
<td>Total count</td>
<td>263</td>
<td>264</td>
</tr>
<tr>
<td>Total motile count</td>
<td>131</td>
<td>105</td>
</tr>
</tbody>
</table>

Van Leeuwen et al. 2004
Ejaculate and HIV

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Motility</th>
<th>Abnormal Mot.</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>HIV pos</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Nicoleikis et al. 2004
Dulicur et al. 2002

HAART and semen quality

- Only few studies
  - Zidovudine monotherapy: no effect
  - HAART: tendency to improvement
    (short follow up) HAART: increase of mitochondrial DNA
  - In vitro: didanosine, zidovudine, saquinavir and indinavir; saquinavir: dose dependent decrease motility, decrease mitochondrial potential, increase acrosome reaction Ahmad et al. 2011

Relevance of stage of disease

- Semen quality normal
- Ovarian function/fertility norm
- AIDS
- Healthy, seropositive
- Menstrual cycle disturbed
- Pregnancy rate
- Motility decreased
- Leukocytospermia
- Testosterone decrease
- Testicular atrophy
- Increased incidence testicular cancer 50x
HIV infection and reproduction

- 80% HIV-pos. Couples had unprotected intercourse to achieve pregnancy
- Unprotected intercourse: 0.1–0.2% risk of HIV-infection per event man/woman
  - 100,000 copies: 1 zu 100
  - 100 copies: 3 zu 10,000

Natural conception?

- > 6 months no HIV-RNA in blood
- 62 serodiscordant couples
  - 22 HIV-pos. women
  - 40 HIV-pos. men
- 76 pregnancies, 68 births
- No seroconversion partner
- 1 HIV-pos. child
- 55/75% HCV-pos: no infection

Barrera et al. 2007

Natural conception?

- Undetectable HIV in blood
- In semen: in 48% (12/25) in more than one control:
  - viral shedding
  - 4/25 isolate > 5000 copies/ml
- 5-6% in other studies

Sheth et al. 2009
Treatment

- Counseling
- Antiretroviral therapy (< 1000 copies/ml) → significant improvement unrelated to CD4-count, ejaculate parameters, stimulation protocol
- Preparation of ejaculate: gradient centrifugation
  2x washes
  Swim-up
- PCR: use if HIV-RNA negative (~95 %)
- Use for IUI/ICSI

Zulkevis 2006

ART in HIV-pos. men

- 18 studies
- 1239 couples: preparation of sperm
- 2794 IUI-cycles
  - 89 IVF-cycles
  - 188 ICSI cycles
- 539 pregnancies
- 474 births
- No seroconversion

Van Leeuwen et al. 2007

ART in HIV-pos. men

- 245 couples, 439 cycles IUI
- 111/245 (45,4 %) pregnancy
- No seroconversion
- Prognostic relevant: maternal age and semen quality, rather than HIV factors
- Frozen sperm: significant negative impact on outcome

Nicopoulos et al. 2010
## Diagnostics of infections and ART

<table>
<thead>
<tr>
<th>Woman</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phasecontrast preparation, vaginal pH</td>
<td>Spermatogram (if leukozytospermia microbiol. diagnostics)</td>
</tr>
<tr>
<td>Chlamydia Gen amplification (cervical smear)</td>
<td></td>
</tr>
<tr>
<td>Rubella-HAH, eventually IgG</td>
<td></td>
</tr>
<tr>
<td>Varicella-antibodies</td>
<td></td>
</tr>
<tr>
<td>HBsAg, event. Anti-HBc Screening</td>
<td>HBsAg, Anti-HBc Screening</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>Anti-HCV</td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>Anti-HIV</td>
</tr>
</tbody>
</table>

AWMF-Leitlinie 15/040, 2004

## STI's and Male Infertility

- **Relevant:**
  - Chlamydia, gonococci, HIV
  - Especially developing countries
- **Unclear**
  - Mycoplasma, Ureaplasmen, Trichomonas, Viruses
- **Consequences**
  - Timely adequate therapy
  - HIV: counseling, assisted reproduction

## References

- [Other references provided in the text]
References


Genetically determined susceptibility to iatrogenic therapies

Yvonne Lundberg Giwercman, Ass Prof.
Faculty of Medicine
Dept. of Clinical Science

ESHRE
Stockholm 2011

Disclosure

The lecturer has no commercial and/or financial relationships with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices.

Learning objectives

• Azoospermia is common after cancer treatment (10-20%)
• Inhibin B is a good marker for recovery of spermatogenesis
• Genetic variants can also be used as predictive tools for azoospermia.
Iatrogenic therapies

- “First, do no harm”
- Conditions do not only result from medical errors e.g. mistakes made in surgery, the prescription or dispensing of the wrong therapy, but sometimes adverse effects of a medical treatment or combinations of treatments may be iatrogenic.
- For example, radiation therapy and chemotherapy frequently produce iatrogenic effects such as hair loss, anemia, vomiting infertility, etc.

High survival rate and risk of infertility

Childhood cancer

Facing heavy treatment.
Cryopreservation of testis tissue or not?

Testicular cancer

Received heavy treatment
Wants to have children when, or if, sperm production is restored.

Can genetic markers predict?

Childhood cancer incidence in Sweden

![Graph showing childhood cancer incidence in Sweden for boys and girls from 1960 to 2010. The incidence decreases over time.](https://example.com/plot.png)
Mortality

- Chemotherapy
- Radiotherapy
- Surgical therapy
- Diagnostic tools

Swedish Childhood Cancer Registry

Yvonne Lundberg Giwercman / ESHRE / Stockholm 2011

Diagnoses

% azoospermia

CCS, Brain, surgery, CT alone, RT alone, both CT and RT


Therapy

% azoospermia

OGS, Brain surgery, Surgery alone, CT alone, RT alone, both CT and RT


If radiotherapy to the testes → 100% azoospermia
High risk treatment

![Graph showing the percentage of azoospermia with and without RT combined with sterilizing doses of cisplatin/alkylating agents.]

Clinical markers

<table>
<thead>
<tr>
<th>OR</th>
<th>p</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subnormal Inhibin B</td>
<td>91</td>
<td>&lt;0.001</td>
<td>98</td>
</tr>
<tr>
<td>Elevated FSH</td>
<td>18</td>
<td>&lt;0.001</td>
<td>96</td>
</tr>
<tr>
<td>Subnormal total testicular volume (≤24 mL)</td>
<td>17</td>
<td>&lt;0.001</td>
<td>94</td>
</tr>
</tbody>
</table>

PPV = positive predictive value: still 35-50% have preserved sperm production
NPV = negative predictive value: risk of azoospermia only 1-2% if normal conc.

Can genetic variants be used in prediction?

AR mutations are rare
1:10 000
Profound effects on phenotype

Polymorphisms are frequent
>1% of the population
Small effects on phenotype
Candidate genes

Testosterone → AR → Prostate functional status

DHT → 5α-reductase II → AR

E2 → (DHT, E2) → Female phenotype (Bone calcification, Expressed in bone and genitals)

Hair → SpERMогenesis

Muscle → Testis

E2 → Aromatase → Female phenotype

Testis and germ cells

Yvonne Lundberg Giwercman / ESHRE / Stockholm 2011

Estrogen receptor α

% Azoospermia

AA/AG (42%) 74

GG (58%) 21

OR = 3.3, p=0.014

Romerius P et al. Pharm Genet & Genom, in press.

High-risk treatment

% azoospermia

AAAG (49%) 79

GG (51%) 29

70% of those with azoospermia can be identified by using this SNP

Romerius P et al. Pharm Genet & Genom, in press.
Summary childhood cancer

- ¾ survive
- 20% will have azoospermia
- FSH and inhibin B are good markers
- More than 70% are carriers of certain oestrogen receptor α variant, could be subjects for cryopreservation if prepubertal

Testicular Germ Cell Cancer

- incidence 6 / 100 000 men
- the most common cancer in men aged 20 – 40 years
- >95% cured
- associated with impaired sperm production and decreased fertility potential before diagnosis

Azoospermia at different time-points

- Frequency (%)
- Time-point after therapy

n=318

Eberhard J et al. Hum Reprod 2004
Azoospermia due to oncological treatment

Inhibin B as predictor of azoospermia

<table>
<thead>
<tr>
<th>Time of Inhibin B analysis</th>
<th>Inhibin B cut off level (ng/mL)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>T6</td>
<td>64.5</td>
<td>100</td>
<td>60</td>
<td>0.84</td>
</tr>
<tr>
<td>T12</td>
<td>60.5</td>
<td>92</td>
<td>74</td>
<td>0.84</td>
</tr>
<tr>
<td>T24</td>
<td>68.4</td>
<td>81</td>
<td>71</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Normal range: <50 ng/mL

- Important for a patient to know whether he will be permanently infertile or sperm production will recover
- Can genetic markers predict?
Genetic markers

Testosterone → AR → Prostate function

Hair → Androgenesis → Female phenotype

Muscle → Testis → E2 → Estrogen receptor α

Prostate function: Hair

Hair → Androgenesis → Female phenotype

Testis: E2 → Estrogen receptor α

Muscle: Testosterone → AR

Female phenotype: Bone maturation, expressed in breast and genitals

GG carriers are safe, also if treated with high dose chemotherapy

rs2077647

General population

Young men from general population
prior to military service, n=305

<table>
<thead>
<tr>
<th>rs2077647</th>
<th>LH (IU/L)</th>
<th>p-value</th>
<th>FSH (IU/L)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA+AG / GG</td>
<td>4.3 / 3.6</td>
<td>0.04</td>
<td>3.6 / 2.9</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Unpublished data.
Summary testicular cancer

• More than 95% survive
• 10% will have azoospermia
• Inhibin B is a good marker
• Estrogen receptor alfa variant predictive in all of cases.

Take home message

• Azoospermia is common after childhood cancer (20%) and testicular cancer treatment (10%)
• Inhibin B is a good marker for recovery of spermatogenesis
• Genetic variants can also be used as predictive tools for azoospermia.

References

• www.barncancerfonden.se
• www.cancerfonden.se
Acknowledgments

• Aleksander Giwercman
• Patrik Romerius
• Jakob Eberhard
• Olof Ståhl
Cancer: impact of disease and therapy on male fertility

Bernard Robaire, Ph.D. and Barbara F. Hales, Ph.D.
Departments of Pharmacology and Therapeutics
And of Obstetrics and Gynecology
McGill University
Montreal, Quebec, Canada

SIG Andrology – Pre-congress course: 3 July 2011
Lifestyle and male reproduction
27th Annual Meeting - ESHRE 2011 - Stockholm, Sweden, 3-6 July 2011

Conflict of Interest

I have no commercial relationships or other activities that might be perceived as a potential conflict of interest.

Learning Objectives

• There is an increasing rate of cancer among young men in developed countries.
• Improving survival of young men with testis cancer and lymphomas is associated with high rates of infertility.
• Men with some cancers have damaged germ cells even prior to initiation of drug treatments.
• Chemotherapeutic agents have a wide range of effects on male germ cells.
• Chemotherapeutic agents can have effects on sperm while residing in the epididymis.
• Sperm returning after chemotherapy show damage to chromatin at least up to two years after drug treatment.
• Therefore sperm banking should be advised prior to treatment.
**Paternal Occupations Affecting Incidence of Malformations in Progeny**

<table>
<thead>
<tr>
<th>Profession</th>
<th>Effect on offspring</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Janitor</td>
<td>hydrocephalus</td>
<td>5.04</td>
</tr>
<tr>
<td></td>
<td>ventricular septal defects</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>other heart defects</td>
<td>2.35</td>
</tr>
<tr>
<td>Forestry and</td>
<td>congenital cataract</td>
<td>2.28</td>
</tr>
<tr>
<td>Logging</td>
<td>atrial septal defects</td>
<td>2.03</td>
</tr>
<tr>
<td>Worker</td>
<td>syndactyly</td>
<td>2.03</td>
</tr>
<tr>
<td>Painter</td>
<td>spina bifida</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>patent ductus arteriosus</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>cleft palate</td>
<td>3.36</td>
</tr>
<tr>
<td>Printer</td>
<td>atresia of the urethra</td>
<td>4.50</td>
</tr>
<tr>
<td>Plywood mill</td>
<td>patent ptylic stenosis</td>
<td>4.12</td>
</tr>
<tr>
<td>Worker</td>
<td>ductus arteriosus</td>
<td>2.52</td>
</tr>
<tr>
<td></td>
<td>dislocated hip</td>
<td>2.71</td>
</tr>
</tbody>
</table>


**Associations between paternal exposures and adverse reproductive outcomes**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Embryofetal loss*</th>
<th>Birth defects*</th>
<th>Childhood cancer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation</td>
<td>0.9-1.5</td>
<td>1.4-5.6</td>
<td>N/T</td>
</tr>
<tr>
<td>Solvents</td>
<td>0.9-2.3</td>
<td>N/T</td>
<td>1.7-7</td>
</tr>
<tr>
<td>Anesthetic gases</td>
<td>1.5-1.8</td>
<td>N/T</td>
<td>N/T</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>0.9-2.3</td>
<td>1.5-249</td>
<td>3.5-7</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.6-1.4</td>
<td>1.3</td>
<td>1.2-3.9</td>
</tr>
<tr>
<td>Herbicides/pes ticides</td>
<td>N/T</td>
<td>5.7-405</td>
<td>2.4-7.1</td>
</tr>
<tr>
<td>Cancer drugs</td>
<td>N/T</td>
<td>4.1</td>
<td>N/T</td>
</tr>
</tbody>
</table>

*Values represent the range of OR/RR (Odds Ratios/Relative Risk) found by different studies

Sawyer and Atkin, 2001

**Chemotherapeutics**

- Male germ cells
- Damage induced in sperm chromatin (genetic and/or epigenetic)
- Germ cell death
  - Partial repair of damaged germ cells
  - Complete repair of damaged germ cells
  - Fertilization
- Effects on the conceptus
Male mediated developmental toxicity: chemotherapy, sperm chromatin, psychosocial and progeny outcome

Project 1: Impact of Combination Chemotherapy on Reproductive Health and Gamete Genetic Integrity in Humans

Project 2: Risk Communication & Psychosocial Impact of Reproductive Sequelae Among Men Treated for Testicular or Lymphatic Cancers

Project 3: Impact of Chemotherapeutic Regimens on Male Reproduction and Progeny Outcome in the Rat Model

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Grant # HGG-62294

Incidence of Testicular Cancer and Survival Rate

Testicular cancer incidence and rates of mortality in the United States.
Incidence of Testicular Cancer and Survival Rate

Paternity following Treatment for Testicular Cancer

Post-treatment Parenthood in Hodgkins Lymphoma Survivors

Brydøy et al., Journal of the National Cancer Institute (2005) 97):1580-1588

C E Kiserud et al., British Journal of Cancer (2007) 96, 1442–1449
Evidence for Male-mediated Developmental Toxicity

- Xenobiotics in the ejaculate can produce developmental toxicity
- Exposure of males to clastogens increases frequency of abnormal germ cells
- Sperm carrying chromatin abnormalities can fertilize an oocyte

Impact of Cancer Chemotherapy

- Actions of a single alkylating agent (cyclophosphamide) on sperm chromatin structure and consequences for progeny outcome
- Actions of combinations of agents used for treating testis cancer (BEP) (bleomycin, etoposide, cisplatin)
- Actions of combination of agents used for treating Hodgkin lymphoma (CHOP) (doxorubicin, cyclophosphamide, vincristine, prednisone) pact of the chemotherapeutic regimens for testis cancer and Hodgkin lymphoma on sperm parameters and progeny outcome.
Anticancer Agent
(Non)-Hodgkin Lymphoma
Lymphocytic Leukemia
Breast Cancer
Ovarian Cancer
Lung Cancer

Immunosuppressive Agent
Lupus Erythematosus
Wegener’s Granulomatosis
Graft-versus-Host Disease

• Spermatogenesis
• Assessing semen: quantity/quality issue
• Animal models to study effects of chemotherapeutic drugs

Effect of CPA on Spermatogenesis
Effect of Cyclophosphamide and Efferent Duct Ligation (7 days) on Pre- and Post-implantation loss

Chronic Paternal CPA affects Progeny

Effects of Paternal CPA on Skeletal Abnormalities in Offspring
Are the effects of paternal CPA exposure on progeny outcome heritable?

- Pre-implantation loss
- Post-implantation loss
- Malformations

Paternal CPA affects the F_{2} generation

Abnormal Progeny
Pre-implantation loss
Post-implantation Death
Aneuploidy
Altered template function
DNA breaks
Nuclear matrix composition changes
F₂ Fetuses after Paternal CPA Exposure

Chronic BEP Treatment of Male Rats

Drugs Used in the Management of Testis Cancer

- Bleomycin: causes DNA strand breaks
- Etoposide: inhibits topoisomerase II
- Cisplatin: alkylates DNA, causing cross-links
Chronic BEP Treatment Regimen

Doses: 0X, 1/3X, 2/3X and 1X; n=10/group
Human equivalent doses were determined for rats by converting for differences in surface area.
- Bleomycin: 1.5mg/kg/week (i.p.)
- Etoposide: 15mg/kg/day (gavage)
- Cisplatin: 3mg/kg/day (gavage)

Mate to control, naturally cycling, females

Effects of BEP on Male Reproductive Organ Weights

Bieber et al., J Androl 27: 189-200, 2006

Effects of BEP on Spermatid-Head Counts in the Testis

Bieber et al., J Androl 27: 189-200, 2006
Quantification of Morphological Abnormalities In Spermatozoa

Effects of BEP on Sperm Quality: TUNEL Assay

Analysis of progeny outcome
Numbers of pups per litter that survived past postnatal day 1

Chronic treatment with BEP results in:
- Decreased weights of the body, testes, and epididymides
- Abnormal testis histology
- Decreased spermatid head counts
- Significant effects on sperm motility, morphology, and quality
- No effects on pre- or post-implantation loss, litter size, or sex ratio
- Decreased post-natal survival

Progeny outcome after recovery
Regimen for Non-Hodgkin Lymphoma (NHL): CHOP

- Cyclophosphamide: alkylates DNA, causing cross links
- Doxorubicin: inhibits topoisomerase II, generates free radicals
- Vincristine: binds tubulin, depolymerizes microtubules
- Prednisone: glucocorticoid

Objectives

1) To evaluate the effects of treatment with CHOP on spermatogenesis, gamete genetic integrity, and progeny outcome in the rat.
2) To determine the effects of CHOP chemotherapy for NHL on sperm chromatin quality and the time course of recovery.
Experimental design

F x mg/kg = mg/m²
F for rats is 6
species surface area
to body weight ratio

Standard regimen for patients
Cyclophosphamide 750 mg/m²
Doxorubicin 50 mg/m²
Vincristine 1.4 mg/m²
Prednisone 40 mg/m²
5-6 cycles
6-8 cycles

Experimental regimen for rats
27 mg/kg
1.8 mg/kg
0.05 mg/kg
One injection i.c. every 3 weeks
One injection i.p. every 3 weeks
p.o., 5 days
p.o., 5 days
4 cycles

Effects of CHOP on Tissue Weights and Sperm Counts

Testis

Epididymis

Effects of CHOP on Testicular Histology
Effects of CHOP on Sperm Quality: SCSA

Effects of Paternal CHOP on Progeny Outcome

Effects of Paternal CHOP on Progeny Outcome
Reversal Study
Male-Mediated Developmental Toxicity

- The treatment regimens for both testis cancer (BEP) and non-Hodgkin lymphoma (CHOP) have adverse effects on male germ cells and progeny outcome in the rat model.
- Both BEP and CHOP affect multiple parameters of sperm chromatin quality and function. BEP, but not CHOP, affects spermatogonial stem cells.

Sperm Quality: Chromatin Biomarkers

- Sperm decondensation
- Breaks and cross-links and integrity of chromatin
- Chromatin template function
- Chromatin structure
- Chromatin epigenome

Delbes et al., Mol. Human Reprod. 2010
**Sperm Quality: Chromatin Biomarkers**

Delbes et al., Mol. Human Reprod. 2010

---

**Male mediated developmental toxicity: chemotherapy, sperm chromatin, psychosocial and progeny outcome**

Project 1: Impact of Combination Chemotherapy on Reproductive Health and Gamete Genetic Integrity in Humans

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---

**Chemo patients (N=30 for each cancer group)**

Clinical Psych Evaluations

Semen analyses

Anti-body

Hormone levels

Testicular biopsy

Ca Status

Sperm genetic integrity

---

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### Time Line

<table>
<thead>
<tr>
<th>Study Gps</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis Ca</td>
<td>Chemo</td>
<td></td>
</tr>
<tr>
<td>HL</td>
<td>Chemo</td>
<td></td>
</tr>
<tr>
<td>Control Gps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infertile Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Semen Parameters

#### Sperm concentration

- Normal forms (%)
  - Control: n=20
  - Infertile: n=21
  - Testicular cancer: n=12
  - Hodgkin lymphoma: n=11

#### Progressive motility

- PMNF index

- PMNF index

#### Normal forms (NF)

- Control: n=20
- Infertile: n=21
- Testicular cancer: n=12
- Hodgkin lymphoma: n=11

### Acridine Orange Assay

Sperm chromatin structure assay® (SCSA®)

- DFI
  - Control
  - Infertile
  - Testicular cancer
  - Hodgkin lymphoma
- Mean DFI
- SD DFI

O’Flaherty et al. Hum Reprod. 2008; 23:1044-1052
Sperm DNA strand breaks

TUNEL assay

Comet assay

Control | Infertile | Testicular cancer | Hodgkin lymphoma

n=20 n=17 n=11 n=9

n=9 n=16 n=12 n=10

Reactive thiol groups

mBBr assay

Control | Infertile | Testicular cancer | Hodgkin lymphoma

n=11 n=15 n=9 n=10

Level of Protamination

CMA3 assay

Control | Infertile | Testicular cancer | Hodgkin lymphoma

n=16 n=18 n=10 n=9

O’Flaherty et al. Hum Reprod. 2008; 23:1044-1052
CONCLUSIONS
* Spermatozoa from cancer or infertile patients have lower sperm chromatin quality than in the control group.
* SCSA®, TUNEL and comet assays similarly predict sperm chromatin quality in infertile patients.
* In cancer patients, sperm chromatin anomalies can be identified best using the comet assay.
* Routine semen parameters fail to predict sperm chromatin quality.

Comet assay (Tail extent moment)

Sperm chromatin structure assay® (SCSA®) %DFI
**CONCLUSIONS**

- Sperm generated post-chemotherapy maintain a significant degree of chromatin damage. Thus, survivors of TC and HL are at risk of having abnormal reproductive outcome.

- Proper counseling to these patients on reproductive risks and fertility preservation prior to chemotherapy is recommended.
Recreational drugs (smoking, alcohol and cannabis)

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Is male reproductive health under threat?

- decreasing sperm counts
- congenital abnormalities
- testicular cancer
- inefficient reproduction
- lifestyle hazards - in the environment
- recreational drugs (smoking, alcohol and cannabis) 

Are Lifestyle factors associated with semen quality?

Factors (evidence ±, ++, +++)
- Smoking (+++)
- Alcohol (++)
- Caffeine (+)
- Drugs (who me? ++)

Endpoints (evidence ±, ++, +++)
- Sperm conc (+++)
- Sperm motility (++)
- Sperm motility by CASA (+)
- Sperm morphology (++)
- Sperm motility and acrosome reactions (++)
- Sperm chromosome abnormalities (+)
Recreational drug number 1: cigarette smoking

- Impotence: 120,000 men/year
- ↓ Sperm counts
- ↓ Sperm motility
- ↓ Normal sperm morphology
- Mutations passed to children
- ↑ Childhood cancers with smoking fathers

The sheer scale of smoke damage to fertility is shocking!

British Medical Association 12 February 2004

Smoking & Spermatogenesis

Smoking has a small –ve impact

(Vine et al, 1994; Vine, 1996)

Mechanism: Hypoxia - high metabolic requirements

50% of arterial blood goes to arterio-venous anastomoses in spermatocord

(Maddocks et al 1993; Piner et al, 2002)

↓40% in sperm counts n sons by maternal smoking in utero

(Ramalau et al, 2007)

↓10-17% in sperm counts of heavy smokers in adults

No effects

Smoking & Semen Quality

- Is smoking an independent risk factor for poor semen quality or fertility?
- Impact may depend on both amount of exposure (cigarettes/day) and duration (pack years)
- Additive or synergistic Lifestyle factors may co-occur.
  - Smoking and drinking? (“Pub” lifestyle, Rubes et al., 1998)
  - Abuse of alcohol and drugs? Unhealthy lifestyles, poor nutrition.
  - Smoking and vitamin C (protective?)

Are fertile men less susceptible to smoking and other lifestyle exposures?

- The “Healthy Men Study” (HMS)
  - Partners of pregnant women in a pregnancy outcome study
  - Exposure of interest: Disinfection byproducts (DBPs) in drinking water
  - Men lived in community with low DBPs, or high chlorinated DBPs or high brominated DBPs.
  - Exposure carefully characterized
  - Semen: Count/conc., morphology, and DNA damage (%SCSA-%DFI) and immaturity (%SCSA-%HDS)
  - No differences were found based on DBP exposures (Luben et al, 2007), adjusting for other factors.

Analysis of Lifestyle Exposure factors in the ‘Healthy Men Study’ HMS

- **Smoking**: current, former or never: 0, 1-10, or >10 cigarettes/day, and years smoked (0, 1-5, 6-10 and >10). Pack years: /day /20 x years
- **Alcohol**: calculated based on average drinks [beers (12 oz), wine (4 oz) and hard liquor (1oz)] and categorized by # drinks/week: 0-7, 8-15 and >15
- **Caffeine**: Based on Coffee (and other caffeinated drinks), mg caffeine/day was calculated and categorized: none, >0 to 150 (low), >150-300 (moderate) and >300 (high = 3 cups coffee).

With kind permission from Lavelle K, Perreault, S, Olshan, A, 2010
Statistical Analysis in HMS

• Lifestyle exposure factors were examined (controlling for study site, age, income, education, abstinence interval, history of chronic or serious illness, body mass index (BMI), with other study exposures (smoking, alcohol, caffeine) as potential confounders.

• Multiple linear regression was used to estimate associations of each lifestyle exposure factor and each outcome. Full model (with all covariates) was evaluated for each covariate and only those that changed the parameter estimate of the exposure variable by at least 10% were retained. Age, sexual abstinence, income and study site were retained as obligate, along with any factor that met the criteria for confounding.

• Semen outcomes were also dichotomized when possible for logistic regression: percent normal forms at <15%; and, SCSA %DFI at >30% according to the literature.

With kind permission from Lavelle K, Perreault, S, Olshan, A, 2010

Conclusion

The HMS findings show that, on average, men in this fertile cohort have above average semen quality and below average consumption of cigarettes and alcohol

Recreational drug number 2: Alcohol

• ↓ testosterone
• ↓ impaired semen volume
• ↓ sperm concentration
• ↓ sperm motility
• ↓ normal sperm morphology
• but the good news- its reversible!

Donnelly, Lewis et al, Andrologia 1 43-47 1999
Muthusami et al, Fertility and Sterility 84 919-924 2005
Oliva et al, Rep Tox 22 565-605, 2006
Alcoholism associated
• with impotence and testicular atrophy
  Royden et al, Endocorhe Rev 1983
• Spermatogenesis decreases ∝ alcohol intake
• XY aneuploidy (RR=1.38, CI 1.2-1)
  Robbins et al, Cytogenet 2005
• Synergistic effects of alcohol and smoking
  Mendola, Agarwal et al, 2008

Most studies do NOT show a significant effect on
• sperm counts with moderate drinking
  (Morielli et al, 2004; Mordi et al, 2004)
in contrast in chronic alcoholics
• impaired spermatogenesis
• reduced sperm counts
• reduced testosterone levels
  (Villalta et al, 1997; Muthusamy and Chinnaswamy, 2005, reviewed by Sharpe, 2010)

Cigarette smoking is strongly associated with adverse reproductive outcomes
High exposure to alcohol, drugs and caffeine are only weakly linked with negative outcomes
Recreational drug number 3
Phosphodiesterase-5 inhibitors
such as sildenafil citrate and tadalafil

• between 1998-2005, 1 billion scripts
• for impotence
• for sexual enhancement
• in treatment of diabetes
• in infertility clinics

Phosphodiesterase-5 inhibitors
Sildenafil citrate

• Use with Serotonin for temporary ejaculation failure during ART
  Lu et al, FS 2009
• Adjunct tool for ↑ Leydig function and contractility of epididymis
  Dimitriadis et al, Curr Pharma Design 2009
• ↑ sperm motility and viability, opp effects at higher doses
  Pomara et al, FS 2007
• ↑ sperm motility and viability with Tadalafil
  Pomara et al, FS 2007
  Contradicted by Hellstrom et al, Eur Uro 2006
• No effects on volume, concentration, integrity or penetration.
• Variable effects on capacitation
  Methods Int J Reprod 2006
• ↑ fertilizing ability
  Dimitriadis et al, Asian J Androl 2008

Methods to determine the direct effects of Viagra on sperm function

➢ Conc of 450ng/mL ~ 100mg oral dose
  generously donated by Pfizer
➢ Quantitative motility 0-135 min using CASA
➢ Acrosome reaction by PNA-FITC

➢ in vitro (n=45)
The direct effect of Viagra on sperm numbers

![Graph showing the effect of Viagra on sperm numbers](image)

Sildenafil slightly improves sperm motility but causes a premature paracrine release of vasoactive substances in vivo (P<0.05).

The direct effects of Viagra on the quality of sperm motility

![Graph showing the effect of Viagra on sperm motility](image)

Sildenafil slightly improves sperm motility but causes a premature paracrine release of vasoactive substances in vivo (P<0.05).

Alterations in sperm motility after acute oral administration of sildenafil or tadalafil in young, infertile men

| Table 1: Survival parameters in basal conditions and after either sildenafil or tadalafil.
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival parameters</td>
</tr>
<tr>
<td>Volume (µL)</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Acidic reserve capacity (vac.)</td>
</tr>
<tr>
<td>Relative progression motility (%)</td>
</tr>
<tr>
<td>Total progressive motility [%]</td>
</tr>
<tr>
<td>Normal-form %</td>
</tr>
</tbody>
</table>

*Vasoactive substances can release vasoactive substances.

**Data from: Infertile male infertile semen parameters, 2009.
The direct effects of Viagra on the acrosome reaction

Burger, Heltstrom et al, 2000- no effects on any sperm parameters

Phosphodiesterase11 regulation of spermatozoa physiology

Wayman et al, Pfizer Global R and D, 2005

- Ejaculated sperm from PDE11-/-
  - reduced sperm conc, prog motility and viability
  - Pre-ejaculated sperm had premature capacitation
  - Data consistent with human data


- Dogs given Tadalafil for 6-12 months
  - alterations in seminiferous epithelium and subsequent effects on spermatogenesis

To determine effects of one dose of Viagra on embryo development

- Case study by Tur Kaspa et al, 1999
- Same action as Pentoxifylline??
- Animal model
- Effects on fertilization
- Effects on embryo cleavage rates
The effects of one dose Viagra on embryo yield

Glenn et al., 2004

The effects of Viagra on embryo numbers and stage at Day 2

Glenn et al., 2004

The effects of Viagra on embryo numbers and stage at Day 3

Glenn et al., 2004
The effects of Viagra on embryo numbers and stage at Day 4

![Graph showing embryo numbers and stage at Day 4](source)

Glenn et al., 2004

---

Developmental toxicity of orally administered sildenafil citrate (Viagra) in SWR/J mice

Abou- Tarboush et al., 2001

Sildenafil citrate to 285 pregnant mice at 1-50mg on 7-9, 10-12 or 13-15 days gestation

- No maternal toxicity
- No external, internal or skeletal malformations
- 40mg → fetal growth suppression at all times
- 25-40mg at 13-15 days → embryo-fetal toxicity

---

Recreational Drug No 4
(Cannabis - δ-9-tetrahydrocannabinol; THC)

![Recreational Drug No 4](source)
Cannabis and Male Fertility

- ↑ sexual behaviour in humans
- ↓ sexual behaviour in animals
- ↔ effects on spermatogenesis
- Highly variable effects on motility
- Increased chromatin condensation
- Damage to developing sperm

Cannabis and endocrine profiles - animal studies

- Suppression of LH and accumulation of THC in testis
  Ho et al, 1970
- Acute and chronic doses both decrease testosterone
  List et al, 1987; Harclerode et al, 1978
- No changes in FSH, no direct oestrogenic effect
  Ruh et al, 1987; Fernandez-Huete et al, 1987
- Reduced nucleic acid and protein synthesis
  Jakubovic et al 1979; Husain et al 1979

Potency of THC on the streets

The Observer 18 January 2004
Are endocannabinoids present in the male reproductive system?

Cannabinoid (CB1) receptors are present in:
- Testis
  - Gerard et al., 1991
- Vas deferens
  - Pertwee et al., 2002
- Epididymis
- Prostate
  - reviewed by Schuel et al., 2002
- Sea urchin sperm
  - Chang et al., 1993
- Human sperm
  - Schuel et al., 2002

The endocannabinoid system in sperm

Sperm have:
- CBR receptors
- Vanilloid (TRPV1) receptors
- AEA
- NAPE-PLD
- AMT
- FAAH

Bull sperm study by Maccarrone et al., 2005

Endocannabinoid Effects on Sertoli Cells

- Sertoli cells have CB1R and CB2R and can degrade AEA
- FAAH activity ↓ with Sertoli cell age
- AMT↓ with age but ↑ by NO donors
- ↑AEA can force Sertoli cells into apoptosis
- FSH activates FAAH via mRNA and PrS to prevent this

Maccarrone et al., 2003
Therapeutic and Recreational Concentrations of THC

- 0.001 µg/mL ~ therapeutic
- 0.01 µg/mL ~ recreational
- 1.5 µg/mL ~ recreational

Direct effects of THC on sperm motility

Progressive motility (%)
- in 90% layer

Progressive motility (%)
- in 45% layer

N=27

Whan et al, 2004

Direct effects of THC on sperm motility

Progressive velocity (um/s) - in 90% layer

Progressive motility (%) - in 45% layer

Whan et al, 2004
Direct effect of THC on the Acrosome Reaction

Acrosome reacted sperm in 90% layer (%) Acrosome reacted sperm in 45% layer (%)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.01</th>
<th>0.1</th>
<th>1.5 THC</th>
</tr>
</thead>
<tbody>
<tr>
<td>90% layer</td>
<td>0.0</td>
<td>2.5</td>
<td>5.0</td>
<td>7.5</td>
</tr>
<tr>
<td>45% layer</td>
<td>0.0</td>
<td>2.5</td>
<td>4.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*Whan et al., 2004*

Direct effect of THC on the Induced AR

Acrosome reacted sperm (%)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+A23187 2.5μM</th>
<th>+THC 1.5 μg/mL</th>
<th>+THC and A23187</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>15.0</td>
<td>10.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*Whan et al., 2004*

What are the mechanisms of action?

i) Reduced ATP by impaired mitochondrial function

- Potent inhibitors of mt O2/min/10^8 sperm
- mt membrane potential by JC-1 uncoupling of electron transport
What are the mechanisms of action?

- 2nd messenger systems- Ca\(^{2+}\) channels

- G protein mediated inhibition of Ca\(^{2+}\) channels
- Shrinkage of neurons
- DNA fragmentation in hippocampus

Conclusions

- Recreational drugs may impair male reproduction, either singly or together
- It is difficult to determine the impact of any one factor separate from other factors as men often use several recreational drugs together
- Very little is currently known about the mechanisms behind observed associations, how lifestyle factors may interact, and whether some men are inherently more vulnerable than others
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Good Sperm, Good Brain?  
The Connection Between Semen Quality & Intelligence

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Learning Objectives

- Understand why intelligence and semen quality may both be influenced by common genetic factors that influence overall survival fitness
- Understand the biologic commonalities of sperm and neuron function that may be influenced by pleiotropic mutations of those common genetic factors that affect overall fitness
Background/Hypothesis

• Survival adaptations
  – Low heritability, low phenotypic and genetic variation between individuals of a species

• Survival fitness ($f$)
  – The statistical propensity to survival and reproductive success, under ancestrally normal conditions
  – In courtship, fitness indicators have higher variation, and advertise highly heritable traits promising good genes and good health
  • These indicators are so costly that only high-fitness individuals can maintain them

During human evolution, mate choice by both sexes focused increasingly on intelligence as a major component of biological fitness – both for its heritable genetic benefits and its relevance to parenting ability. Many human-specific behaviors (such as conversation, music production, artistic ability, and humor) may have evolved principally to advertise intelligence during courtship.”

Miller GF, 2000
Background/Hypothesis

• If intelligence is a prominent component of survival fitness, is there a correlation between sperm quality and intelligence?

Subjects

• The Center for Disease Control Vietnam Experience Study
  — Multidimensional health assessment of American veterans of the Vietnam War
  — A random sample of enlisted men who joined the U.S. Army from 1965 to 1971, 7,024 Vietnam and 7,564 non-Vietnam veterans participated in a telephone interview.
  — A random subsample of 2,490 Vietnam and 1,972 non-Vietnam veterans also underwent a comprehensive health examination, including medical examination, laboratory tests, and a psychological evaluation.
  — A subset of 425 men submitted semen samples
  • Mean Age: 36
  • 355 white
  • 48 black
  • 16 Hispanic
  • 4 Asian/Pacific Islander
  • 2 Native American/Native Alaskan
  — Data published in the Journal of the American Medical Association in numerous publications circa 1988

Intelligence metrics (g)

• g = the general factor of mental ability
• Principal axis factoring of five tests to extract g
  — Verbal and Arithmetic tests
    • Army Classification Battery
  — Spatial awareness tests
    • Information and Block Design subtests of the Wechsler Adult Intelligence Scale – Revised
  — Reading comprehension
    • Subtest of the Wide Range Achievement Test
Semen quality metrics

- Sperm concentration (millions of sperm per ml of semen), log$_{10}$ transformed
- Sperm count (millions of sperm in the total ejaculate) log$_{10}$ transformed
- Sperm motility (percentage of motile sperm)

Results

- Significant but modest positive correlations between intelligence and 3 key indices of semen quality:
  - Log sperm concentration ($r=0.15$, $p=0.002$)
  - Log sperm count ($r=0.19$, $p<0.001$)
  - Sperm motility ($r=0.14$, $p=0.002$)
- Correlations controlled for:
  - Sexual abstinence (no. of days prior to sample)
  - Age
  - Body Mass Index
  - Drinking alcohol (drinks per month)
  - Smoking (cigarettes per day)
  - Drugs: marijuana or hard drugs (past & current use examined separately)
  - Service in Vietnam

Proof of Concept: Strikingly similar constellations of genes are involved in the regulation of sperm and neural function (Pierce et al., 2009)
SNAREs are concentrated at presynaptic terminals of neurons, as well as the acrosomal region of sperm (Kuo et al. 2002, Tomesani et al. 2005).

SNAREs also mediate hypothalamic release of gonadotropin releasing hormone, thyrotropin releasing hormone, and growth hormone.

Potential effect on other traits of fitness.

Other commonalities

- Glial cell line derived neurotrophic factor (GDNF) (Meng et al., 2000)
- Tight genetic regulation of polyunsaturated fatty acids of plasma membrane
  - Spatial compartmentalization of membrane microdomains of lipids and proteins (lipid rafts) coordinates the sequences of signal transduction required for spermatogenesis, maturation, capacitation, acrosomal reaction (AR) and ultimately fertilization (Lien et al., 2006).

More commonalities

- Sperm, retinal photoreceptor cells, and olfactory sensory neurons employ conserved cyclic nucleotide gated calcium ion channels
  - Many of these channels in sperm are T-type voltage-gated Ca2+ ion channels involved in AR regulation (Korman et al., 1998; Stamboulis et al., 2004).
More commonalities

- Other odorant gene family receptors are sperm-specific, directly regulating sperm motility and chemotaxis via activation of Ca\textsubscript{\textalpha 1H}2 (\textalpha 1H) Ca\textsuperscript{2+} ion channels (Spehr et al. 2003, Babcock 2003)
  - The CACNA1H gene encodes this ion channel, which is heavily expressed in the neocortex as well. Various mutations have been implicated in case studies of childhood absence seizures (Chen et al., 2003) and idiopathic generalized epilepsy (Heron et al., 2006)

Implications

- If most genes have pleiotropic effects on several traits (e.g. sperm and neuron function), then most mutations will harm several traits in parallel and create positive genetic correlations among traits, as manifest in an $f$ (fitness) factor (Arden, 2009)

The real question

- Is the $g$ factor a special case of a more general fitness factor $f$ that captures individual differences in general phenotypic quality? (Houle, 2000)
**f → g?**

- Numerous studies link g with better longevity, attractiveness, health, etc. (Batty et al., 2007; Bates, 2007)
- f, like g, is likely not traceable to single genetic loci with Mendelian inheritance
  - “The data imply that the genes causing the high heritability of IQ do not code for different levels of psychometric intelligence per-se but are pleiotropic—expressed in many systems, and acting on fitness in the same direction, positive or negative in all the systems in which they are expressed” (Bates, 2007)

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### Reference

- Bates TF, Saffren EH, Abdolrazaghi MT. Intelligence differences may mediate family and laterality risk. Psychological Science 2006; 17(12):1213-1216
- Chen HJ, Nieuwdorp M, Smith K. The CACNA1H-CEK2 genetic architecture links social and cognitive function and the male reproductive system to educational achievement in humans. Journal of Biomedical Science 2009; 16(1):1-9
- Chen HJ, Nieuwdorp M, Smith K. The CACNA1H-CEK2 genetic architecture links social and cognitive function and the male reproductive system to educational achievement in humans. Journal of Biomedical Science 2009; 16(1):1-9
Exercise: Fit Sperm???

1. Prior considerations
2. Physical Exercise vs. Physical Activity
3. Physical Exercise/Training Load Variables
4. Background: Exercise vs. reproductive system
5. Endocrine system and reproductive system
6. Recent research (Intensity, Volume, and Modality)
7. Exercise: bad sperm???
8. Take home message and challenges

Is Exercise Health???
Physical Exercise vs. Physical Activity

Both terms refer to the voluntary movements you do that burn calories (energy expenditure)

- Physical activities are activities that get your body moving.
- Exercise is a form of physical activity that is planned, structured and done to improve at least one aspect of physical fitness*** that is, strength, flexibility or aerobic endurance.

Physical Exercise/Training Load Variables

- Intensity:
- Volume:
- Frequency:
- Type of exercise:
  - Strength
  - Endurance

Background: Exercise vs. Reproductive System
Elite FEMALE Athletes

- Hormonal decrease
- Amenorrhea
- Delayed menarche
- Inadequate luteal phase
- Oligomenorrhea
- Anovulatory cycles

Shangold, 1984; Cumming et al., 1985; De Souza et al., 1991, 1994; Loucks, 2001

Endocrine system and Reproductive System

Morphological Sciences Department · University of Cordoba
Disrupting agents of endocrine and reproductive homeostasis

An extenuating physical exercise may provoke alterations on the reproductive system.
(Cumming et al., 1985; Shangold, 1984)

Adequate assessment of male reproductive potential

- Hormonal analysis
- Semen analysis
- Fertilizing capacity assessment

Semen Analysis

- Complete sexual abstinence: 3–6 days
- Questionnaire.
- Time between sample collection and delivery: under 30 minutes
- Physical parameters: volume*
- Microscopical qualitative parameters:
  - Sperm concentration and total sperm number*
  - Sperm Velocity (a, b, c, d)*
  - Sperm Morphology *
What is known…

• Hormonal Responses
  – Marathon
  – Cyclists
  – Swimmers
• Semen Response
  – Marathon
  – Cyclists
  – Swimmers
• VOLUME THRESHOLD (De Souza and Miller, 1993)

Recent research

Intensity
Volume
Modality

**Intensity**

Training parameter: 2 weeks of cycle ergometer exercise to exhaustion.

<table>
<thead>
<tr>
<th>Design</th>
<th>AP</th>
<th>HA</th>
<th>PE</th>
<th>SA</th>
<th>1st week</th>
<th>2nd week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HA</td>
<td></td>
<td>SA</td>
<td>Pre-Tests</td>
<td>Post-Tests</td>
</tr>
<tr>
<td>Training</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Hormonal and semen analysis**

**Testosterone and Cortisol**

**FSH and LH**

*Los valores en Azul representan los vatios.*

Vaamonde et al., 2006.
Sperm Concentration, ejaculate volume, total sperm number

Type “a” Velocity

Vaamonde et al., 2006.
**Type “d” Velocity**

![Graph showing Type “d” Velocity over time.](image)

Vaamonde et al., 2006.

**Sperm Morphology**

![Graph showing sperm morphology over time.](image)

Vaamonde et al., 2006.

**Modalities**

![Image of modalities.](image)
Response of semen parameters to three training modalities.

Modality

• Exclusion criteria
  - Any condition possibly impairing reproduction

• Inclusion criteria
  - Not exclusion criteria,
  - Minimum practice of 3 hours/week
  - VO2max ≥ 40 ml/min/kg.

45 Subjects

Physically active 16
Waterpolo 14
Triathlon 15

Training volume analysis
Semen analysis
Physically Active Water Polo Triathletes

Subjects

| Age (years) | 19.0 ± 1.8 | 25.5 ± 3.2 | 33.1 ± 3.5 |
| Weight (kg) | 73.1 ± 8.3 | 79.9 ± 10.7 | 74.5 ± 7.6 |
| Height (cm)  | 175.9 ± 4.2 | 180.1 ± 5.2 | 175.3 ± 3.7 |
| Body fat (%) | 15.6 ± 3.0 | 13.2 ± 3.5 | 7.0 ± 2.9 |
| VO2max (mL/min/kg) | 45.2 ± 4.2 | 54.2 ± 4.9 | 64.0 ± 5.1 |

| Years of training | 1.8 ± 0.7 | 4.0 ± 1.1 | 8.1 ± 3.2 |
| Number of sessions/week | 3.3 ± 0.4 | 5.0 ± 0.0 | 9.9 ± 1.8 |
| Duration of session (min) | 60.0 ± 0.0 | 90.0 ± 0.0 | 122.6 ± 32.3 |

| Sports category | Local | Regional | International |

*a* Significant differences (p< 0.05) compared to physically active subjects

*b* Significant differences (p< 0.05) compared to water polo players

*c* Significant differences (p< 0.05) compared to triathletes

# Mean of all sessions (cycling, swimming, running)

---

<table>
<thead>
<tr>
<th>Methods</th>
<th>Modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>Physically Active</td>
</tr>
<tr>
<td>Age (years)</td>
<td>14</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.1 ± 8.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.9 ± 4.2</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>15.6 ± 3.0</td>
</tr>
<tr>
<td>VO2max (mL/min/kg)</td>
<td>45.2 ± 4.2</td>
</tr>
<tr>
<td>Years of training</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>Number of sessions/week</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Duration of session (min)</td>
<td>60.0 ± 0.0</td>
</tr>
</tbody>
</table>

*a* Significant differences (p< 0.05) compared to physically active subjects

*b* Significant differences (p< 0.05) compared to water polo players

*c* Significant differences (p< 0.05) compared to triathletes

ANOVA with repeated measures and Sidak post hoc test for multiple comparisons

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<table>
<thead>
<tr>
<th>Results</th>
<th>Modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>Sperm concentration (10^6/mL)</td>
<td>61.8 ± 23.0</td>
</tr>
<tr>
<td>Total sperm number (10^6)</td>
<td>191.6 ± 73.4</td>
</tr>
<tr>
<td>% Type &quot;a&quot; Vel.</td>
<td>31.1 ± 9.7</td>
</tr>
<tr>
<td>% Type &quot;b&quot; Vel.</td>
<td>25.6 ± 9.1</td>
</tr>
<tr>
<td>% Type &quot;a+b&quot; Vel.</td>
<td>56.7 ± 6.5</td>
</tr>
<tr>
<td>% Type &quot;c&quot; Vel.</td>
<td>16.4 ± 5.0</td>
</tr>
<tr>
<td>% Type &quot;d&quot; Vel.</td>
<td>33.0 ± 7.1</td>
</tr>
</tbody>
</table>

*a* Significant differences (p< 0.05) compared to physically active subjects

*b* Significant differences (p< 0.05) compared to water polo players

*c* Significant differences (p< 0.05) compared to triathletes

ANOVA and Sidak post hoc test for multiple comparisons

---

<table>
<thead>
<tr>
<th>Results</th>
<th>Modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>Sperm concentration (10^6/mL)</td>
<td>61.8 ± 23.0</td>
</tr>
<tr>
<td>Total sperm number (10^6)</td>
<td>191.6 ± 73.4</td>
</tr>
<tr>
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*a* Significant differences (p< 0.05) compared to physically active subjects

*b* Significant differences (p< 0.05) compared to water polo players

*c* Significant differences (p< 0.05) compared to triathletes

ANOVA with repeated measures and Sidak post hoc test for multiple comparisons

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Correlation between cycling kilometers and sperm morphology in elite triathletes

Vaamonde D¹, Da Silva-Grigoletto ME², Cunha Filho JS³, Garcia-Manso JM⁴, Suarez Serra R⁵.

¹School of Medicine- Universidad de Córdoba, Spain
²Andalusian Center of Sports Medicine – Junta de Andalucía, Córdoba, Spain
³Insemine Centro de Reprodução Humana and Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil
⁴School of Physical Education- Universidad de Las Palmas de Gran Canaria, Spain
⁵Centro Iberoamericano de Reproduccion Asistida, Uruguay

Presented at ESHRE 2009

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Fifteen male triathletes.

<table>
<thead>
<tr>
<th>Subjects' demographics</th>
<th>Value (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.1 ± 3.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.5 ± 7.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.3 ± 3.7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>7.8 ± 2.9</td>
</tr>
<tr>
<td>VO2max (ml/min/kg)</td>
<td>64.0 ± 5.1</td>
</tr>
<tr>
<td>Years of training</td>
<td>8.1 ± 3.2</td>
</tr>
<tr>
<td>Number of sessions/week</td>
<td>99 ± 1.8</td>
</tr>
<tr>
<td>Duration of session (min)</td>
<td>122.6 ± 62.7</td>
</tr>
</tbody>
</table>

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Training Volume

Methods

Training was carefully analyzed, especially with regards to weekly volume expressed as total volume or volume in each modality.

Results

\[ y = -17.76x + 405.4 \]

\[ R^2 = 0.498 \]

\[ p < 0.05 \]
Training Volume

A high cycling volume, especially over 300km/week, is detrimental to sperm morphology.

Training Volume, Intensity and Modality

High-load physical exercise, whether intensity or volume may alter male reproductive function

- De Souza et al. (1994,1997), Hackney (1996); Vaamonde et al., 2009: volume
- Vaamonde et al., 2006: intensity

Triathletes show worse semen parameters than physically active subjects or water polo players.

- Vaamonde et al., 2009: modality

Exercise, Bad Sperm ???

The right amount of exercise is good...
PHYSICALLY ACTIVE SUBJECTS SHOW BETTER SEMINOLOGICAL PARAMETERS THAN SEDENTARY SUBJECTS

METHODS · Criteria

• Exclusion criteria

• Inclusion criteria
  – Not exclusion criteria
  – Minimum practice of 2-4 hs/week
  – VO₂max ≥ 40 ml/min/kg
  – Not practicing any physical activity
  – VO₂max < 40 ml/min/kg

METHODS · Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sedentary</th>
<th>Physically Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>19.0 ± 1.8</td>
<td>19.2 ± 1.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.1 ± 8.3</td>
<td>73.8 ± 9.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.9 ± 4.2</td>
<td>176.1 ± 5.2</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>15.6 ± 3.0</td>
<td>13.2 ± 3.5</td>
</tr>
<tr>
<td>VO₂max (ml/min/kg)</td>
<td>36.9 ± 3.2</td>
<td>51.1 ± 4.9</td>
</tr>
</tbody>
</table>

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RESULTS

<table>
<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th>Physically Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Type “a+b” Velocity</td>
<td>56.7 ± 4.5</td>
<td>60.94 ± 5.83</td>
</tr>
<tr>
<td>% normal forms (morphology)</td>
<td>14.40 ± 1.15</td>
<td>15.54 ± 1.38</td>
</tr>
</tbody>
</table>

Unpaired student T-test
Values are Mean and Standard Deviation

CONCLUSION

Moderate exercise seems to benefit endocrine and sperm production processes with regards to sedentary subjects

- Regular endurance exercise
  - Catabolic and stress-related hormones (Habib et al. 1987b; Rivier & Rivier 1991, Susah et al. 1988)
  - Anabolic hormones
- Moderate exercise favors a more anabolic state

What’s happening?

High-load physical exercise, seems to interfere with endocrine and spermatogenic processes

Manna et al. (2004): exercise alters sperm cell lineages and antioxidant enzymes. Antioxidant administration
Training characteristics
- Modality
- Volume
- Intensity
- Frequency

Athlete's history
- Age
- Years of practice
- Reproductive system disorders
- Others...

Mechanical stress, oxidative stress, genetics, energetic imbalance

Endocrine profile
- Anabolic/Catabolic State (T/C)
- Gonadotrophins State (FSH and LH)

Seminological profile
- Quantitative parameters (semen volume, sperm number)
- Qualitative parameters (velocity and morphology)

TAKE HOME MESSAGE AND CHALLENGES
- Moderate exercise seems to improve hormonal milieu and semen
- High-load exercise (volume/intensity) may have adverse effects
  - Key mechanism
  - How to palliate
    - Antioxidants
    - Training modification

Bibliography
Bibliography


Many thanks...

fivresearch@yahoo.com
Mark your calendar for the upcoming ESHRE campus workshops!

- Early pregnancy disorders: integrating clinical, immunological and epidemiological aspects  
  23-26 August 2011 - Copenhagen, Denmark

- The management of infertility – training workshop for junior doctors, paramedics and embryologists  
  7-8 September 2011 - St. Petersburg, Russia

- Basic genetics for ART practitioners  
  9 September 2011 - Bucharest, Romania

- The whole man  
  22-23 September 2011 - Sevilla, Spain

- Accreditation of a Preimplantation Genetic Diagnosis Laboratory  
  3-4 October 2011 - Athens, Greece

- Human reproductive tissues, gametes and embryos: Innovations by science-driven culture and preservation systems  
  9 October 2011 - Cairns, Australia

- Comprehensive preimplantation screening: dynamics and ethics  
  13-14 October 2011 - Maastricht, The Netherlands

- Endometriosis and IVF  
  28-29 October 2011 - Rome, Italy

- Endoscopy in reproductive medicine  
  23-25 November 2011 - Leuven, Belgium

- What you always wanted to know about polycystic ovary syndrome  
  8-10 December 2011 - Sofia, Bulgaria

www.eshre.eu  
(see “Calendar”)  

Contact us at info@eshre.eu