“Evaluation of the man in the infertile couple”
SPECIAL INTEREST GROUP ANDROLOGY

28 June 2009
Amsterdam
The Netherlands
**PRE-CONGRESS COURSE 8**

Organised by the Special Interest Group Andrology

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Evaluation of the man in the infertile couple

Organised by the Special Interest Group Andrology

Course co-ordinators: Lars Björndahl (Sweden) and Roelof Menkveld (South Africa)

Course description: A critical update of the investigation and evaluation of the man in the infertile couple

Target audience: Clinicians working with investigations of infertile couples, but also other professionals involved in the evaluation and treatment of the infertile couple. The aim of the course is to give a broad base for better understanding and treatment of male factors in subfertility.

08:45 - 09:00  Introduction - Isn’t semen analysis enough? - Lars Björndahl (Sweden)

09:00 - 09:30  Practicalities, client groups and utilisation of sperm cryopreservation - Mathew Tomlinson (United Kingdom)

09:30 - 09:45  Discussion

09:45 - 10:15  What is the risk for hypogonadism and testicular cancer among infertile men? - Aleksander Giwercman (Sweden)

10:15 - 10:30  Discussion

10:30 - 11:00  Coffee break

11:00 - 11:30  Erectile dysfunction among infertile men - does it exist? - Jose M. Pomerol (Spain)

11:30 - 11:45  Discussion
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:45 - 12:15</td>
<td>Ejaculatory dysfunction. What can go wrong? How to treat? – Wallace Dinsmore (United Kingdom)</td>
</tr>
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<td>12:15 - 12:30</td>
<td>Discussion</td>
</tr>
<tr>
<td>12:30 - 13:30</td>
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<td>13:30 - 14:00</td>
<td>Male accessory gland infection. Diagnosis and treatment - Gerhard Dohle (The Netherlands)</td>
</tr>
<tr>
<td>14:00 - 14:15</td>
<td>Discussion</td>
</tr>
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<td>14:15 - 14:45</td>
<td>Late Onset Hypogonadism. Who should be investigated and treated for early and late onset hypogonadism? - Eric Meuleman (The Netherlands)</td>
</tr>
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<td>Discussion</td>
</tr>
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<td>Coffee break</td>
</tr>
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<td>What does poor sperm DNA quality mean? A critical review of methods, interpretation and clinical value - Ulrik Kvist (Sweden)</td>
</tr>
<tr>
<td>16:00 - 16:15</td>
<td>Discussion</td>
</tr>
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<td>16:15 - 16:45</td>
<td>How much assistance does a man need? ART for male factors – David Mortimer (Canada)</td>
</tr>
<tr>
<td>16:45 - 17:00</td>
<td>Discussion</td>
</tr>
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<td>17:00 - 17:30</td>
<td>Conclusions - how to give best help to the man - All speakers</td>
</tr>
<tr>
<td>17:30 - 18:30</td>
<td>Business Meeting -Special Interest Group in Andrology</td>
</tr>
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Course 8
Evaluation of the Man in the Infertile Couple
Organised by
the Special Interest Group in Andrology
SIGA

Special Interest Group in Andrology
• Standardization and quality improvement of laboratory investigations of the man
  – Methods, training, quality control

• Training in clinical andrology
  – To be aware of causes for disorders
  – To be prepared for exchange with clinical andrologists

Andrology Activities at ESHRE 2009
• SIGA Business meeting
  – Today, in this room at 17:30-18:30

• SIGA Basic Semen Analysis EQAP
  – Users’ Meeting
    • Tuesday June 30, at 15:00 in Room R+S

• Main Programme
  – Monday 11:45 - 12:45
    • Small RNAs in the male germline:
      René Ketting (The Netherlands)
Isn’t semen analysis enough?

Lars Björndahl, M.D. Ph.D.
Centre for Andrology and Sexual Medicine
Karolinska University Hospital, Huddinge
Stockholm, Sweden

Conflicts of Interests
Lars Björndahl
• I have no commercial relationships or other activities that might be perceived as a potential conflict of interest

Pre Congress Course 2009
• Evaluation of the Man in the Infertile Couple
  – Sperm cryopreservation
    • patients, efficacy, and safety
      Dr Mathew Tomlinson (PhD)
      Nottingham University Hospital, Nottingham, UK
  – Hypogonadism and testicular cancer among infertile men
    Aleksander Giwercman, MD, PhD,
    Malmö University Hospital, Lund University, Sweden
Pre Congress Course 2009

• **Evaluation of the Man in the Infertile Couple**
  – Erectile dysfunction among infertile men
    José Mª Pomerol, MD
    Instituto Valenciano de Infertilidad (IVI)
    Instituto de Andrología y Medicina Sexual
    Barcelona, Spain
  – What can go wrong with ejaculation?
    Professor Wallace Dinsmore
    University Of Ulster
    Northern Ireland

Pre Congress Course 2009

• **Evaluation of the Man in the Infertile Couple**
  Diagnosis and treatment of urogenital tract infections to improve treatment results in ART?
    Gert Dohle, MD, Ph.D
    Erasmus MC, Rotterdam, The Netherlands
  – Late Onset Hypogonadism
    • who should really be treated?
    Prof Dr Eric JH Meuleman
    Urologist, Free University Medical Centre,
    Amsterdam, The Netherlands

Pre Congress Course 2009

• **Evaluation of the Man in the Infertile Couple**
  – What does poor sperm DNA quality mean?
    • methods, interpretation and clinical value
    Ulrik Kvist, M.D. Ph.D.
    Karolinska University Hospital, Huddinge
    Stockholm, Sweden
  – How much assistance does a man need?
    • ART for male factors
    Dr David Mortimer, PhD
    Ootoa Biomedical Inc, Vancouver, BC, Canada
Pre Congress Course 2009

• Evaluation of the Man in the Infertile Couple
  – How to give best help to the man
  • Panel discussion

Reference

Sperm cryopreservation: Practicalities, client groups and utilisation

Dr Mathew Tomlinson (PhD)
Nottingham University Hospital, Nottingham, UK
www.nuh.nhs.uk/andrology

Disclosures

- The author has no commercial or financial interest in any of the laboratory products, materials or equipment cited in this presentation

Objectives

- Requirement for cryopreservation
- Reasons for referral
- Process of referral and consent
- Obtaining a specimen
- Processing and storage
- Use in Assisted Reproduction
- Risk Analysis
Introduction

Cryopreservation - what for?

- Sperm storage for fertility preservation
- Tissue preservation (Ovarian/Testicular)
- Sperm Donation
- Assisted Reproduction

Why cryopreserve?

- **Fertility Preservation**  
  sterilising (potentially) treatments  
  - Surgery  
  - Chemotherapy  
  - Radiotherapy

- **Prior to assisted conception**  
  - Absent partners  
  - Anxiety related anejaculation  
  - Elective surgical retrieval

- **Quarantine**  
  During donation e.g. sperm donation

Referrals for cryopreservation
### Fertility Preservation

#### Surgery
- Transgender realignment
- Vasectomy / Vasovasostomy
- Urinary Tract e.g. Bladder Neck
- Cancer Surgery e.g. lymph node dissection (RPLND)

#### Chemotherapy

<table>
<thead>
<tr>
<th>Malignant Disease</th>
<th>Non-malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>Autoimmune diseases</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Nephritis/Nephrotic syndrome</td>
</tr>
<tr>
<td>Germ cell</td>
<td>SLE</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Rheumatoid Arthritis</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>Multiple sclerosis</td>
</tr>
</tbody>
</table>

### Agents (cumulative dose for effect)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorambucil (1.4 g/M²)</td>
<td>Prolonged azoospermia</td>
</tr>
<tr>
<td>Cyclophosphamide (19 g/M²)</td>
<td>Likely to cause prolonged azoospermia, but given with other highly sterilizing agents</td>
</tr>
<tr>
<td>Procarbazine (4 g/M²)</td>
<td>Likely to cause prolonged azoospermia, but given with other highly sterilizing agents</td>
</tr>
<tr>
<td>Melphalan (140 mg/M²)</td>
<td>Prolonged azoospermia</td>
</tr>
<tr>
<td>Cisplatin (500 mg/M²)</td>
<td>Likely to cause prolonged azoospermia, but given with other highly sterilizing agents</td>
</tr>
<tr>
<td>BCNU (1 g/M²), CCNU (500 mg/M²)</td>
<td>Prolonged azoospermia</td>
</tr>
<tr>
<td>Busulfan (600 mg/M²)</td>
<td>Likely to cause prolonged azoospermia, but given with other highly sterilizing agents</td>
</tr>
<tr>
<td>Ifosfamide (42 g/M²)</td>
<td>Likely to cause prolonged azoospermia, but given with other highly sterilizing agents</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>Causes only temporary reductions in sperm count alone</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>Causes only temporary reductions in sperm count alone</td>
</tr>
<tr>
<td>Adriamycin (770 mg/M²)</td>
<td>Causes only temporary reductions in sperm count alone</td>
</tr>
<tr>
<td>Thiotepa (400 mg/M²)</td>
<td>Causes only temporary reductions in sperm count alone</td>
</tr>
<tr>
<td>Cytosine arabinoside (1 g/M²)</td>
<td>Causes only temporary reductions in sperm count alone</td>
</tr>
<tr>
<td>Vinblastine (50 mg/M²)</td>
<td>Causes only temporary reductions in sperm count alone</td>
</tr>
</tbody>
</table>

## Testicular Radiation Dose and Effect on Sperm Count

<table>
<thead>
<tr>
<th>Radiation Dose</th>
<th>Effect on Sperm Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.15 Gy</td>
<td>None detectable</td>
</tr>
<tr>
<td>0.15 - 0.6 Gy</td>
<td>Transient oligospermia</td>
</tr>
<tr>
<td>0.6 - 2 Gy</td>
<td>Azoospermia (usually reversible)</td>
</tr>
<tr>
<td>&gt;2.5 Gy</td>
<td>Azoospermia (generally permanent)</td>
</tr>
<tr>
<td>8 Gy</td>
<td>Permanent azoospermia in 85% of men</td>
</tr>
</tbody>
</table>

*Given as fractionated radiation over 4 weeks
*Given as a single dose or in up to 6 fractions

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## Cryopreservation for ART

- No specimen available - IUI, IVF, ICSI
  - Working schedule
  - Performance anxiety
  - Retrograde ejaculation
- Increasing fertility impairment
  - Successive semen analysis
  - Endocrinology
  - Obstruction e.g. vasovasostomy
- Sperm donation - quarantine
  - Anonymous/known donation
  - Surrogacy arrangements

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## Informed Consent - Patient Storage

- The appointment system
- Semen analysis/freezing process
- What tests are needed prior to storage (HIV/Hep B/C
- Duration of Storage
- Consent
- Use of sperm in the future
- Fate of sperm in the event of death or mental incapacitation
- Counselling (offered)
- Contraception
- Repeat sperm tests
- Where is the storage centre?

Adolescents - separate specific information and in understandable language

---

Adolescents
Assessment of maturity and capacity

Mental Capacity

Gillick Competence – UK, in Australia, Canada and New Zealand.
- Can absorb and understand the information related to consent
- Can use this information to consider whether to consent or not
- Is able to communicate their wishes

UK DH guidance
- Families should be involved (where possible)
- Free from coercion
- Right to confidentiality must be respected

Adolescents
Assessment of maturity and capacity

Maturity
Tanner staging or (Tanner scale) - physical development

- Tanner I
  - Prepubertal, undeveloped genitalia, no pubic hair
  - Typical age <9 and younger

- Tanner II
  - Testicular volume (TV) increases up to 6ml, small amount of long, downy hair with slight pigmentation at the base of the penis (typical age 9 to 11)

- Tanner III
  - TV 6-12 ml; scrotum enlarges; penis lengthens to about 6 cm; pubic hair becomes coarser/curl, begins to extend inferriorly (typical age 11.5-13)

- Tanner IV
  - TV 12-20 ml; scrotum enlarges further and darkens; penis to 10 cm; adult-like hair, extends across pubis (typical age 12.5-14)

- Tanner V
  - TV 20 ml; adult scrotum and penis of 15 cm in length; hair extends to medial surface of the thighs (typical age 14+)

Sperm Storage - Consent

Specify
- Storage period
- Fate of the sperm
  - Death
  - Mental incapacity

Man with partner
- Consent to treatment
Sperm Storage - Consent

Specify

- Partner identity
- Treatment
  - Partner
  - Creating embryos
  - Use of embryos
  - Storage of embryos
  - Fate of embryos in death or mental incapacity
  - Treatment of others
  - Use of sperm or embryos in research projects
  - Posthumous birth registration

Semen Sample Production

- Sterile container
- Conducive producing room
- Adult literature/editing/clean
- Movies??

Problems
- Pain on Production
- Illness
- Anxiety (clinical area)
- Children/Young adults (literature)
- Cancer patients - relatively high failure rate

Alternatives
- Vibrostimulation
- Electro-ejaculation*
- Testicular Biopsy*
- Carry risk/Require anaesthetic*
**Processing - Semen Analysis**

Validated methods
- Sterile Analysis (storage)
- Phase contrast microscopy
- Heated stage (37°C) for motility analysis
- Haemocytometer for concentration
- Morphology on stained smear x100 magnification
- Remove aliquot for analysis
- Process one patient at a time

**Processing Cryoprotectants**

Addition of 6-7% Glycerol
- Removes water - prevents ice
- Dilutes intracellular toxic solutes

Rate of addition - controversial
- osmotic shock - glycerol toxicity
- small volumes, added gradually larger volumes
- 10 minutes maximum
- 4 step addition over 4 minutes (Gao et al, 1995)
- Rapid addition of 'cool' cryoprotectant (Clarke et al, 2004)

**Semen Processing - Can we do better?**

Embryo:Sperm survival >80% : <50%*  
*End point measurement - variable  
Tendency to over-estimate motility  
Incentive to improve sperm freezing

Gradient Preparation Prior to freezing

<table>
<thead>
<tr>
<th>Number of sperm (millions)</th>
<th>Number of motile sperm in the neat ejaculate</th>
<th>Number of motile sperm after gradient</th>
<th>Number of motile sperm after freezing, thawing and wash</th>
<th>Number of motile sperm after freezing, thawing, gradient and wash.</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.7</td>
<td></td>
<td>12.3</td>
<td>11.6</td>
<td>8.5</td>
</tr>
<tr>
<td>21.7</td>
<td></td>
<td>3.6</td>
<td>2.5</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>
Semen Processing – Can we do better?
Sperm Preparation prior to freezing

- Provides clean (lower risk sample)
- Improves quality of individual treatment units
- Reduces operator time post thaw

Table 1. Mean donor sperm yields from 5 donors (10 specimens) prepared using Puresperm™ prior to cryopreservation

<table>
<thead>
<tr>
<th>Donor</th>
<th>Pre Freeze</th>
<th>Post Prepared</th>
<th>Post Thaw</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.16</td>
<td>3.2</td>
<td>14.15</td>
<td>45.05</td>
</tr>
<tr>
<td>2</td>
<td>6.9</td>
<td>5.0</td>
<td>14.87</td>
<td>22.9</td>
</tr>
<tr>
<td>3</td>
<td>4.29</td>
<td>3.3</td>
<td>13.0</td>
<td>33.44</td>
</tr>
<tr>
<td>4</td>
<td>3.31</td>
<td>2.8</td>
<td>8.8</td>
<td>25.8</td>
</tr>
<tr>
<td>5</td>
<td>2.57</td>
<td>3.9</td>
<td>11.2</td>
<td>43.6</td>
</tr>
</tbody>
</table>

Table 1. Yields from 5 donors prepared using Puresperm density gradients prior to freezing.

Packaging - for long term storage

Safe, sterile and suitable container in LN2
Used for a single ART treatment
Permit uniform cooling of sample
Easily filled and sealed
Label clearly/easily
Batch traceable
Robust and impermeable at -196°C

Permit uniform cooling/warming
Best sample survival
- Optimum heat transfer
- Cooling rate reflected inside package
- Rapid warming during thaw
- Vials
  - Large volume:surface area
  - Large lag behind theoretical cooling rate
  - One advantage slow thaw when auditing

Mortimer, RBM online (2004)
### Requirements of sperm packaging

<table>
<thead>
<tr>
<th>Requirement</th>
<th>PTEG</th>
<th>VIALS</th>
<th>CBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safe, sterile and suitable container in LN2</td>
<td>✔</td>
<td>✔</td>
<td>✔/✔</td>
</tr>
<tr>
<td>Used for a single ART treatment</td>
<td>✔/✔</td>
<td>✔/✔</td>
<td>✔/✔</td>
</tr>
<tr>
<td>Permit uniform cooling of sample</td>
<td>✔/✔</td>
<td>✔/✔</td>
<td>✔/✔</td>
</tr>
<tr>
<td>Easily filled and sealed</td>
<td>✔/✔</td>
<td>✔/✔</td>
<td>✔/✔</td>
</tr>
<tr>
<td>Label clearly/easily</td>
<td>✔/✔</td>
<td>✔/✔</td>
<td>✔/✔</td>
</tr>
<tr>
<td>Batch Traceable</td>
<td>✔/✔</td>
<td>✔/✔</td>
<td>✔/✔</td>
</tr>
<tr>
<td>Robust and impermeable at -196°C</td>
<td>✔/✔</td>
<td>✔/✔</td>
<td>✔/✔</td>
</tr>
</tbody>
</table>

### Sperm Cooling

- Optimum -10°C/minute (Mazur, 1962)
- Liquid nitrogen vapour
- Static vapour cooling
- Controlled rate freezer

### Sperm Cooling

**controlled rate freezing**

- Verifiable (validated)
- Cooling rate
- Repeatable
- Quality Assurance

- Blown vapourised nitrogen (Planar)
- Chamber immersed in LN2 (Cryologic)
- Nitrogen free (Stirling engine)
**Sperm Cooling - static vapour methods**

- Numerous methods published – 5,10,15, 25cm from N2 surface.
- Single height/or several positions
- Uncontrolled suspension of sperm
- May be historic/inherited
- May have no validation

- **BUT**
- **Cheap!**
- **Can work!!**

---

**Controlled rate vs vapour cooling**

Post-thaw motile concentration for each sample in both methods a standardised (Plano) cooling protocol

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Post-thaw motile concentration</th>
<th>CRF</th>
<th>VF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td></td>
<td></td>
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<tr>
<td>30</td>
<td>60</td>
<td></td>
<td></td>
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<tr>
<td>40</td>
<td>60</td>
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<tr>
<td>50</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CRF 12.3 vs VF 10.5 NS

---

**Sperm Storage**

- Long term (40 years) storage requires temperatures <-137°C.
- Below the ‘glassy transformation’ temperature of water (136 Kelvin)
- Above this temperature crystalline structure of ice exists

- Liquid nitrogen -196°C
- Nitrogen vapour -145-192°C
- -140°C mechanical freezer
Sperm Storage - Liquid storage

Dewars

- Disadvantages
  - Health and Safety
  - Biocontainment
  - Take up floor space
  - Individually alarmed

- Advantage
  - stable -196°C
  - ok for small banks
  - Use little nitrogen
  - Relatively maintenance free

Sperm Storage - Vapour storage

- Disadvantages
  - High cost
  - Increase nitrogen consumption
  - Increased monitoring
  - Temperature gradients?

- Advantages
  - Automated filling systems
  - Safer for operator
  - Safer for samples?
  - Integrated alarms

Sperm Storage - Racking

- What does it need to do?
  - Keep samples safe - long term
  - Be easily accessed (without damaging samples or operator)
  - Conduct (vapour)
Sperm Thawing and Preparation

Sperm Thawing
Thaw rate

- Even and rapid thaw (37°C).
- Straws being handled warm above - storage requires temperatures <-135°C within 5-6 seconds (audit)
- Cryovials more slowly and more uneven
- Addition of warm wash buffer - stepwise to prevent osmotic shock

AFFECTED BY
1. Packaging
2. Diluent
3. Thaw environment

Risk
- Injury to personnel
- Loss of stored material
- Damage to stored material
- Misidentification of material

 área of service
- Process
- Procedure
- Area or room

Causative factors
- Natural events (Floods/Fire/terrorism)
- Technical/training
- Professional liability/Human error
- Infection Control
- Staffing Issues
- Facilities (nitrogen transport and storage)
- Product and equipment liability
- Security (specimens, facilities, data)
- Resources

Treatment using stored sperm

simplest least expensive
IUI (10-20%)
IVF (20-30%)
ICSI (30-40%)
Highly technical/most expensive

- May be the only chance of conception
- Careful balance between available straws/vials and post thaw quality
- Many opt for the Rx which gives the best chance of conception i.e. ICSI
Safety of treatment

- Persistently raised aneuploidy levels 24 months post chemotherapy (Tempest et al., 2008)
- Natural conception should be avoided
- Genetic counselling
- Risk analysis - includes: relative safety of own samples, relative quality of sperm (fresh v frozen), age of partner

Summary

- Sperm cryopreservation: fertility preservation, ART and donation
- Regulation, informed consent
- Special consideration for adolescents
- Validated methods for semen analysis, processing and cooling
- Store long term in liquid nitrogen or vapour
- Risk analysis with regard to sample safety/quality, regulation, staff safety is essential
- Use of sperm in ART needs to balance, quality of sample post thaw, quantity stored and fertility of partner

Bibliography

What is the risk for hypogonadism and testicular cancer among infertile men?

ESHRE 2009 Precongress Course
"Evaluation of the Man in the Infertile Couple"

Aleksander Giwercman, MD, PhD, Chairman
Reproductive Medicine Centre, Malmö University Hospital
Lund University, Malmö, Sweden

Conflict of interest declaration

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

Learning objectives

This presentation aims to set focus on following aspects of male infertility:
1. To show an increased risk of hypogonadism and testicular malignancy in subgroups of men seeking for infertility treatment;
2. Based on information obtained under item 1 to stress the need of careful clinical investigation of men from infertile couples;
3. Suggestion of guidelines for follow up of certain groups of men from infertile couples, after completion of the infertility treatment.
Different options of approaching males from infertile couples

1. To focus on deciding the best way of utilising sperms in semen/testes;
2. To try to find the reason of male subfertility;
3. As 2 + look for associated conditions

Testicular Dysgenesis Syndrome (TDS) ?

We should expect (at least some) men with poor semen quality to be at increased risk of:

- Hypogonadism;
- Testicular Germ Cell Cancer (TGCC)

But do we see that in the clinics?
Hypogonadism

- Can be defined as:
  - S-Testosterone < 10 nmol/L and/or
  - S-LH > 10 IU/L

Male infertility and Leydig cell function

For some subgroups of infertile men, hypogonadism is known/expected
Klinefelter’s syndrome

Romerius et al, submitted

Risk of hypogonadism in Childhood Cancer Survivors

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Hypogonadal</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>141</td>
<td>6 (4.3%)</td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>CCS</td>
<td>144</td>
<td>33 (23%)</td>
<td>6.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leukemias</td>
<td>26</td>
<td>8 (31%)</td>
<td>10</td>
<td>0.001</td>
</tr>
<tr>
<td>Brain tumors</td>
<td>31</td>
<td>6 (19%)</td>
<td>5.4</td>
<td>0.006</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>32</td>
<td>10 (31%)</td>
<td>10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>9</td>
<td>2 (22%)</td>
<td>6.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Wilms’ tumor</td>
<td>11</td>
<td>1 (9.1%)</td>
<td>2.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Others</td>
<td>35</td>
<td>6 (17%)</td>
<td>4.7</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Increased proportion of hypogonadal men among those with unexplained male subfertility

Giwercman et al, unpublished
Hypogonadism in unexplained male subfertility

- 10% subfertile men (4.4% fertile);
- OR=3.0; 95%CI 1.17-7.82;
- Risk factors:
  - High BMI (>25 kg/m²)
  - but not related to
  - Sperm concentration, FSH, testis volume

Hormones by Age

| Total Testosterone (nmol/L) | β  | P
|----------------------------|----|---
| 0.0 | 0.0652 | <0.001
| 0.2 | 0.256  | <0.001
| 0.4 | 0.118  | <0.001
| 1.0 | -0.041 | <0.001
| 0.0 | -3.381 | <0.001

A high proportion of hypogonadal men may become hypogonadal by age

| OR=3.0; 95%CI 1.17-7.82 |
Conclusion 1

- Male coming for infertility investigation are at increased risk:
  - Being hypogonadal;
  - Becoming hypogonaal by age;

- Due to relatively unspecific symptoms, the patient and the doctor may not be aware of the hypogonadism at least hormone values are assessed.

Sperm number prior to c testis treatment

[Graph showing median sperm count and sperm density in patients with testicular cancer before orchiectomy.]

Petersen et al, JCO 1999

Fertility prior to c testis treatment

[Graph showing mean cumulative age-specific fertility in men with testicular cancer.]

Waller & Ulreich. Medic, 1867, 1999
Male subfertility and risk of testicular cancer

- Men with male factor infertility were nearly 3 times more likely to develop testicular cancer compared with those without (hazard ratio, 2.8; 95% confidence interval, 1.3-6.0) (Walsh et al, Arch Intern Med. 2009);

- The standardized incidence ratio of testicular cancer was 22.9 (95% CI 22.4-23.5) when comparing our infertile group to the control population (Raman et al, J Urol 2006).

Testicular cancer can be prevented

Ultrasound in diagnosis of testicular cancer or carcinoma-in-situ
Testicular malignancy in biopsies from subfertile men

- 4/38 (10%) with NOA – Mancini et al, Hum Reprod 2007;
- 13/534 (2.4%) biopsy because of infertility – McLachlan et al, Hum Reprod 2007

Conclusion 2

- Male coming for infertility investigation are at increased risk for having testicular malignancy at invasive or pre-invasive stage;
- Early diagnosis of testicular malignancy may not only save some lives but implies a less intensive therapy and, thereby, a better life quality (incl. fertility) of the survivors.

How can we utilize this knowledge

- Semen analysis is not sufficient investigation of men seeking for infertility;
- Standard andrological examination of men from infertile couples should include:
  - Hormone assessment (T; SHBG; LH);
  - Scrotal palpation;
  - Scrotal ultrasound;
- TESE tissue should be histologically examined for presence of carcinoma-in-situ
Follow-up of subgroups of subfertile men

- In case of hypogonadism – androgen replacement after completion of infertility treatment;
- Borderline testosterone/LH levels – hormone assessment after 1-2 year;
- Testicular microlithiasis – testicular biopsy should be considered.

List of references

Erectile dysfunction among infertile men – does it exist?

José María Pomerol, MD

Instituto Valenciano de Infertilidad (IVI)
Barcelona, Spain

Instituto de Andrología y Medicina Sexual (IANDROMS)
Barcelona, Spain

Precongress ESHRE 25th Annual Meeting. Amsterdam, June 28, 2009

Statement of disclosure

Speaker (Spain)
Lilly
Bayer-Shering
Pfizer

Advisory board (Spain)
Lilly
Bayer-Shering
Janssen-Cilag

Objectives

To understand:

• The different situations of infertile patients with erectile dysfunction (ED)
• Epidemiology and etiology of ED
• Diagnosis and treatment of ED
• What to do in expected and unexpected ED cases during assisted reproductive techniques
- Infertility clinic
  - Patients with ED secondary to infertility
  - Patients with ED secondary to different causes

- Infertility clinic
  - Patients with persistent ED
  - Patients with temporary / occasional / circumstantial ED
    - during the ovulatory cycle
    - when they have to provide a semen sample

- Patients with ED secondary to the infertility
INFERTILITY

FEMALE SEXUAL DYSFUNCTION

MALE SEXUAL DYSFUNCTION

Anxiety
Stress
Distress
Depression

Familiar and social pressure

Gestational
well-being
Self-esteem
Self-confidence

Male sexual function
- Sexual desire
- Frequency of sexual activity
- Erectile dysfunction
- Premature/delayed ejaculation
- Sexual satisfaction

Couple relationship

Quality of life

INFERTILITY

FEMALE SEXUAL DYSFUNCTION

MALE SEXUAL DYSFUNCTION
Infertility clinic

Severe and persistent ED

Inability to penetrate

Loss of erection before ejaculation

Mechanical cause of male infertility

FEMALE SEXUAL DYSFUNCTION

INFERTILITY

MALE SEXUAL DYSFUNCTION

ED in infertile men

It is advisable to study and treat erectile dysfunction before applying treatments for infertility
How often is erectile dysfunction among infertile men?

cross-sectional study
100 infertile couples

• Sexual Function Questionnaire (SFQ)
• International Index of Erectile Function (IIEF) questionnaire


International index of ED (IIEF)
- Internationally validated in 30 languages
- Questionnaire of 15 questions
- It evaluates 5 sexual function areas

Erectile function (6 questions)

<table>
<thead>
<tr>
<th>Score</th>
<th>ED classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-10</td>
<td>severe</td>
</tr>
<tr>
<td>11-16</td>
<td>moderate</td>
</tr>
<tr>
<td>17-25</td>
<td>mild</td>
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<tr>
<td>26-30</td>
<td>normal</td>
</tr>
</tbody>
</table>

Rosen RC et al, 1997
• The SFQ score was within the normal range in all five domains in only 7% of women

• Only 2% of male participants have had severe erectile dysfunction (ED)


Study to evaluate the hypothesis that infertility may result in a decrease in quality of life and an increase in marital discord and sexual dysfunction

18 infertile couples
12 couples seeking elective sterilization

Monga M et al. Urology 2004; 63:126

Quality of life
Quality of Well-Being Scale-Self Administered Test
Sexual function
Brief Index of Sexual Functioning for Women
International Index of Erectile Function for men
Marital adjustment
Locke-Wallace Marital Adjustment Test

Monga M et al. Urology 2004; 63:126
No statistically significant impact on sexual functioning in women was noted; however, the men in the infertile couples had lower total International Index of Erectile Function scores (P = 0.05) and intercourse satisfaction scores (P = 0.03).

Monga M et al. Urology 2004; 63:126

**Instituto Valenciano de Infertilidad (IVI)**

3787 infertile couples

<table>
<thead>
<tr>
<th>Male age</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>20-29</td>
<td>137</td>
<td>3.6</td>
</tr>
<tr>
<td>30-39</td>
<td>1957</td>
<td>51.6</td>
</tr>
<tr>
<td>40-49</td>
<td>1443</td>
<td>38.2</td>
</tr>
<tr>
<td>&gt;49</td>
<td>249</td>
<td>6.6</td>
</tr>
</tbody>
</table>

IVL 2009

**Instituto Valenciano de Infertilidad (IVI)**

500 infertile couples

**Male sexual function**

- Low sexual desire 14%
- Decrease of sexual activity 19%
- Erectile dysfunction 3%
- Premature ejaculation 16%
- Delayed ejaculation 1%

IVL 2009
### Instituto Valenciano de Infertilidad (IVI)

#### Male sexual function

<table>
<thead>
<tr>
<th>Erectile dysfunction</th>
<th>3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>mild</td>
<td>50%</td>
</tr>
<tr>
<td>moderate</td>
<td>45%</td>
</tr>
<tr>
<td>severe</td>
<td>5%</td>
</tr>
</tbody>
</table>

IVI, 2009

---

**Patients with ED secondary to different causes**

---

**Penile anatomy and physiology**
Erectile dysfunction

ED is defined as the consistent or recurrent inability of a man to attain and/or maintain a penile erection sufficient for sexual activity.

The diagnosis of ED is based in patient’s self-report.

How often is erectile dysfunction among young men?

Epidemiology ED

MEMAS USA 40-70 yrs 52%

Feldman HA et al, 1994
EDEM study
Spain, 1998

25-70 yrs 12 %
40-70 yrs 26 %

Age
- 25-39
- 40-49
- 50-59
- 60-70

ED according to EIEF

Prevalence of ED among a large-scale young adult population

5836 men aged 25-50 years

SHIM self-administrated questionnaire

26.9% ED
19% mild
7% moderate
1% severe

Heruti R et al, 2004

Couples are delaying pregnancy

There is an increase in the age of men trying to conceive
Instituto Valenciano de Infertilidad (IVI)
3787 infertile couples

<table>
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<tr>
<th>Male age</th>
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<td>249</td>
<td>6.6</td>
</tr>
</tbody>
</table>

IVI, 2009

ED in young men

Organic 20 - 40%
Psychogenic 60 - 80%
Erectile dysfunction
Mixed

Psychogenic factors
- Prior life experiences
- Cultural/educational/religious
- Performance anxiety
- Relationships conflicts
- Inadequate sexual information or stimulation
- Psychiatric disorders
- Fear of intimacy
- Impaired self-image or self-esteem
Psychogenic factors in cases of male infertility

- Adverse feelings towards paternity
- Anxiety during the partner’s ovulation or when he has to provide a semen sample

Etiology and risk factors of ED

- Lifestyle factors and individual health conditions
  - Sedentary life-style
  - Nicotine
  - Alcohol abuse
  - Drug addictions
  - Obesity
  - Age

Cardiovascular risk factors
- Hypertension
- Dyslipemia
- Coronary arterial disease (CAD)
- Peripheral arterial occlusive disease

Post-traumatic ED
- Neural and vascular lesions

Endocrine factors
- Hypogonadism
- Hyperprolactinemia and prolactinoma
- Thyroid disorders

Cavernous factors
- Cavernous veno-occlusive dysfunction
- Cavernous myopathy
- Cavernous fibrosis after priapism
- Peyronie’s disease
- Penile fracture

Iatrogenic ED
- Drug induced
- Post-operative
- Post-radiation

Other medical disorders
- LUTS and BPH
- Hepatic insufficiency
- Respiratory disorders and sleep apnea
- Renal insufficiency
- Neurogenic disorders

Diabetes mellitus
- Diabetes type 1
- Diabetes type 2
Medications/RD associated with ED

<table>
<thead>
<tr>
<th>Antihypertensives</th>
<th>Antiarrhythmics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazide diuretics</td>
<td>Digoxin</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>Amiodarone</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>Disopyramide</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antidepressants/Neuroleptics</th>
<th>Phenytoins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricyclic antidepressants</td>
<td></td>
</tr>
<tr>
<td>Selective serotonin reuptake inhibitors</td>
<td></td>
</tr>
<tr>
<td>Phenothiazines</td>
<td></td>
</tr>
<tr>
<td>Butyrophenones</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medications with hormonal influence</th>
<th>Anti-androgens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GnRH agonists</td>
</tr>
<tr>
<td></td>
<td>Fluotamide</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
</tr>
<tr>
<td></td>
<td>Spironolactone</td>
</tr>
<tr>
<td></td>
<td>H2 blockers</td>
</tr>
<tr>
<td></td>
<td>Cimetidine</td>
</tr>
<tr>
<td></td>
<td>Estrogens</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antiarrhythmics</th>
<th>Recreational substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxin</td>
<td>Marijuana</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>Cocaine</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>Alcohol</td>
</tr>
</tbody>
</table>

Diagnosis

Sexual and medical history

ED

- Onset (suddenly, gradual)
- Circumstances (partner, masturbation)
- % occurrence
- Hardness of erections
- Maintenance of erections
- Possibility to penetrate
- Nocturnal and morning erections (frequency and quality)

Erection characteristics

- Absent
- Tumescence
- Incomplete rigidity
- Loss of rigidity before/after penetration
International index of ED (IIEF)

- Internationally validated in 30 languages
- Questionnaire of 15 questions
- Evaluates 5 sexual function areas

**Erectile function (6 questions)**

<table>
<thead>
<tr>
<th>Score</th>
<th>ED classification</th>
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<tbody>
<tr>
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<td>mild</td>
</tr>
<tr>
<td>26-30</td>
<td>normal</td>
</tr>
</tbody>
</table>

Rosen RC et al, 1997

---

**Diagnosis**

**Sexual and medical history**

**Other sexual aspects**

- Sexual desire
- Frequency of sexual activity
- Ejaculation (premature, delayed,..)
- Orgasm
- Sexual satisfaction

Female sexual dysfunctions

Couple relationship

---

**Diagnosis**

**Medical disorders**

- Endocrinologic Diseases
- Vascular Risk factors
- Neurologic Surgeries
- Morphologic
- Psychiatric

Medications and recreational drugs

Psychological factors
Diagnosis
Physical examination

- Thyroid
- Mammary glands
- Male: Penis, Testes
- Rectal examination
- Blood pressure
- Waist circumference
- BC reflex
- Peripheral pulses
- Weight

Diagnosis
Blood tests

- Fasting glucose
- Fasting lipid profile
- Testosterone
- Prolactine
- Other (according to the history)

Hypogonadism

Testosterone
FSH/LH
Pathologies
Hypothalamus
Hypophysis

FSH/LH
Pathologies
Testicles
FSH
LH

Secondary hypogonadism
Secondary sexual characteristics

Primary hypogonadism
Cortex
Hypogonadism

Low Testosterone

- Low sexual desire
- Erectile dysfunction

Klinefelter's syndrome

- 1:500 – 1:1000
- 47 XXY (90%)
- XXY/XY (10%)
- ↑ FSH, LH
- ↓ T

Hypogonadotropic hypogonadism

- ↓ T, LH, FSH

Magnetic resonance imaging (MRI)

- Tumors / other pathologies
Neurogenic ED

- Supraspinal
  - Suprasacral: Reflexogenic erection is maintained
    - Erection of short duration, requiring continuous stimulation
    - Incomplete lesion: can maintain erection
  - Sacral: No reflexogenic erection
    - No response to psychogenic stimulation
- Spinal
- Peripheral: Disruptions sensory afferent/efferent nerves

Neurogenic ED
Potential causes

- Pelvic injury, or surgery
- Injuries or lesions to the spinal cord
- Diabetic neuropathy
- Multiple sclerosis
- Stroke
- Alzheimer’s disease

Erectile dysfunction

- History + physical examination
- Laboratory work-up

- Evident psychogenic etiology
  - Psychological evaluation
- Evident organic etiology
  - Evaluation therapeutic alternatives
- Non-evident etiology
  - Studies to assess the erection
Diagnosis
Intracavernous injection test combined with Doppler/duplex ultrasound

Alprostadil (PGE1)

Penile Doppler ultrasound

Normal values
- MSV > 30 cm/seg
- FDV < 4 cm/seg
- Resistance index > 0.75

$$IR = \frac{MSV - FDV}{MSV}$$

Nocturnal penile tumescence (NPT)

Rigiscan®

1 - 3 nights
rigidity (%) / diameter (cm)
No / episodes duration

> 10 min
> 60% rigidity
**Diagnosis**

Pudendal arteriography

- Arterial insufficiency
- Arterial obstruction

**Diagnosis**

Cavernosography

**Treatment**

Alteration of modifiable risk factors

- Smoking
- Alcohol
- Substance abuse
- Lifestyle
- Illness (control)
- Medications (alterations drug dosages or classes)
Psychogenic factors

Psychological evaluation
Psychotherapy
+ / - PDE5 inhibitors

Hypogonadism

Hypogonadotropic
Hypergonadotropic
FSH + HCG
After fertility therapy
Testosterone replacement treatment
Gel 50 mg/day T undecanoate 1000 mg/3 months
+ / - PDE5 inhibitors

First line therapy

PDE5 inhibitors
Sildenafil Viagra®
Tadalafil Cialis®
Vardenafil Levitra®
**SEXUAL STIMULATION**

Cavernous nerve

Nonadrenergic
Noncholinergic

L-Arginine

O$_2$

Endothelial cell

Smooth-muscle cell

NO

Decreased Ca$^{2+}$

Smooth muscle relaxation

Guanylyl cyclase

cGMP

GTP

cGMP specific protein kinase

5'GMP

PDE 5 inhibitors

<table>
<thead>
<tr>
<th></th>
<th>Time to action</th>
<th>Time of effectivity</th>
<th>Interference with food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sildenafil (Viagra ®) 25 / 50 / 100 mg</td>
<td>14 min</td>
<td>4-5 hs</td>
<td>Yes</td>
</tr>
<tr>
<td>Vardenafil (Levitra ®) 10 / 20 mg</td>
<td>11 min</td>
<td>4-5 hs</td>
<td>Yes</td>
</tr>
<tr>
<td>Tadalafil (cialis ®) 10 / 20 mg 5 mg once a day</td>
<td>16 min</td>
<td>24-36 hs</td>
<td>No</td>
</tr>
</tbody>
</table>

Initiate with higher doses

Instructions

**PDE5 inhibitors are effective in more than 75% of patients**

-Sildenafil: Montorsi et al 1999, n = 514
-Tadalafil: Brock et al 2002, n = 1112
-Vardenafil: Porst et al 2001, n = 590
### Adverse events (%)

<table>
<thead>
<tr>
<th>Event</th>
<th>Viagra 100</th>
<th>Cialis 20</th>
<th>Levitra 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>16</td>
<td>15</td>
<td>15.3</td>
</tr>
<tr>
<td>Flushing</td>
<td>10</td>
<td>3</td>
<td>11.3</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>7</td>
<td>8</td>
<td>6.3</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>4</td>
<td>2</td>
<td>7.3</td>
</tr>
<tr>
<td>Vision alterations</td>
<td>3</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td></td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Discontinuations due to: 2 - 4%

---

### PDE5 inhibitors

**Tadalafil 5 mg**

*Continous treatment*

1 tablet daily

*Reduce anxiety*

---

### Historical Comparison: IIEF EF Domain

**Pooled Daily vs. Integrated On Demand**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean IIEF EF Domain Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td></td>
</tr>
<tr>
<td>Tadalafil 5 mg</td>
<td>14.9</td>
</tr>
<tr>
<td>Placebo</td>
<td>15.3</td>
</tr>
<tr>
<td>Tadalafil 2.5 mg</td>
<td>19.2</td>
</tr>
<tr>
<td>Placebo</td>
<td>15.3</td>
</tr>
<tr>
<td>Placebo</td>
<td>21.9</td>
</tr>
<tr>
<td>Placebo</td>
<td>21.1</td>
</tr>
<tr>
<td>Placebo</td>
<td>23.2</td>
</tr>
</tbody>
</table>

* p<0.001 versus placebo

---

* Data on file (pooled data from LVCV and LVFP after 12 weeks of treatment); Eli Lilly and Company, Indianapolis, IN.
  * Carson et al. BJU Int. 2004;93:1276-1281.
### Treatment

#### Intracavernous injection with alprostadil

<table>
<thead>
<tr>
<th>Effectivity</th>
<th>73%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse events</td>
<td>5%</td>
</tr>
<tr>
<td>Discontinuation</td>
<td>40%</td>
</tr>
</tbody>
</table>

Linet, 1994

---

#### Treatment

#### Vacuum devices

---

#### Treatment

#### Penile prosthesis

- **Maleable**
- **Inflatable**

---
Patients with known ED secondary to severe anxiety during attempts to masturbate and during sexual contact with their partners

Treatment

1. Psychological advise  
2. Treatment with PDE5 inhibitors (on demand, daily dose)  
3. Intracavernous injection with PGE1  
4. Vibratory stimulation  
5. Testicular sperm retrieval

Treatment with PDE5 inhibitors

- Continuous treatment with Tadalafil 5 mg / day beginning at least 4 days before sexual activity
- On demand treatment with Tadalafil / Vardenafil / Sildenafil
A cohort observational study

405 men undergoing infertility evaluation

Severe anxiety during attempts to masturbate and during sexual contact with their partners

11% failed to collect semen by masturbation for a second semen analysis after repeated attempts at 2-to 3-day intervals

20% of these men were able to collect semen using vibratory stimulation

Ramadan A et al, 2003

Patients with previously unknown ED who are unable to get erection and ejaculation during a scheduled assisted reproductive technique

Treatment

1. Psychological advises (change location, sexual stimulation,..)
2. Treatment with fast acting PDE5 inhibitors (vardenafil 20 mg)
3. Intracavernous injection with PGE1
4. Vibratory stimulation
5. Testicular sperm extraction (TESE) or aspiration (TESA)
Conclusions

- ED in infertile patients may be secondary to the infertility itself or to other psychogenic or organic causes.
- It is advisable to study and treat ED before infertility treatments.

Conclusions

- It is important to know the ED causes and its management in order to offer the patient the best therapeutic options.
- PDE5 inhibitors constitute the first ED therapeutic line.

References (1)

## References (2)

Disclosure

1. I have no commercial and/or financial relationships with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices to disclose.

2. G.R. Dohle, April 2009

LEARNING OBJECTIVES

Explain the relationship between male accessory gland infection and male infertility

Explain the diagnostic process of male accessory gland infection

Explain the appropriate treatment of male accessory gland infection
INTRODUCTION

- A history of urogenital infection is present in 1.6-10.3% of men attending fertility clinics.
- Urogenital infections may influence sperm parameters, especially motility.
- Leucocytes produce reactive oxygen species (ROS) and cytokines.
- In some men male accessory gland infection (MAGI) becomes chronic and may result in obstruction of the male genital tract and loss of function of the accessory glands.

DOES MAGI INFLUENCE MALE FERTILITY?

- Most isolated bacteria show no impact on sperm parameters in vitro.
- Low bacteria counts are often found in semen of asymptomatic fertile men.
- No clear correlation is found between the number of leucocytes in semen and MAGI.
- Urethral and foreskin contamination can be expected in seminal cultures.
- 80% of "prostatitis patients" have no bacteria in their ejaculates.
Infection and the male reproductive tract

- Temporary inflammatory episodes in the male reproductive tract are common.
- Caution should be exercised in the use of leukocytospermia or bacteriospermia as parameters for MAGI.
- Rectal ultrasound indicates that a number of men with poor semen quality have a non-symptomatic, chronic prostatovesiculitis.
- Oxyuris trachomatis may be a major cause of chronic prostatitis, especially in young men.
- The male accessory glands function as a reservoir for chlamydia and other organisms, increasing the probability of infection of the female.
- Ureaplasma urealyticum is a commensal in the male reproductive tract.
- One of the manifestations of MAGI is sperm antibodies.


THE PROSTATITIS SYNDROME

Classification of prostatitis according to the NIDDK/NIH

I. Acute bacterial prostatitis (ABP) RARE
II. Chronic bacterial prostatitis (CBP) 5-15%
III. Chronic pelvic pain syndrome (CPPS) MAJORITY
   A. Inflammatory CPPS: WBC in semen/EPS/voided bladder urine-3 (VB3)
   B. Non-inflammatory CPPS: No WBC in semen/EPS/VB3
IV. Asymptomatic inflammatory prostatitis (histological prostatitis)

EPS = expressed prostatic secretion; WBC = white blood cells.
Leucocytospermia

- Leucocytospermia is a common finding in men without obvious signs of urogenital infection and with negative cultures.

- An increased number of leucocytes in semen may indicate mase, chemical prostatitis (CPPS) and auto-immune disease.

- Leucocytes are the main source of reactive oxygen species (ROS) and cytokines.

- Leucocytospermia does not seem to influence conception rates and the results of ART.
CHLAMIDIA AND PROSTATITIS/MALE INFERTILITY

- Chlamidia is rarely present in healthy asymptomatic men.
- Studies suggest that Chlamidia Trachomatis is responsible for most MAGI in young men.
- Chlamidia may also cause epididymitis with functional impairment and obstruction, but clear evidence is lacking.
- There are no conclusive studies showing that men infected with Chlamidia are less fertile than uninfected men.

TRANSRECTAL ULTRASOUND OF THE PROSTATE

- TRUS is indicated in infertile men with a low seminal volume (<1.0 ml) and in men with a history of MAGI.
- Abnormalities associated with infertility are:
  - Midline (Mullerian) prostatic cysts.
  - Dilatation of the seminal vesicles.
  - Calcifications after prostatitis with obstruction of the ejaculatory ducts.
  - Hypoplasia or absence of the seminal vesicles.
Ejaculatory duct obstruction

- Calcifications and dilatation of the peri-prostatic plexus and seminal vesicles are the most consistent findings in transrectal ultrasound investigations in men with genital infections (Schipper et. al., Fert Steril, 2001).

- These signs of infections are found in at least 50% of men with EDO (Paick et. al., BJU, 2000)
EPIDIDYMITIS

- Etiology:
  - Usually idiopathic
  - Due to obstruction
  - Ascending infection with urethritis/prostatitis
  - In young men usually caused by STD’s (Chlamydia, Gonorrhoea)
  - In older men usually caused by bacteria from the bladder and the prostate due to obstructive voiding
  - In African men epididymitis is sometimes caused by tuberculosis and Schistosomiasis
REACTIVE OXYGEN SPECIES (ROS)

- The deleterious effects of ROS on semen quality has been documented and reviewed.
  - (Tremellen K. Hum. Reprod. update 2008 14:243-258)

- Spermatozoa are more vulnerable to ROS than other cells because:
  - Spermatozoa have a limited repair system: Antioxidants are absent in spermatozoa
  - Mitochondria are particularly vulnerable to ROS stress, which may influence sperm motility
  - ROS can alter sperm DNA
  - Especially in the epididymis ROS exposure time is much longer and the amount of scavengers is limited

(Subtotal) Obstruction in Men with Severe Oligozoospermia

- In 78 men with severe oligozoospermia a testicular biopsy was performed under local anaesthesia.
  - 39/78 (50%) men showed normal spermatogenesis

- The medical history showed:
  - Childhood hernia repair 11(14.1%)
  - Cryptorchidism/orchidopexy 10(12.8%)
  - Male accessory gland infection 10(12.8%)

- Dohle G. R., Andrologia 2003, 35,321-324
Signs of a (Partial) obstruction of the seminal path

- Decline in sperm quality in an episode of infection.
- Low seminal volume, low fructose, low Alfa-glucosidase.
- Normal testicular volume, normal FSH/Inhibin-B.
- Signs of infection on transrectal ultrasound (calcifications, dilatation of the seminal vesicles)


TREATMENT 1

- ANTIBIOTICS OFTEN ONLY EREADICATED MICROORGANISMS BUT DO NOT ALTED ROS PRODUCTION AND WILL NOT ALTER FUNCTIONAL DEFICITS CAUSED BY THE INFLAMMATORY PROCESS.
- A TWO-WEEKS REGIMEN OF A FLUOROQUINOLONE IS RECOMMENDED TO TREAT MAGI.
- CHLAMIDIA CAN BE TREATED WITH TETRACYCLINE OR AZITROMYCINE

TREATMENT 2

- In case of obstructive azoospermia: scrotal exploration - vasography - vaso-epididymostomy
  - Success rate: 25-40% pregnancies.
- In case of failure: Sperm aspiration and ICSI can be performed.
  - Success rate: 25% pregnancies per treatment cycle
KEY REFERENCES


Percutaneous sperm aspiration for ICSI
Disclosures

• Member of advisory boards of
  – Lilly Netherlands

• Clinical research sponsored by
  – GSK
  – Bayer Schering
  – Prostrakan

Who should be investigated and treated for early and late onset hypogonadism?

Prof Dr Eric JH Meuleman
Urologist, Free University Medical Centre

Learning Objectives

• Male hypogonadism several different clinical entities
  – Reproductive medicine ↔ Men’s health
• Pathophysiology of male hypogonadism
• Benefits and risks of (testosterone) treatment
• Alternatives
Male hypogonadism in reproductive medicine

Two rules of thumb
1. Endocrine disorders (0.6 – 8.9) in subfertile males are rare but is higher than in general population
2. The poorer the sperm quality the higher the chance

Endocrinological investigation in subfertile men

When?
- In extreme oligo- and azoospermia

Why?
- Detection of endocrine disorders
- Differentiation between testicular failure and obstruction

How?
- LH – FSH - Testosterone
- Prolactine on indication
- Anosmia
- Visual disturbances
- Low Testosterone
- MRI scan of sella turcica

Two diagnostic groups

Hypergonadotrope Hypogonadism
- LH, FSH ↑
- Kallmann
- Klinefelter (KH)
- Pelvic Tumor
- Anabolic steroids
- Morbid obesity
- Granulomatous diseases
- Haemochromatosis

Hypergonadotrope Hypogonadism
- LH, FSH ↑
- Testicular dysgenesis
- Klinefelter
- Anorchia
- Castration
- Cystic medication
- Radiation
Morbid obesity and fertility

- Increase oestradiol
- Increase aromatisation
- Decrease LH levels and pulse amplitude
- Decrease testosterone
- Visceral adipocytes

Treatment of hypogonadal men in reproductive medicine

- In men with hypogonadotropic hypogonadism proven effectivity of:
  - Pulsatile GnRH, iv or sc, starting at 5, if necessary 10 – 20 mg per 90 minutes. If insufficient response 1500 IU HCG (LH) and 150 IU HMG (FSH) twice weekly im.
  - Prolactinoma: Dopamine agonist or surgery
- In men with idiopathic OAT no evidence of effectivity of androgens, HMG/HCG, anti-estrogens (clomiphene, tamoxifen), prolactine inhibitors (bromocriptine) and steroids in the literature

Late Onset Hypogonadism
Late onset hypogonadism

Testosterone Deficiency Syndrome

- Sexual problems
- Diminished energy, sense of vitality or well-being
- Increased fatigue
- Depressed mood
- Impaired cognition

Factors associated with increased risk of hypogonadism

- DM type 2
- HIV
- Hypothyroidism
- End stage renal disease
- Chronic Obstructive lung disease
- ED and PDE5 inhibitor failure
- Depression
- Parkinson’s disease

Contributing factors
- Stress
- Obesity
- Lack of exercise
- Excessive alcohol consumption
- Medications
Hypogonadism is associated with the metabolic syndrome and accelerated atherosclerosis


Correlation between bioavailable testosterone and waist circumference

\[ r = -0.21 \quad p < 0.001 \]
Total testosterone is correlated with number of risk factors for metabolic syndrome

Indices of metabolic syndrome increase in GnRH agonist-treated men with prostate cancer

Do men with low levels of testosterone have accelerated atherosclerosis?

Case control study
- 60 men with 1 or more coronary stenoses >75%
- 30 men with normal coronary angiograms

Free testosterone -7.3 (-15.3 to +0.6) p=0.07
Bioavailable testosterone -0.5 (-0.9 to -0.11) p=0.01
Total testosterone -1.2 (-3.3 to +0.95) p=ns

Multiple linear regression adjusted for age and BMI

Matthew R et al. JCEM 2006; 91(4): 1305-1308
Correlation between serum testosterone and maximum intima-media thickness of the carotid bulb in 236 middle-aged men

\[ r = 0.21 \]
\[ p = 0.001 \]

Endogenous Sex Hormones and Progression of Carotid Atherosclerosis in Elderly Men

Progression of mean IMT of common carotid artery in tertiles of serum total and free testosterone concentrations (nmol/L)

Hypogonadism – male sexual functions

The literature

- Decrease of sexual desire
- Decrease of sexual activity
- Decrease of fantasy / sleep induced erection

Hypogonadism

ED
Indirect effect of testosterone on arousal

Initiation of Arousal: “Dual Pathway”

Testosterone

- Internal stimuli
  - Erotic Imagery

- External stimuli
  - AudioVisual
  - Olfactory
  - Tactile

Direct hemodynamic effects of testosterone on erectile function

Intracavernous pressure / Systemic Arterial Pressure

Changes in NO and PDE-5 transcriptional regulation following castration

Histological changes in CC following castration

Histological changes

Proposed mechanism of regulation of cellular differentiation by androgens in penile corpus cavernosum
Medications associated with T-deficiency

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased T-production</td>
<td>Alcohol, Ketconazole,</td>
</tr>
<tr>
<td></td>
<td>Opioids, LHRH Agonists</td>
</tr>
<tr>
<td>T-Antagonisten</td>
<td>Cimetidine</td>
</tr>
<tr>
<td>Increased prolactin</td>
<td>Spironolactone</td>
</tr>
<tr>
<td>Increased SHBG-levels</td>
<td>Barbiturates</td>
</tr>
<tr>
<td>Decreased DHT Levels</td>
<td>Dutasteride</td>
</tr>
</tbody>
</table>

Questions

- Physiological process of aging or disease?
- Does testosterone therapy improve signs and symptoms?

Potential risks of Testosterone Therapy

- Benign prostate hyperplasia and LUTS
- Prostate cancer
- Cardiovascular disease
- Lipid alterations
- Erythrocytosis
**BPH and LUTS**

- Prostate volume ↑ during T-replacement during the first 6 months
- Flow-rates, post-voiding residual urine volumes and LUTS do not change

---

**Prostate cancer**

<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>Increase in PSA, µg/L</th>
<th>Prostate Cancer</th>
<th>Method of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hulger et al. (1997)</td>
<td>24 days</td>
<td>1.05</td>
<td>N/A</td>
<td>Intranasal</td>
</tr>
<tr>
<td>Sih et al. (2000)</td>
<td>12 days</td>
<td>0.13</td>
<td>N/A</td>
<td>Micronized implant</td>
</tr>
<tr>
<td>Dolo et al. (1990)</td>
<td>24 days</td>
<td>0.13</td>
<td>N/A</td>
<td>Intranasal</td>
</tr>
<tr>
<td>Sperling et al. (2000)</td>
<td>26 days</td>
<td>0.13</td>
<td>N/A</td>
<td>Micronized implant</td>
</tr>
<tr>
<td>Ng et al. (2000)</td>
<td>6 days</td>
<td>0.13</td>
<td>N/A</td>
<td>Micronized implant</td>
</tr>
<tr>
<td>Hulger et al. (1997)</td>
<td>24 days</td>
<td>0.13</td>
<td>N/A</td>
<td>Intranasal</td>
</tr>
</tbody>
</table>

---

**The saturation model**

The traditional model of testosterone (T)-dependent prostate cancer (PCa) growth suggests that higher serum T concentrations lead to some degree of greater PCa growth (curves a and b). The saturation model (curve c) describes a steep T-dependent curve at low serum T concentrations or below the near-castrate region, with a plateau representing little or no further growth above this concentration.
The effect of exogenous testosterone (T) administration on the prostate tissue levels of T and dihydrotestosterone (DHT) in hypogonadal men.


Polycytemia
- 2.8 percent
  5 mg T per day by non scrotal patches
- 11.3 percent
  gel preparations delivering 5 mg per day
- 17.9 percent
  gel preparations delivering 10 mg per day

No testosterone-associated tromboembolic events have been reported

Potential benefits of testosterone Therapy
- Metabolic syndrome
- Sexual function
- Quality of (sexual) live
Testosterone replacement therapy may be beneficial for some components of the metabolic syndrome in overweight men with low T-levels.

Cardiovascular benefits

Improvement in distance achieved in the shuttle walk test

T-supplementation may improve insulin resistance

Reverse relationship between fasting insulin and testosterone levels
A placebo controlled study of the effects on insulin sensitivity and sexual function of transdermal testosterone gel in hypogonadal men with type II diabetes and/or metabolic syndrome

TIMES 2 Study
J Buvat S Arver, H Behre, E Meuleman, I Moncada, M Morales, Chevallier

**P & M**
- 220 hypogonadal men (T < 11nmol/l) with T2D or MetS
- 12 months Metered-dose of topical 2% T-gel (Tostran®)

**Results**
- Improvement insulin sensitivity
- Significant improvement sexual desire and intercourse satisfaction domain

The addition of T replacement after failure of sildenafil alone

<table>
<thead>
<tr>
<th>T gel 1% + sildenafil 12 weeks</th>
<th>PBO + sildenafil 12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIEF Domain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Delta baseline</td>
</tr>
<tr>
<td>Erectile function</td>
<td>5.65</td>
</tr>
<tr>
<td>Orgasmic function</td>
<td>1.53</td>
</tr>
<tr>
<td>Sexual desire</td>
<td>0.44</td>
</tr>
<tr>
<td>Intercourse satisfaction</td>
<td>1.21</td>
</tr>
<tr>
<td>Satisfaction</td>
<td>1.62</td>
</tr>
<tr>
<td>Total score</td>
<td>10.44</td>
</tr>
</tbody>
</table>

ESHRE 280609


Hormonal supplementation: A matter of timing?

“You have to treat these cells in the brain at a time when they are healthy.”

- Roberta Brinton

NATURE 445:25 jan 2007

Life-style: Physical activity: An alternative!

Metabolic factors

\[ \text{LDL-C} \uparrow \]
\[ \text{HDL-C} \downarrow \]
\[ \text{Insulin resistance} \]

Vascular factors

\[ \text{Vascular delivery} \]
\[ \text{Peripheral vasodilation} \]
\[ \text{Blood volume} \]

Sexual function
Physical activity and progression/regression of coronary atherosclerosis

N=52 men with CAD

Progression No change Regression

The feasibility of a physical activity program for men with LUTS and/or ED who visit the urology OPD VUmc: A pilot study C. Martis

Conclusions
- 41% of men visiting a urological OPD with LUTS and/or ED demonstrate a lack of physical activity
- A high percentage (70%) of sedentary men are willing to participate in a PA program
- Only 20% started to work-out of whom nobody completed the full program
- There is a need for an increase of lifestyle-awareness amongst urological patients and urological health-care providers
- The success of a programs aimed at life-style improvement depends on close professional coaching

References
What does poor sperm DNA quality mean?
A critical review of methods, interpretation and clinical value

Ulrik Kvist, M.D. Ph.D.
Centre for Andrology and Sexual Medicine
Karolinska University Hospital, Huddinge
Stockholm, Sweden

Disclosures of commercial and/or financial relationships

• I have no commercial and/or financial relationships with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices scrutinized in this lecture.

Learning objectives

• To mediate insight into the organization of the sperm chromatin structure of DNA, protamines and histones
• To mediate the evolutionary aspects of a sperm chromatin closed for the environment until fertilization
• To mediate the consequences for an investigator facing a sperm chromatin evolved to refuse to take up substances exposed to.
What is sperm DNA quality?

Sperm DNA quality
Sperm quality
Sperm integrity are "political terms" an increase.

Methods give

RESULTS

Investigators should tell about the method used and focus on RESULTS.

The spermatozoon is a messenger cell carrying messages for healthy grandchildren.

The \textit{spermatozoon} is a messenger cell carrying messages for healthy grandchildren.

Messages are

• The \textit{intact DNA – the genome}
• Structural defects
• Numerical defects
• DNA strand – breaks
• The \textit{"normal" epigenetics}
• Protamines in place protecting and silencing > 95\% of the genome
• "The normal" Methylation of paternal DNA
• "The normal" Acetylation and Methylation of Sperm Histones
• The sperm RNA
• The sperm nuclear Proteins
• The paternal centrosome
• The Factors initiating the placenta
What does poor sperm DNA quality mean?

DNA strand breaks

A hit in a spermatozoon in the epididymis or the test tube now!

May result in a grand-child with an unbalanced translocation with impaired psychomotor development and malformations

The protamin covered sperm DNA.

- The "rope" of sperm chromatin is composed by three strings
- The two DNA-strands and the third is the string of protamine-monomers.
Protamine free Toroid linker DNA attaching the protamine covered DNA in the toroids to the sperm nuclear matrix

1 zinc/1 protamine/ 10 bp DNA

<table>
<thead>
<tr>
<th>Condition</th>
<th>Zinc/Sulfur x 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fortile men</td>
<td>150 (97-182)</td>
</tr>
<tr>
<td>Childless men without prostatic affection</td>
<td>134 (110-201)</td>
</tr>
<tr>
<td>Childless men with prostatic affection</td>
<td>62 (48-77)</td>
</tr>
</tbody>
</table>
SDS exposed after EDTA pre-exposed at ejaculation

Organized in loops forming doughnuts
The Sequence of Ejaculation

- Man offers the woman spermatozoa in prostatic fluid

Chromatin zinc is retained by prostatic fluid

- The physiological ejaculate is spermatozoa suspended (emitted) in prostatic fluid and expelled in the very first split-ejaculate fraction onto the cervix.

- Spermatozoa in prostatic fluid retain chromatin zinc.
Chromatin zinc is depleted by Seminal vesicular fluid

- Seminal vesicular fluid contains High molecular weight proteins (seminogelins) trapping zinc.
- HMW-Zn
- Increased pH increase the binding of zinc to citrate.

Liquefied ejaculate can act zinc-chelating, % HMW-Zn

- 20 fertile men 13% (Arver 1982)
- 13 fertile donors < 10% (Kjellberg, 1993)
- 115 infertile men 2-67% (Kjellberg 1993)

Liquefied whole ejaculate can act a zinc-chelating medium, especially in men with low zinc concentration, indicating abundancy of seminal vesicular fluid

Vesicular fluid chelates chromatin zinc

- Spermatozoa expelled in vesicular fluid at ejaculation reveal lower zinc content in the chromatin (Björndahl, 1990).
- Spermatozoa incubated in seminal vesicular fluid loose zinc
Chromatin zinc

- Fertile donors have higher zinc content in chromatin than infertile men.
- Men with signs of prostatic inflammation had the lowest chromatin zinc content (Kvist, 1988).

Dual actions by Zinc: (1) stabilizes the structure and (2) prevents oxidation

- Removal of zinc gives two possibilities:
  - 1) immediate decondensation
  - 2) otherwise develops superstabilization in air atmosphere.

Sodium Dodecyl Sulphate introduces negative repulsive forces
Totally Resistance vs Fast delivery of DNA

• Sulfonuklein

• 90% decondensed < 5min after ejaculation
  If exposed also to zinc-chelating EDTA
Exposed to SDS-EDTA
one resistant, one decondensed

Exposed to SDS alone (upper left and lower left) and SDS after pre-exposure to EDTA.
SDS exposed after EDTA pre-exposed at ejaculation

Methods

- Principles of methods and limitations:
  - Acridine orange staining
  - Toluidine staining
  - Anilinic blue staining
  - Sperm-Halo SCD Sperm chromatin dispersion
  - TUNEL
  - AO FACS (SCSA©)
  - Sperm swelling in SDS
  - Sperm swelling in SDS-EDTA
  - Sperm swelling in SDS-DTT
  - Sperm swelling in SDS-Cysteine; Albumine; Histidine

Some methods
A sperm comet
Comet head and comet tail

Calculation of results CASP –
Comet Assay Software Project www.casplab.com

- HeadArea: Area of the comet head
- TailArea: Area of the comet tail
- HeadDNA: Sum of intensities of pixels in the head
- TailDNA: Sum of intensities of pixels in the tail
- HeadDNA%: Percent of intensity of pixels in the comet head
- TailDNA%: Percent of intensity of pixels in the comet tail
- HeadRadius: Radius of the comet head
- TailLength: Length of the comet tail
- CometLength: Length of the entire comet from head area to end of tail
- HeadMeanX: Center of gravity of intensity in the head (x coordinate)
- TailMeanX: Center of gravity of intensity in the tail (x coordinate)
- TailMoment: TailDNA% x TailLength
- Olive/TailMoment: TailDNA% x (TailMeanX-HeadMeanX)
• **Principle**

• **Lysis:** Take away all proteins binding DNA leaving the naked and free DNA

• **Electroforesis:** Put current on it and small pieces will move.

The lysis does not reveal all

• Cysteine (zinc-chelating and S-S cleaving) increased the amount of DNA available to the assay.

• The effect was significantly related to the the conc of zinc in the ejaculate

After cysteine treatment the structure re-stabilizes (S-S)
Conclusion:

- In toxicology studies clear differences between controls and exposed.
- In human standard lysis protocol does not reveal all DNA.
- The lysis response related to zinc concentration in seminal plasma.
- Zinc removal stabilizes the chromatin towards the lysis protocol.

A Comet tail means broken DNA - no good sign.
- DNA remaining in the head can be intact or broken, but caught by S-S crosslinked protamines.
- Thus there is the risk of false negatives!

TUNEL = TdT-mediated dUTP Nick End Labeling
- Fluoresceine coupled dUTP (deoxyuracil monophosphate)
- TdT enzyme (Terminal deoxynucleotidyl transferase)
- Single strand DNA break
- DNA breaks
TUNEL assay and chromatin stability

- Using In Situ Cell Detection Kit (Fluorescein)
  - Positive controls only stained 15-55% of sperm
  - High percentage TUNEL-positive spermatozoa related to
    - Low seminal zinc concentration
    - Long abstinence time
    - Long time between ejaculation and start of TUNEL preparation

<table>
<thead>
<tr>
<th>% TUNEL (Pos. control)</th>
<th>P</th>
<th>r</th>
<th>Pbf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc conc. (mM)</td>
<td>0.73</td>
<td>0.53</td>
<td>0.020</td>
</tr>
<tr>
<td>Abstinence time (days)</td>
<td>0.80</td>
<td>0.63</td>
<td>0.01</td>
</tr>
<tr>
<td>Analysis delay (min)</td>
<td>0.99</td>
<td>0.90</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

From Björk et al. 2009, Poster presentation at the American Society of Andrology, Philadelphia, PA, USA, April 4-7.


DNA cross-linking of the TUNEL assay

DNA cross-linking of the TUNEL assay

John Aitken: Sperm DNA: organization, protection and vulnerability – from basic science to clinical application Stockholm 19-22 May 2009

<table>
<thead>
<tr>
<th>% TUNEL (Pos. control)</th>
<th>P</th>
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<td>Abstinence time (days)</td>
<td>0.80</td>
<td>0.63</td>
<td>0.01</td>
</tr>
<tr>
<td>Analysis delay (min)</td>
<td>0.99</td>
<td>0.90</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% TUNEL (Pos. control)</th>
<th>P</th>
<th>r</th>
<th>Pbf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm conc. (10^6/mL)</td>
<td>0.17</td>
<td>0.03</td>
<td>ns (0.67)</td>
</tr>
</tbody>
</table>


Conclusion TUNEL. Intelligent!

- Identifies breaks but only if given access!!
- All spermatozoa did not respond to the standard protocol. They did not take part!
- Thus, No stained dots can mean intact DNA or broken and closed up! i.e the enzyme etc did not get access to the chromatin that is superstabilized?
- Thus there is the risk of false negatives!
Acridine orange is prepared from coal tar and creosote oil.

The rhetorical view!
- Intact DNA looks green
- Damaged DNA looks red
- Red is bad!
- Increased Red/Red+Green is said to be due to fragmented sperm DNA

Acridine Orange and somatic cells
- Nucleic acid selective fluorescent cationic dye useful for cell cycle determination (i.e. the shift between 2n and 4n cells).
- Cell-permeable, interacts with DNA and RNA by intercalation or electrostatic attractions respectively.
- Green: When bound to DNA, an excitation maximum at 502 nm (e.g. 488 nm) and an emission maximum at 525 nm (green). (SAB: >300 nm)
- Red: With RNA, the excitation maximum shifts to 460 nm (blue) 488 nm and the emission maximum shifts to 650 nm (red) (i.e. >630 nm).
- Orange: Acridine Orange will also enter acidic compartments such as lysosomes and become protonated and sequestered.
- In these low pH conditions, the dye will emit orange light when exited by blue light.
AO bound to ssDNA by electrostatic forces
+ forming aggregates with other AO molecules

AO binds to dsDNA by intercalation

Procedure flow cytometry with AO

200 ul. frozen sample

Thaw - one at a time

Why?

-20°C, -196 -80

Why?

Dilute to 1-2 ml/ml

Freeze

Why?

5000 events = spermatozoa to be measure in duplicates, 200 spermatozoa per second if not, dilute the sample.
Dilution

- TNE (Tris-NaCl-EDTA) buffer
- 0.01M Tris-HCl (Sigma),
- 0.15 M NaCl (Sigma),
- 1 mM EDTA (ethylene diamine tetraacetic acid) (Sigma), pH 7.4

Add "acid"

400 uL acid detergent solution to 200 uL TNE diluted sample

Why?
To produce single stranded DNA

Denaturation (dsDNA into ssDNA) can be induced by elevated temperature alkali acid solvents some drugs
In somatic cells:
Acid denatures DNA.
Plus the idea that this will be enhanced at strand break points.

NB the denaturation increases with time

Production of single stranded DNA is stopped and AO given!
At 30 s exactly add solution pH 6.0 with AO

AO 6 mg/L in pH 6.0:
0.1 M citric acid (Sigma),
0.2 M Na2PO4 (Sigma),
1 mM EDTA (Sigma),
0.15 M NaCl, pH 6.0
Collection of green fluorescens

The proportion of red light of all light emitted. (red/red+green)
AO itself can induce strand-breaks and cause single stranded DNA

- AO emits light (= energy) that can
- 1) induce strand breaks
- 2) Cause DNA-denaturation and AO is then stacked on emitting red

Consequence:
- Green light decrease
- Red light increase, but fades more rapidly than green

Consequences for measurements?

- Bound to ds DNA green G+
- Bound to ss DNA red R+
- The amount (intensity) of red a question of stacking R+

- + light
  - Decreases ds DNA G-
  - And increases ssDNA R+
  - Red fades > green R-

Experiments: One

- How is the AO-assay influenced by sperm storage in seminal plasma
- From 30 min post ejaculation until 24 hours post-ejaculation.
- (NB Time interval 0 to 30 minutes is not covered here)
DFI changes (increases) upon sperm storage in seminal plasma

Figure 1: Defragmentation index (DFI%) increased significantly during 24 hour storage in original seminal plasma (N=13, P<0.01). No significant difference was observed in the proportion High Green (HG%).

Experiments: Two
Can DFI% diminish by induced S-S-superstability?

DFI changes (decreases) after sperm zinc depletion

Conclusion AO-staining

- Red staining (High DFI%) is no good and tells about a combination of
- Accessability to DNA,
- Ability to denaturate DNA and
- the relative amounts of ss and ds stranded DNA
- Low red could be either a good sign or mask bad sign due to SS-superstabilization.
- Thus there is the risk of false negatives!

Aniline Blue staining of ejaculated spermatozoa exposed to buffer and buffer with DTT 5 min

Superstabilization results in low accessibility

False negatives

Calls for more methodological work and standardisation
Consequences of Reported DNA damage in the male germ line

- Reduced pregnancy rates following natural or assisted conception
  [Chohun et al., 2006; Duran et al., 2002; Bungum et al., 2004]

- Impaired fertilization
  [Sakkas et al., 1998; Morris et al., 2002; Virro et al., 2004]

- Disrupted preimplantation development
  [Sakkas et al., 1998; Morris et al., 2002; Virro et al., 2004]

- Increased rates of abortion
  [Saleh et al., 2003; Carrell et al., 2003]

- Increased rates of disease in children and young adults – eg cancer, complex neurological conditions
  [Ji et al., 1997; Aitken and Krausz, 2001; Edwards and Ludwig, 2003; Aitken, 2004]

Summary from Armand Zini: Usefulness of Sperm Chromatin Tests in the Context of Infertility Treatment: IUI, IVF, ICSI

in Sperm DNA organization, protection and vulnerability – from basic science to clinical application

Stockholm 19‐22 May 2009

Sperm DNA damage and

IUI pregnancy: strong negative impact (OR = 9.9)
Positive predictive value: 97% no PR (2% PR)
Negative predictive value: (24% PR)
One valid study: Bungum 07

IVF pregnancy: modest negative impact (OR = 1.6)
Positive predictive value (PPV median): 74% no PR (26% PR)
Negative predictive value (NPV median): (34% PR)
Clinical significance of an 8% difference in PR?

ICSI pregnancy: no effect

IVF-ICSI pregnancy loss: moderate impact (OR = 2.5)
What do we learn?

• >30% DFI in ejaculates had low (1/9th) pregnancy rate after insemination with gradient separated spermatoza (Bungum et al 2007).
• One study- no rush!!!
• The gradient selected spermatoza used, had mean DFI of some 4% (Bungum et al 2008).
• Thus, the bad IUI results were obtained with spermatoza having "very good" DFI but coming from ejaculates with high DFI.
• They carry the problem without showing it in the assay.
• False negatives? Superstabilized? Or is the lesson to never run AD FACS if not on whole semen?

What do we learn more?

• Samples with sperm DFI%> 30 in semen show 
  • after gradient centrifugation DFI% 4-5% and results in (Bungum et al 2007).
  • 3 % PR after IUI
  • 27% PR after IVF
  • and significantly best
  • 40,5% PR after ICSI.

Severely damaged sperm DNA should be injected in oocytes -- makes poor physiology

• Does it mean that in samples with "damaged DNA-messages" also have comprimised performance never reaching the oocyte in IUI or IVF.
• Or that DFI mostly tells about an open vulnerable chromatin that will be damaged upon a long journey and benefits from direct injection?

Therefore, as clearly stated in the SICRA guidelines (Bowen et al, 2005), SICRA analysis should be performed on raw semen or sperm.
Final remarks 1

- Many methods have been designed to characterize the “integrity of the sperm DNA”.

- The original protocols developed and validated on somatic cells and not on sperms.

Final remarks 2

- None of the protocols take into consideration that the availability to sperm chromatin undergoes severe changes with respect to its degree and type of stabilization (disulfide-bridge dependent, zinc-dependent).

- Nor that these changes are influenced by the ejaculatory sequence and the time of exposure to seminal plasma.

Final remarks 3

- A positive signal (pos TUNEL, AO-red, Increased DFI%, Toluidine+,Sperm swelling in SDS, positive COMET) tells that the sperm chromatin is available and susceptible to damage and probably damaged.

- A negative signal can be true or false due to superstabilization. A zinc-deficient chromatin is both vulnerable and likely to undergo excess superstabilization by S-S, which in turn decreases its availability and gives a false negative signal.
Clinical importance and the future

- Conclusions about the clinical value of the methods discussed. Few studies, mechanisms mainly unknown, changes after ejaculation not taken in account resulting in false negatives.
- Which are the future questions to be asked to the sperm chromatin? Is DNA damaged? Is DNA vulnerable? Direct measurement of Oxidative DNA damaged 8-OHdG. Incorporation of CMA3?
- Where do we go? Increase our knowledge about sperm chromatin organization and how it is affected after ejaculation. Learn how to select spermatozoa in physiological ways. Standardize methods.

And...what about

- "The normal" Methylation of paternal DNA
- "The normal" Acetylation and Methylation of Sperm Histones
- The sperm RNA
- The sperm nuclear Proteins
- The paternal centrosome
- The Factors initiating the placenta
Superstabilization results in low accessibility

False negatives

Calls for more methodological work and standardisation

Or choose physiology!
The first split ejaculate fraction at ejaculation
How Much Assistance Does A Man Need?  
ART for Male Factors

Dr David Mortimer, PhD  
Oozoa Biomedical Inc  
Vancouver, BC, Canada

Commercial Conflicts of Interest Disclosure

David Mortimer has been a full-time freelance consultant since October 1999 and has no commercial or financial interest (e.g. commissions or royalties) in any of the products mentioned in this presentation; royalties from sales of the Cook Sydney IVF culture media go to Sydney IVF. No commercial or financial interest has influenced the statements made in this presentation.

LEARNING OBJECTIVES

1. To understand how laboratory tests of sperm functional potential can:
   (a) identify which men require assistance via ART for sperm dysfunction; and
   (b) provide information pertinent to managing a subfertile couple's treatment options in a cost-effective manner.

2. To understand that ICSI:
   (a) is not necessary for all ART cases; and
   (b) can be disadvantageous if used when not needed.
Subfertility and Getting Pregnant

100 couples commence trying to get pregnant
85 achieve a pregnancy in their 1st year of trying
15 present for infertility investigations:

5 treatable female factors:
- 2 dysovulation
- 2 blocked tubes
- 1 cervical factor
  → endocrine Rx ± IUI
  → surgery or IVF
  → IUI

5 treatable male factors:
- 2 moderate sperm problems
- 1 severe sperm problem
- 1 ASABs
- 1 azoospermia
  → IUI
  → IVF
  → ICSI
  → (MESA/TESE)

5 idiopathic infertility
  → IUI
  → IVF
  → ICSI

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PROCESSES LEADING TO CONCEPTION

Spermatogenesis & spermiogenesis
Epididymal sperm maturation & storage
Ejaculation
Insemination
Penetrate cervical mucus
Sperm transport (& reservoir?)
Capacitation & Hyperactivation

Oogenesis
Folliculogenesis
Oocyte maturation
Ovulation
Oocyte pickup
Oocyte transport

Sperm penetration of cumulus and corona
Sperm binding to zona pellucida
Induction of the acrosome reaction
Sperm penetration of the zona pellucida
Sperm binding to the oolemma
Sperm incorporation into the oocyte
Male pronucleus formation
Syngamy

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Why Not Just Use ICSI On Everybody?

Pros:
• Will minimize the risk of fertilization failure.
• No need to investigate the male or his sperm.

Cons:
• Usually more expensive to the patients.
• More time consuming for the lab (workload / workflow).
• More invasive, with inherent risk of oocyte damage.
• Fertilization rate is lower than can be achieved by IVF in the absence of sperm dysfunction.
• Bypasses the natural fertilization process.
• The male is often not investigated at all (poor medical practice).

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The Argument Against “ICSI For All”

What happens if you use ICSI on someone for whom IVF would work?

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IVF (%)</th>
<th>ICSI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maturity</td>
<td>85%</td>
<td>90%</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>85%</td>
<td>90%</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>90%</td>
<td>90%</td>
</tr>
<tr>
<td>Utilization rate</td>
<td>75%</td>
<td>90%</td>
</tr>
<tr>
<td>Cumulative implantation</td>
<td>114%</td>
<td>91%</td>
</tr>
<tr>
<td>Total failure for patient</td>
<td>23%</td>
<td>23%</td>
</tr>
</tbody>
</table>

The Man Is NOT Just A “Source of Sperm”

Am I not more than this?

• Responsible medical care must consider both partners; it is more than just getting the woman pregnant.

• A “male factor” (i.e. isolated or combined) is present in 50% of subfertile couples.

• There could be co-existing or underlying medical issues in the male partner (e.g. testicular cancer) that will affect his future health.

“STRUCTURED MANAGEMENT”

ISS8, Montreal, August 1998
Workshop 2: Structured Management of Male Infertility
Speakers: David Mortimer, Chris de Jonge
Denny Sakkas, Gabor Huszar, Chris Barratt

Definition:
Structured management protocols for infertile couples determine the appropriate level of medical intervention required to achieve a reasonable chance of pregnancy according to available diagnostic information and the female partner’s age. “Appropriate” is judged in terms of cost, likelihood of a successful outcome (birth of a healthy baby), and all associated risk factors, thereby allowing more effective use of healthcare and personal funds by reducing the application of the most invasive techniques until they have been shown to be necessary.
SPERM ASSESSMENTS

- Semen Analysis
  - Quantitative & qualitative sperm production as indicators of potential sperm (dys)function
  - Anti-sperm antibodies (e.g. Immunobead test)

- Sperm Function Tests
  - Sperm-mucus interaction: Kremer / SCMC / HMT / CASA
  - Capacitation: Hyperactivation (HAmas) / CASA
  - Acrosome reaction: ARIC (A23187 + rhZP3?)
  - Zona binding: Hemi-zona assay / ZBT (rhZP3 beads?)
  - Oocyte penetration: Zona-free hamster eggs (HEPT/SPA)

- Sperm DNA / Chromatin Tests
  - Sperm Chromatin Structure Assay (SCSA™)
  - TUNEL / COMET / Halosperm test / chromomycin A2

SEMEN ANALYSIS

Still the initial basic investigation for all male partners of subfertile couples, but it must be standardized.

Purpose:
- Diagnosis of sterility.
- Diagnosis of infertility.
- Prognosis for fertility.
- Identify treatment options:
  - Surgical treatment;
  - Medical treatment; or
  - Assisted reproductive technology treatment.
- A screening test to help direct the couple’s management.
A Modern View of Sperm Assessments

Principles:
1. The specific diagnosis of sperm dysfunction is of little use without specific treatment options.
2. We cannot expect to be able to predict pregnancy just from looking at the man’s sperm.
3. We are not interested in trying to predict the likelihood of treatment success which are the “customary” outcomes (e.g. fertilization at IVF).
4. Our real interest is in identifying specific risks of failure using particular therapeutic modalities.
   In other words: Will gamete approximation and interaction be successful or not?

SPERM FUNCTIONAL ASSESSMENT (“SFA”)

- Semen Analysis:
  * Comprehensive, as per ESHRE/NAFA (c.f. WHO’99)
  * Detailed sperm morphology, including TZI

- Trial Wash:
  * PureSperm gradient: determine quantitative & qualitative yields

- Anti-Sperm Antibodies:
  * Direct IBT with “GAM” bead, + isotypes if &gt;20% bead-binding

- Computer-Aided Sperm Analysis: (IVOS v12)
  * Mucus penetration-capable sperm population in semen
  * Hyperactivation: “HAmax” assay (includes spontaneous control)

SFA-Based Treatment Recommendations

OK for anything
No apparent sperm dysfunction, hence no treatment is required based on the man’s perceived sperm quality.

IUI recommended
Minor sperm dysfunction likely to affect mucus penetration / migration only, impaired fertilizing ability not suspected.

IVF recommended
Sperm dysfunction likely to affect sperm transport and/or a possible minor impact on fertilization identified.

ICSI needed
Severe sperm dysfunction likely to reduce or prevent fertilization, even in vitro, identified.
Clinical Value of the SFA

“SpermScreen” package (equivalent to the SFA)
( Genesis Fertility Centre, Vancouver, Canada )

- SpermScreen assessment was applied to 485 new referrals.
- Of 266 patients with “normal” WHO semen analysis, 103 (39%) had abnormal results in the other SpermScreen tests, so ICSI recommended.
- But 1267 men with poor semen analysis results had good post-wash motility and hyperactivation (“OK for IVF”).
- Incidence of low/failed fertilization in IVF fell from 6% to just 1% of cycles.


SFA and IUI

“SpermScreen” (Genesis Fertility Centre, Vancouver, Canada)

69 couples treated by IUI (128 cycles) c.f. 56 contemporaneous non-SpermScreen cases (90 cycles); clomiphene or FSH in only 28% of cases.

<table>
<thead>
<tr>
<th></th>
<th>IUI Recommended</th>
<th>IUI Not recommended</th>
<th>SFA Not done</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy per cycle (%)</td>
<td>26/69 10/90</td>
<td>28/128 10/90</td>
<td></td>
</tr>
<tr>
<td>Fecundity rate per cycle (%)</td>
<td>25/69 15/90</td>
<td>25/128 15/90</td>
<td></td>
</tr>
</tbody>
</table>

* by Fisher’s exact test

P < 0.05*

P < 0.01*


SFA and IUI

“Simplified SFA” (no CASA)
Victoria Fertility Centre, BC, Canada

Pregnancy Rate

<table>
<thead>
<tr>
<th></th>
<th>IUI Recommended</th>
<th>IUI Not recommended</th>
<th>SFA Not done</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per IUI cycle</td>
<td>36% (8/21)</td>
<td>20% (9/45)</td>
<td>21% (8/38)</td>
</tr>
<tr>
<td>Per couple</td>
<td>57% (8/14)</td>
<td>31% (9/29)</td>
<td>31% (8/26)</td>
</tr>
</tbody>
</table>

SFA and ICSI Usage

Sydney IVF (Australia), 1998–1999:
- >90% male factor by WHO criteria
- ~35% ICSI / ~65% IVF with <5% failed fertilization
SOURCE: Dr D Mortimer (unpublished data)

Genesis Fertility Centre (Canada), 2000–2003:

<table>
<thead>
<tr>
<th>Period</th>
<th>stims/yr</th>
<th>IU/yr</th>
<th>ICSI</th>
<th>IVF</th>
<th>R/ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>550–600</td>
<td>minimal</td>
<td>60%</td>
<td>40%</td>
<td>active programme</td>
</tr>
<tr>
<td>2001–2002</td>
<td>650–700</td>
<td>~600</td>
<td>40%</td>
<td>60%</td>
<td>almost none</td>
</tr>
</tbody>
</table>
SOURCE: Dr ST Mortimer (personal communication)

Halifax AART (Canada), 2008:
- ICSI recommended: ~40% of couples
- ICSI performed: ~50% of cycles with <2% failed fertilization
SOURCE: Dr D Mortimer (unpublished data)

OPTIMIZING ART LAB SYSTEMS FOR SPERM

- Use a simple, broadly applicable (minimum variations/alternatives), safe, and efficient sperm processing method.
- Select the best spermatozoa: motility / morphology / DNA content.
- Protect from natural & iatrogenic ROS-induced damage that can affect their fertilizing ability and/or DNA integrity.
  * Use safe, optimized sperm preparation methods, e.g. optimized density gradient centrifugation.
- Support sperm function:
  * Use media that are optimized for sperm physiology (not mouse embryos!).
  * Provide adequate glucose, calcium, bicarbonate, and albumin for capacitation and fertilization.
- Optimize ICSI (if required): Technique / Correct timing

Origins of Human IVF Culture Media

- Media for somatic cells: e.g. EBSS, MEM, Tyrode's, TALP
- Media based on oviduct fluid:
  * Tervit's "SOF" (1972)
  * Ménézo’s “B2” (1976)
  * Quinn’s “HTF” (1985) | “Advantage” sequential media system
  * Mortimer’s “STF” (1986) | “M91” | Cook SIVF sequential media
- De novo formulations: e.g. BWW, Bavister's HECM, Biggers' KSOM
- Research animal embryo culture media: e.g. CZB
- Most human IVF media were developed for culturing in-vivo produced mouse zygotes (i.e. not even IVF-derived mouse zygotes) . . .
Sperm capacitation in vitro requires:

- Separation from seminal plasma (decapacitation factor)
- A "capacitating" culture medium needs to have:
  - Physiologically balanced salts to be isotonic and support general sperm homeostasis.
  - Glucose, usually ~5 mM (range 2.8–6.7 mM)
  - Bicarbonate ions, usually 25 mEq/l
  - Calcium ions (range 1.7–3.0 mEq/l)
  - Albumin as a sterol acceptor: minimum of 10 mg/ml (mid-cycle oviduct fluid contains about 30 mg/ml, serum ~45 mg/ml)

Culture Conditions for Capacitation

Seminal Plasma and Fertilizing Ability

The need for prompt and efficient sperm preparation:

- max 30 min

Sperm Hyperactivation

<14% HA indicative of need for high sperm numbers at IVF

Hyperactivation agonists include progesterone and pentoxifylline.

- "HAmax" assay:
  - Agonist” gives within 10% of the maximum spontaneous hyperactivation for >90% of men after 1 hr incubation.
  - 1 µg/ml P4 + 3.6 mM POF in IVF Medium

Spontaneous hyperactivation is very variable both between men and over time.
SPERM PREPARATION: Basic Principles

- Spermatozoa must be separated from seminal plasma:
  - Prolonged exposure to SP declines in motility and vitality.
  - Washing removes decapacitation factor(s) and prostaglandins.
  - Culture media support/promote capacitation (if required).
- Separate the functional spermatozoa in semen from:
  - Abnormal, senescent and dead spermatozoa.
  - Germinal line cells, leucocytes, other cells.
  - Residual cytoplasmic masses, particulate debris, etc.
- Select a highly motile “more functional” sperm population:
  - Selection for normal sperm morphology.
  - Minimize seminal microbiological/viral contaminants.
  - Avoid iatrogenic damage to sperm function/DNA; use a “safe” washing method (2-layer density gradient + 1 wash).

Sperm Survival and Senescence

- All sperm have a finite lifespan, which varies between men and within an ejaculate (also normal vs abnormal sperm).
- Prolonged exposure to seminal plasma is deleterious.
- Avoid exposure to deleterious conditions during and after processing:
  - Sperm have a high metabolic rate under capacitating conditions: can lead to ROS generation / “burn-out”.
- Once capacitated, sperm are highly labile: can lead to spontaneous and induced acrosome reactions:
  - Acrosome-reacted sperm cannot fertilize.
  - Sperm die soon after the acrosome reaction in vitro / in vivo.
Sperm Preparation & Optimizing Outcome

• Collect semen specimens for therapeutic uses at the clinic to control conditions & minimize processing delay (ideally <30 min).

• For IUI:
  • process as soon as possible after liquefaction;
  • use a non-capacitating “holding” medium (e.g. HEPES); and
  • hold at room temperature to slow sperm metabolism.

• For IVF/ICSI:
  • Ideally collect after the OPU and then process immediately after liquefaction.
  • ICSI: same as for IUI.
  • IVF: need to be in a capacitating medium at 37°C. (If semen must be obtained early, can hold prepared sperm as for IUI sperm until 2 h before insemination, then wash into IVF medium.)

CONCLUSIONS

THE MAN IS NOT JUST A SOURCE OF SPERM!

Is the appropriate level of technology (and hence cost) employed for each couple?

• Appropriate use of IUI as a first-line treatment?
  — Fecundity rates of ~25% in suitable patients.

• Unnecessary use of ICSI?
  — Rarely needs to exceed 40% of cases.
  — In optimized labs ICSI generates fewer embryos/cycle than IVF.

• Appropriate use of IVF?
  — Requires robust andrology lab workup before treatment.

BIBLIOGRAPHY


