eshre

SCIENCE MOVING
PEOPLE
MOVING SCIENCE

"Evaluation of the man in the infertile couple"

SPECIAL INTEREST GROUP
ANDROLOGY

8

28 June 2009
Amsterdam
The Netherlands

PRE-CONGRESS COURSE 8

Organised by the Special Interest Group Andrology

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PRE-CONGRESS COURSE 8 - PROGRAM

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

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

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
    Oozoa 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

- Jequier, A. M. (2006) The importance of diagnosis in the clinical management of infertility in the male. *Reproductive biomedicine online*, **13**, 331-335.

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

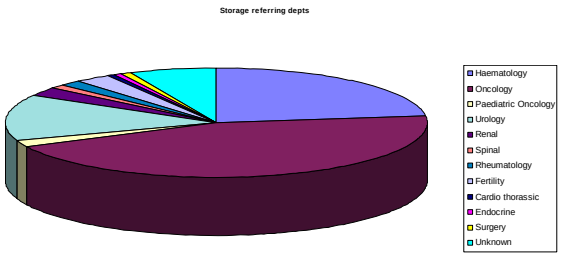
Cryopreservation - what for?

- 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

Malignant Disease

- Carcinoma
- Sarcoma
- Germ cell
- Lymphoma
- Leukaemia

Non-malignant

Autoimmune diseases

- Nephritis/Nephrotic syndrome
- SLE
- Rhematoid Arthritis
- Multiple sclerosis
- Myelodysplastic syndrome
- Aplastic anaemia

Chemotherapy

Bone Marrow***** Transplantation

Lymphoma***
Leukaemia***
Sarcoma***
Carcinoma**
Testicular Teratoma**
Testicular Seminoma*

Agents (cumulative dose for effect)	Effect
Chlorambucil (1.4 g/M^2)	Prolonged azoospermia
Cyclophosphamide (19 g/M^2)	
Procarbazine (4 g/M^2)	
Melphalan (140 mg/M^2)	
Cisplatin (500 mg/M^2)	
BCNU (1 g/M^2),	Azoospermia in adulthood after treatment prior to puberty
CCNU (500 mg/M^2)	
Busulfan (600 mg/M^2)	Likely to cause prolonged azoospermia, but given with other highly sterilizing agents
Ifosfamide (42 g/M^2)	
Nitrogen mustard	
Actinomycin D	
Adriamycin (770 mg/M^2)	Reported to be additive with above agents in causing prolonged azoospermia, but cause only temporary reductions in sperm count alone
Thiotepa (400 mg/M^2)	
Cytosine arabinoside (1 g/M^2)	
Vinblastine (50 mg/M^2)	

Taken from: Pacey & Tomlinson (2009). Sperm banking: theory and practice. Cambridge University Press

Testicular Radiation Dose	Effect on Sperm Count
<0.15 Gy	None detectable
0.15 - 0.6 Gy	Transient oligospermia
0.6 - 2 Gy	Azoospermia (usually reversible)
>2.5 Gy[a]	Azoospermia (generally permanent)
8 Gy[b]	Permanent azoospermia in 85% of men

[a] Given as fractionated radiation over 4 weeks

[b] Given as a single dose or in up to 6 fractions

Seminoma, HL para-aortic nodes

Lymphoma (pelvic)

BMT

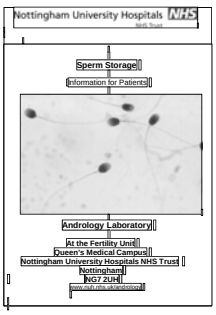
Taken from: Pacey & Tomlinson (2009). Sperm banking: theory and practice. Cambridge University Press

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

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 9-11)
- Tanner III
  - TV 6-12 ml; scrotum enlarges; penis lengthens to about 6 cm, pubic hair more coarse/curly, begins to extend laterally (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


CONSENT TO THE STORAGE OF SPERM

Specify

- Storage period
- Fate of the sperm
  - death
  - mental incapacity

Man with partner

- Consent to treatment

Sperm Storage - Consent


CONSENT TO THE USE OF SPERM AND USE AND STORAGE OF EMBRYOS IN OWN TREATMENT OR RESEARCH

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

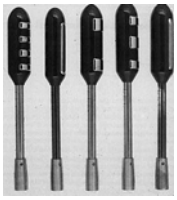
Semen Sample Production

- 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

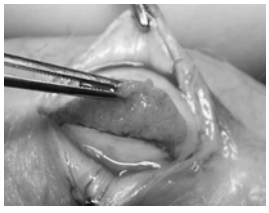


Alternatives

- 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

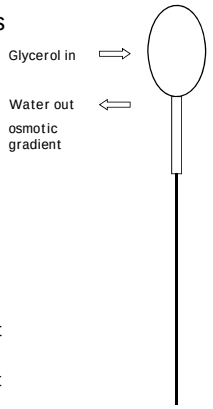
Addition of 6-7% Glycerol

- 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)



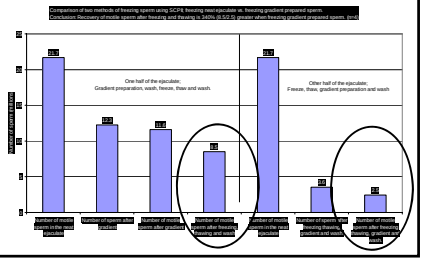
Semen Processing – Can we do better?

- Embryo:Sperm survival >80% : <50%*

*End point measurement - variable
Tendency to over-estimate motility
Incentive to improve sperm freezing

Gradient Preparation

Prior to freezing



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

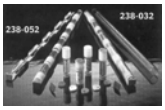
Table 1. Mean donor sperm yields from 5 donors (10 specimens) prepared using Puresperm™ prior to cryopreservation

Donor	Pre Freeze	Post Prepared	Post thaw	% yield
N=10	Concentration of progressively motile sperm x10^6/ml			
1	31.6	35.2	14.15	45.05
2	69.9	50.6	14.87	22.9
3	42.9	32.9	13.6	33.44
4	33.1	28.6	8.8	25.8
5	25.7	39.9	11.0	43.6

Table 1. Yields from 5

Packaging - for long term storage

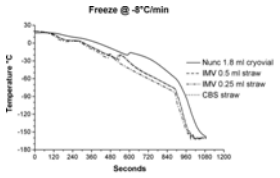
Safe, sterile and suitable container in LN2
Used for a single ART treatment
Permit uniform cooling of sample
Easily Filled and sealed
label clearly/easily
Batch Traceable
Robust and impermeable at -196°C

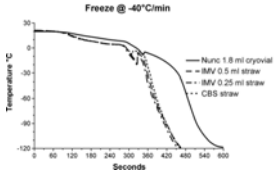


Permit uniform cooling/warming

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





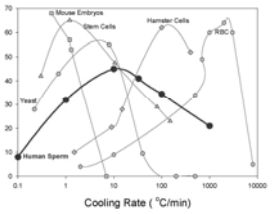
Mortimer, RBM online (2004)

Requirements of sperm packaging

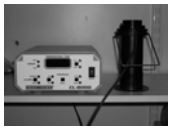
	PTEG	VIALS	CBS
Safe, sterile and suitable container in LN2	☑	☑	☑☑☑
Used for a single ART treatment	☑☑☑	☑☑☑	☑☑☑
Permit uniform cooling of sample	☑☑☑	☑	☑☑☑
Easily Filled and sealed	☑	☑☑☑	☑☑
label clearly/easily	☑	☑☑☑	☑☑☑
Batch Traceable	☑	☑☑☑	☑☑☑
Robust and impermeable at -196°C	☑	☑	☑☑☑

Sperm Cooling

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



Sperm Cooling controlled rate freezing

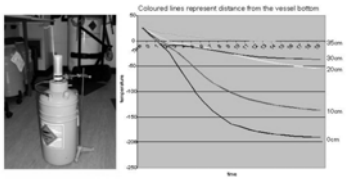


- Verifiable (validated)
- cooling rate
- Repeatable
- Quality Assurance

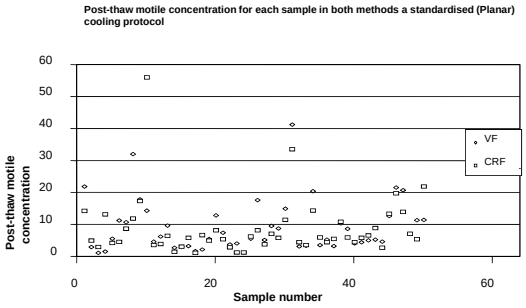
- Blown vaporised nitrogen (Planar)
- Chamber immersed in LN2 (Cryologic)
- Nitrogen free (Stirling engine)

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 (patient samples)

CRF 12.3 vs VF 10.5 NS

Sperm Storage

- Long term (40years) storage requires temperatures <-137°C.
- Below the 'glassy transformation' temperature of water (136Kelvin)
- 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

- Disadvatages
  - 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

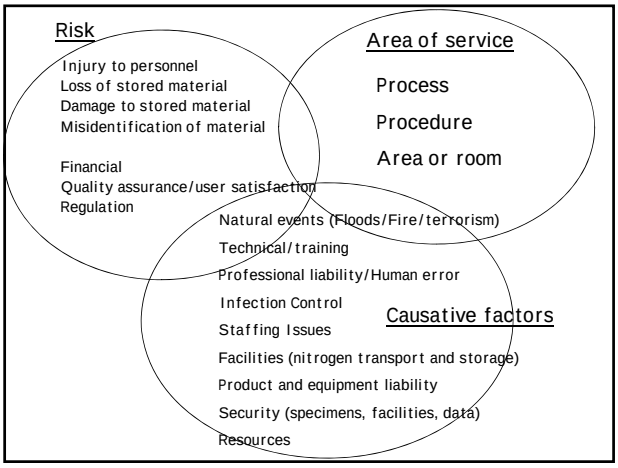
Sperm Thawing

Thaw rate

Affected by

1. Packaging
2. Diluent
3. Thaw environment

- 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



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

- Esteves SC, Sharma RK, Thomas AJ et al. Improvement in motion characteristics and acrosome status in cryopreserved human spermatozoa by swim-up processing before freezing. Hum Reprod 2000; 15: 2173-79.
- Clarke GN, Liu DY, Baker HW. Improved sperm cryopreservation using cold cryoprotectant. Reprod Fertil Dev 2004; 15: 377-81.
- Crister JK, Huse-Benda AR, Aaker DV et al. Cryopreservation of human spermatozoa I I I The effect of cryoprotectants on motility. Fertil Steril 1988; 50: 314-20.
- Devireddy RD, Swanlund DJ, Roberts, KP et al. The effect of extracellular ice and cryoprotective agents on the water permeability parameters of human sperm plasma membrane during freezing. Hum Reprod 2000; 15: 1125-35
- Gao DY, Liu J, Liu C et al. Prevention of osmotic injury to human spermatozoa during addition and removal of glycerol. Hum Reprod 1995; 10: 1109-22.
- Mortimer, D. (2004). Symposium: Cryopreservation and assisted human conception Current and future concepts and practices in human sperm cryobanking. RBM online 9, No 2.
- Meistrich M (2009) Effects of antineoplastic and other medical treatments on sperm production: in Sperm Banking Theory and Practice, Pacey AA and Tomlinson MJ (2009)., (Eds). Cambridge University Press
- Pacey AA and Tomlinson MJ (2008). Sperm Banking Theory and Practice, (Eds). Cambridge University Press
- Nijs M, Ombelet W. Cryopreservation of human sperm. Hum Fertil 2001; 4: 158-63.
- sperm better than standard vapour freezing. Fertil Steril 1990; 53:1072-75.
- Royere D, Barthelemy C, Hamamah S and Lansac J (1996). Cryopreservation of spermatozoa: a 1996 review. Hum Reprod Update 2, 6, 553-559
- Tomlinson MJ, Morroll D. Risks associated with cryopreservation: a survey of assisted conception units in the UK and Ireland. Hum Fertil 2008; 11:33-42

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

ESHRE 2009 Precongress Couse
"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

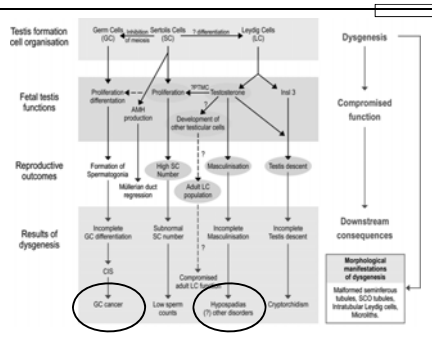
This presentation aims to set focus on following aspects of male infertility:

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) ?



Sharpe and Skakkebaek, Fertil Steril 2008

TDS

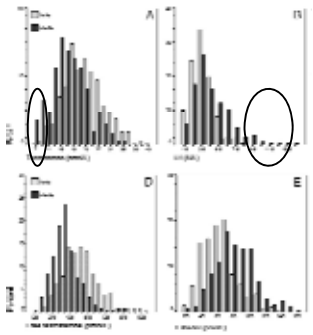
- We should expect (at least some) men with poor semen quality to be at increased risk of:
  - Hypogonadism;
  - Testicular Germ Cell Cancer (TGCC)

  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

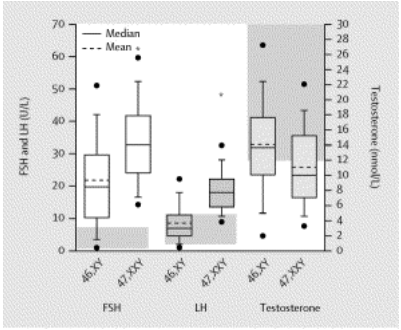
Male infertility and Leydig cell function



Andersson *et al,* JCEM 2004

For some subgroups of infertile men, hypogonadism is known/expected

Klinefelter's syndrome



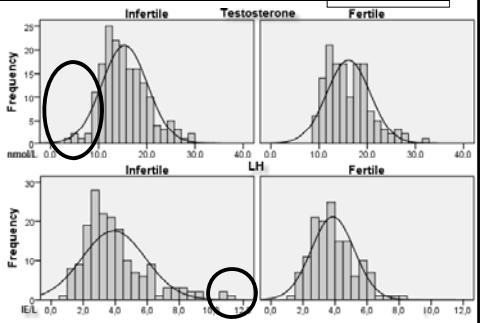
Lanfranco *et al,* Lancet 2004

Risk of hypogonadism in Childhood Cancer Survivors

	N	Hypogonadal	OR	p
Controls	141	6 (4.3%)	1.0	Reference
CCS	144	33 (23%)	6.7	<0.001
Leukemias	26	8 (31%)	10	0.001
Brain tumors	31	6 (19%)	5.4	0.006
Lymphomas	32	10 (31%)	10	<0.001
Testicular cancer	9	2 (22%)	6.4	0.04
Wilms' tumor	11	1 (9.1%)	2.3	0.47
Others	35	6 (17%)	4.7	0.012

Romerius *et al*, submitted

Increased proportion of hypogonadal men among those with unexplained male subfertility



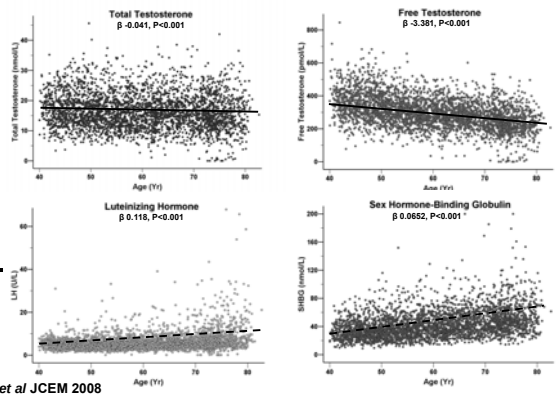
Giwercman *et al*, unpublished

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^2/m)

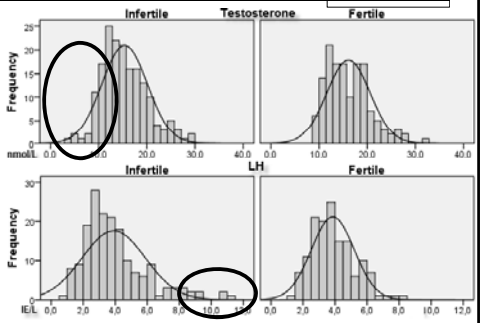
  but not related to
  - Sperm concentration, FSH, testis volume

Hormones bys Age (n = 3369)



Wu *et al* JCEM 2008

A high proportion of hypogonadal men may become hypogonadal by age

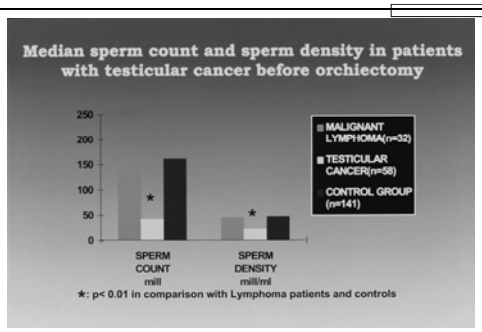


OR=3,0; 95%CI 1,17-7,82

Conclusion 1

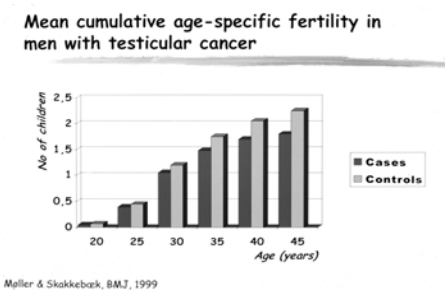
- Male coming for infertility investigation are at increased risk:
  - Being hypogonadal;
  - Becoming hypogonaal by age;
- Due to relatively unspecific symptoms, the patient and the doctor may not be aware of the hypogonadism at least hormone values are assessed

Sperm number prior to c testis treatment



Petersen *et al*, JCO 1999

Fertility prior to c testis treatment

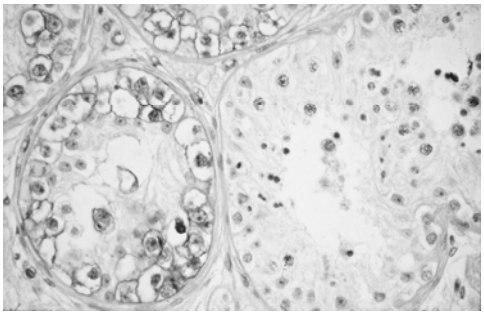


Møller & Skakkebæk, BMJ, 1999

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

Normal

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;
- Eraly 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 assessmen after 1-2 year;
- Testicular microlithiasis – testicular biopsy should be considered.

List of references

- 1. Andersson AM, Jorgensen N, Frydelund-Larsen L, Rajpert-De Meyts E, Skakkebaek NE. Impaired Leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. J.Clin.Endocrinol.Metab 2004;89(7):3161-67.
- 2. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. Lancet 2004;364(9430):273-83.
- 3. Mancini M, Carmignani L, Gazzano G, Sagone P, Gadda F, Bosari S, et al. High prevalence of testicular cancer in azoospermic men without spermatogenesis. Hum Reprod 2007;22(4):1042-6.
- 4. McLachlan RI, Rajpert-De Meyts E, Hoei-Hansen CE, de Kretser DM, Skakkebaek NE. Histological evaluation of the human testis--approaches to optimizing the clinical value of the assessment: mini review. Hum Reprod 2007;22(1):2-16.
- 5. Moller H, Skakkebaek NE. Risk of testicular cancer in subfertile men: case-control study. BMJ 1999;318(7183):559-62.
- 6. Petersen PM, Giwercman A, Hansen SW, Berthelsen JG, Daugaard G, Rorth M, et al. Impaired testicular function in patients with carcinoma-in-situ of the testis. J Clin Oncol 1999;17(1):173-9
- 7. Raman JD, Nobert CF, Goldstein M. Increased incidence of testicular cancer in men presenting with infertility and abnormal semen analysis. J Urol 2005;174(5):1819-22; discussion 22. '
- 8. Sharpe RM, Skakkebaek NE. Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects. Fertil Steril 2008;89(2 Suppl):e33-8.
- 9. Walsh TJ, Croughan MS, Schembri M, Chan JM, Turek PJ. Increased risk of testicular germ cell cancer among infertile men. Arch Intern Med 2009;169(4):351-6.
- 10. Wu FC, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neill TW, et al. Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. J Clin Endocrinol Metab 2008;93(7):2737-45.

Many thanks for your attention

Evaluation of the Man in the Infertile Couple

Erectile dysfunction among infertile men – does it exist?

José Mª 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

Speeker (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**

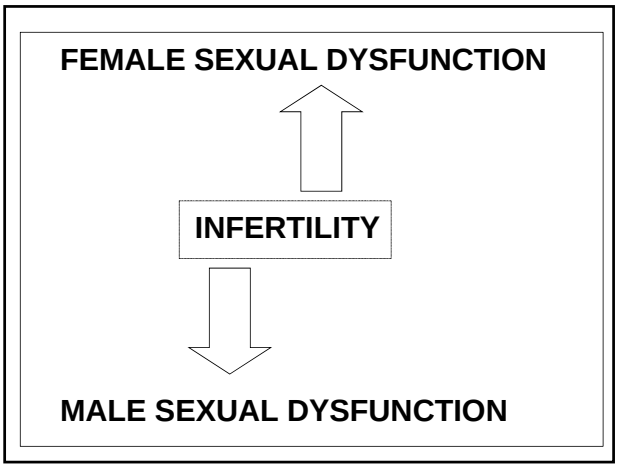
Infertility clinic

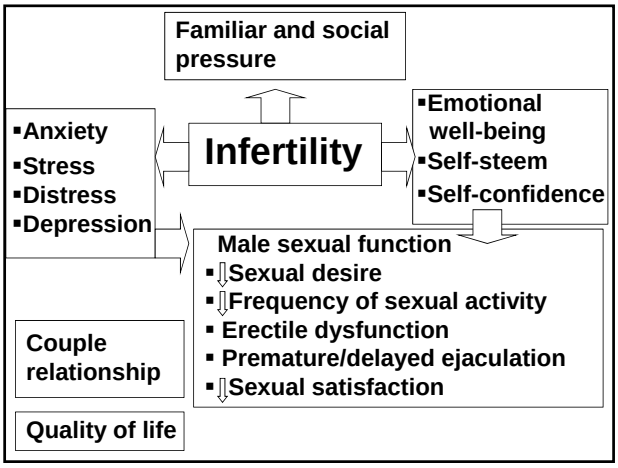
- Patients with ED secondary to infertility
- Patients with ED secondary to different causes

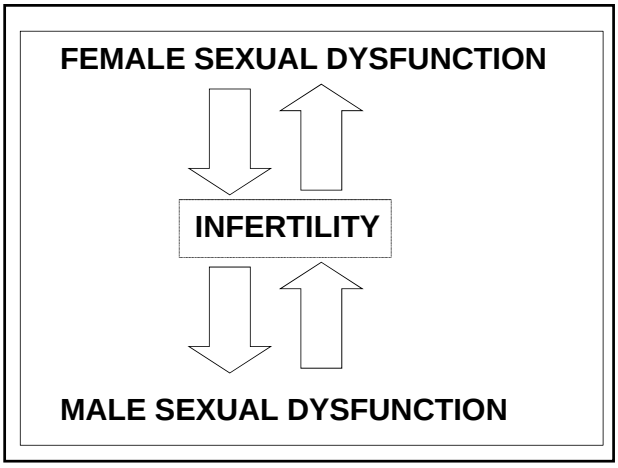
Infertility clinic

- 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


FEMALE SEXUAL DYSFUNCTION
INFERTILITY
MALE SEXUAL DYSFUNCTION


Familiar and social pressure
Infertility
▪Anxiety
▪Stress
▪Distress
▪Depression
▪Emotional well-being
▪Self-steem
▪Self-confidence
Male sexual function
▪ ⇩Sexual desire
▪ ⇩Frequency of sexual activity
▪ Erectile dysfunction
▪ Premature/delayed ejaculation
▪ ⇩Sexual satisfaction
Couple relationship
Quality of life


FEMALE SEXUAL DYSFUNCTION
INFERTILITY
MALE SEXUAL DYSFUNCTION

Infertility clinic

Severe and persistent ED

Inability to penetrate

Loss of erection before ejaculation

Mechanical cause of male infertility

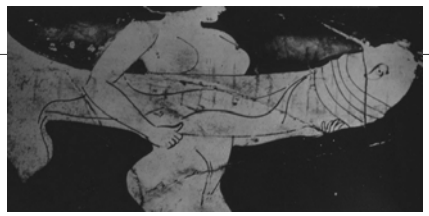
FEMALE SEXUAL DYSFUNCTION

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

Khademi A et al. J Sex Med 2008; 5:1402

International index of ED (IIEF)

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

Erectile function (6 questions)

Score	ED classification
6-10	severe
11-16	moderate
17-25	mild
26-30	normal

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)

Khademi A et al. J Sex Med 2008; 5:1402

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

Male age	No.	%
<20	1	0.02
20-29	137	3.6
30-39	1957	51.6
40-49	1443	38.2
>49	249	6.6

IVI, 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%

IVI, 2009

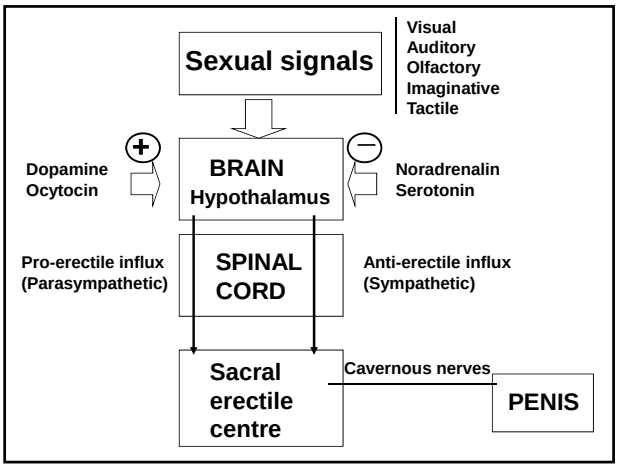
Instituto Valenciano de Infertilidad (IVI)

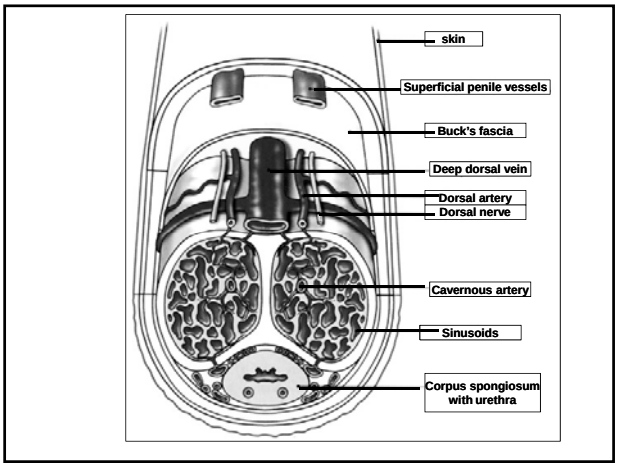
Male sexual function		
Erectile dysfunction		3%
mild	50%	2%
moderate	45%	1.8%
severe	5%	0.2%

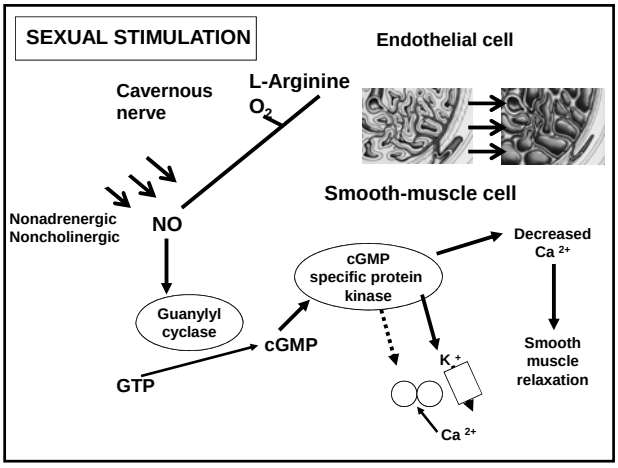
IVI, 2009

Patients with ED secondary to different causes

Penile anatomy and physiology


Sexual signals
Visual
Auditory
Olfactory
Imaginative
Tactile
Dopamine
Ocytocin
BRAIN
Hypothalamus
Noradrenalin
Serotonin
Pro-erectile influx
(Parasympathetic)
SPINAL
CORD
Anti-erectile influx
(Sympathetic)
Sacral
erectile
centre
Cavernous nerves
PENIS


skin
Superficial penile vessels
Buck's fascia
Deep dorsal vein
Dorsal artery
Dorsal nerve
Cavernous artery
Sinusoids
Corpus spongiosum
with urethra


SEXUAL STIMULATION
Endothelial cell
Cavernous
nerve
L-Arginine
O2
Smooth-muscle cell
Nonadrenergic
Noncholinergic
NO
Decreased
Ca 2+
cGMP
specific protein
kinase
Guanylyl
cyclase
cGMP
K +
Smooth
muscle
relaxation
GTP
Ca 2+

Erectile dysfunction

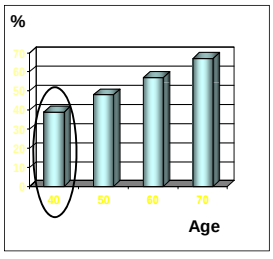
ED is defined as the consistent or recurrent inability of a man to attain and / or maintain a penile erection sufficient for sexual activity

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

How often is erectile dysfunction among young men?

Epidemiology ED

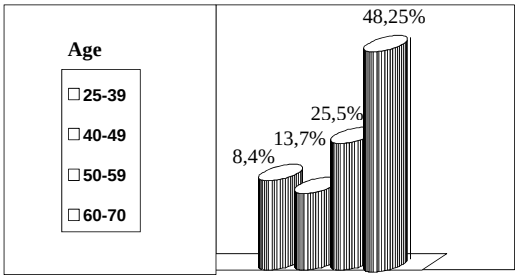
MMAS USA
40-70 yrs 52%



Feldman HA et al, 1994

EDEM study
Spain, 1998

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



ED according to EF-IIEF

Martín Morales A et al, 2001

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

Male age	No.	%
<20	1	0.02
20-29	137	3.6
30-39	1957	51.6
40-49	1443	38.2
>49	249	6.6

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

Etiology and risk factors of ED

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

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

Diabetes mellitus
- Diabetes type 1
- Diabetes type 2

Post-traumatic ED
- Neural and vascular lesions

Endocrine factors
- Hypogonadism
- Hyperprolactinemia and prolactinoma
- Thyroid disorders

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

Medications/RD associated with ED

Antihypertensives
- Thiazide diuretics
- Beta blockers
- Calcium channel blockers

Antiarrhythmics
- Digoxin
- Amiodarone
- Disopyramide

Antidepressants/Neuroleptics
- Tricyclic antidepressants
- Selective serotonin reuptake inhibitors
- Phenothiazines
- Butyrophenones

Recreational substances
- Marijuana
- Cocaine
- Alcohol

Medications with hormonal influence
- Anti-androgens
- GnRH agonists
- Flutamide
- Ketoconazole
- Spirinolactone
- H2 blockers
- Cimetidine
- Estrogens

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
- Tumescense
- 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)

Score	ED classification
6-10	severe
11-16	moderate
17-25	mild
26-30	normal

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

Sexual and medical history

Medical disorders

- Endocrinologic
- Vascular
- Neurologic
- Morphologic
- Psychiatric

Diseases

Risk factors

Surgeries

Medications and recreational drugs

Psychological factors

Diagnosis
Physical examination

Thyroid

Penis

Rectal examination

BC reflex

Weight

Secondary sexual characteristics

Mammary glands

Testes

Blood pressure

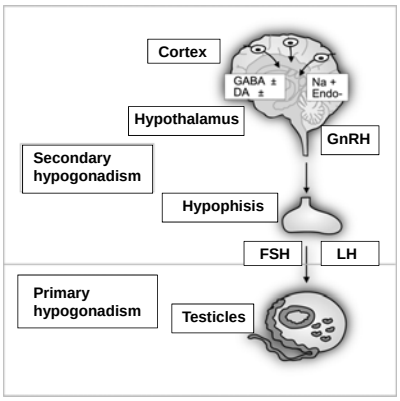
Peripheral pulses

Waist circumference

Diagnosis
Blood tests

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

Hypogonadism



⇩ Testosterone

⇩ FSH/LH

Pathologies
Hypothalamus
Hypophisis

⇧ FSH/LH

Pathologies
Testicles

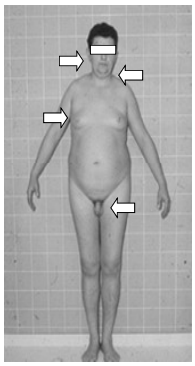
Hypogonadism

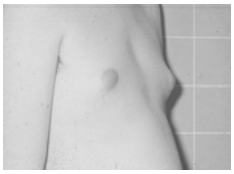
Low Testosterone

Low sexual desire

Erectile dysfunction

Klinefelter's syndrome





1:500 – 1:1000

47 XXY (90%)

XXY/XY (10%)

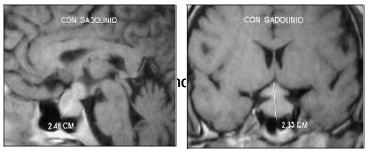
⇧ **FSH, LH**

⇩ **T**

Hypogonadotropic hypogonadism

⇩ **T, LH, FSH**

Magnetic resonance imaging (MRI)



Tumors / other pathologies

Neurogenic ED

- Supraspinal
- Spinal
  - suprasacral >Reflexogenic erection is mantained
    >Erection of short duration, requiring continuous estimulation
    >Incomplete lesion: can maintain erection
  - sacral >No reflexogenic erection
    >No response to psychogenic stimulation
- 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

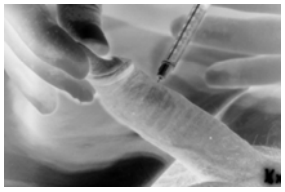
Erectile dysfunction

- History + physical examination
- Laboratory work-up

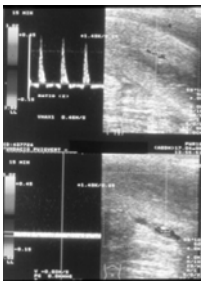
Evident psychogenic etiology	Evident organic etiology	Non-evident etiology
Psychological evaluation	Evaluation therapeutic alternatives	Studies to assess the erection

Diagnosis

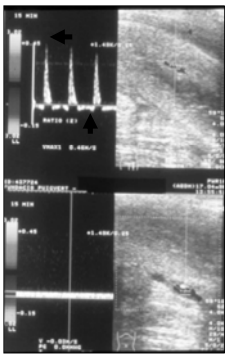
Intracavernous injection test combined with Doppler/duplex ultrasound



Alprostadil (PGE1)



Penile Doppler ultrasound



Normal values

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

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

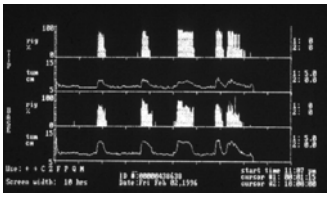
Diagnosis

Nocturnal penile tumescense (NPT)

1 - 3 nights

rigidity (%) / diameter (cm)

No / episodies duration

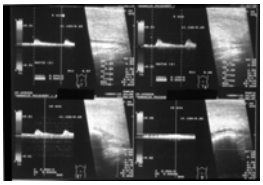


> 10 min

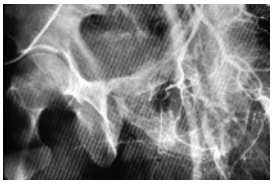
> 60% rigidity

Diagnosis

Pudendal artheriography



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)

Treatment

Psychogenic factors

Psychological evaluation
Psychotherapy

+ / - PDE5 inhibitors

Treatment

Hypogonadism

Hypogonadotropic | **Hypergonadotropic**

FSH + HCG

After fertility therapy

Testosterone replacement treatment

Gel 50 mg/day | **T undecanoate 1000 mg/3 months**

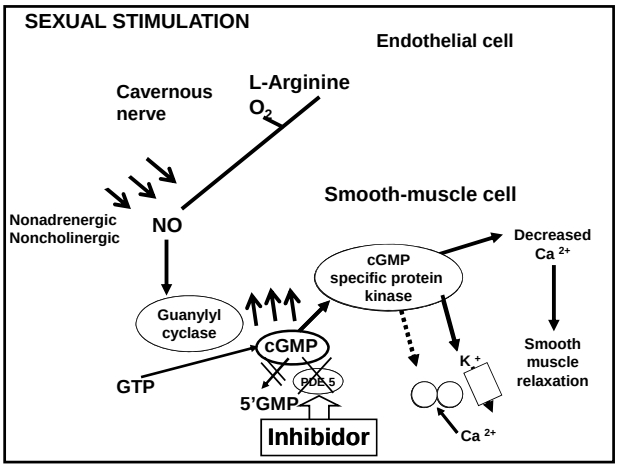
+ / - PDE5 inhibitors

Treatment

First line therapy

PDE5 inhibitors

Sildenafil	**Viagra ®**
Tadalafil	**Cialis®**
Vardenafil	**Levitra®**



PDE5 inhibitors

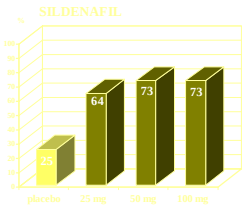
	Time to action	Time of effectivity	Interference with food
Sildenafil (Viagra ®) 25 / 50 / 100 mg	14 min Optimal 1 h	4-5 hs	Yes
Vardenafil (Levitra ®) 10 / 20 mg	11 min Optimal 1 h	4-5 hs	Yes
Tadalafil (cialis ®) 10 / 20 mg 5 mg once a day	16 min Optimal 2 hs	24-36 hs	No

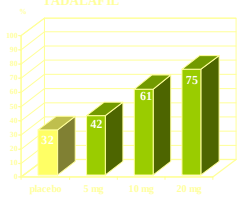
Iniciate with higher doses

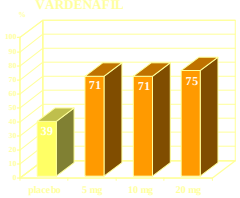
Instructions

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 (%)

Event	Viagra 100	Cialis 20	Levitra 20
Headache	16	15	15.3
Flushing	10	3	11.3
Dyspepsia	7	8	6.3
Nasopharyngitis	4	2	7.3
Vision alterations	3		2.8
Back pain		5	

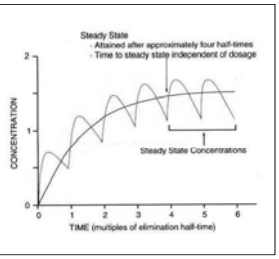
Discontinuations due to : 2 - 4%

PDE5 inhibitors

Tadalafil 5 mg

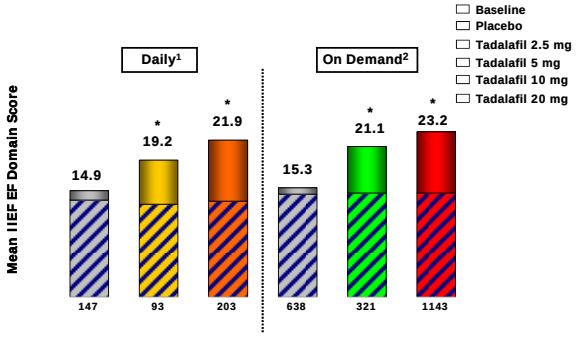
Continous treatment

1 tablet daily



Reduce anxiety

Historical Comparison: IIEF EF Domain Pooled Daily vs. Integrated On Demand

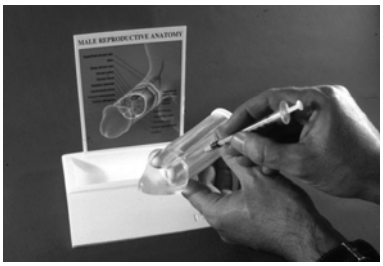


* p<0.001 versus placebo

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

Treatment

Intracavernous injection with alprostadil



10353 patients

Effectivity 73%

Adverse events 5%

Discontinuation 40%

Linet, 1994

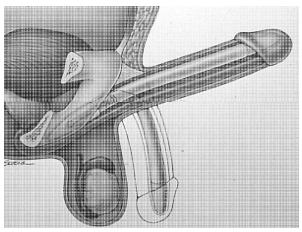
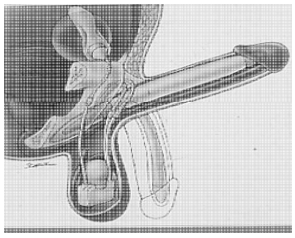
Treatment

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

- Continous treatment with Tadalafil 5 mg / day begining 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 schedduled 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 infrequent in infertile patients, however will increase as the population each day is older**
- **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)

Feldman HA, Goldstein I, Hatzichristou DG et al. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol 1994;151:54

Heruti R, Shochat T, Tekes-Manova D et al. Prevalence of erectile dysfunction among young adults: results of a large-scale survey.
J Sex Med 2004;1: 284

Khademi A, Alleyassin A, Amini M et al. Evaluation of sexual dysfunction prevalence in infertile couples. J Sex Med 2008; 5:1402

Martín Morales A, Sánchez-Cruz JJ, Sáenz de Tejada I et al. Prevalence and independent risk factors for erectile dysfunction in Spain: results of the Epidemiologia de la Disfuncion Erectil Masculina Study. J Urol 2001;166:569

Monga M, Alexandrescu B, Katz SE et al. Impact of infertility on quality of life, marital adjustment, and sexual function. Urology 2004; 63:126

Rosen RC, Riley A, Wagner G et al. The International Index of Erectile Function (IIEF): a multidimensional scale for assessment of erectile dysfunction. Urology 1997; 49:822

References (2)

Montorsi F, McDermott TE, Morgan R et al. Efficacy and safety of fixed-dose oral sildenafil in the treatment of erectile dysfunction of various etiologies. Urology 1999;53:1011

Brock GB, McMahon CG, Chen KK et al. Efficacy and safety of tadalafil for the treatment of erectile dysfunction: results of integrated analyses. J Urol 2002; 168;1332

Porst H, Rosen R, Padma-Nathan H et al. The efficacy and tolerability of vardenafil, a new, oral, selective phosphodiesterase type 5 inhibitor, in patients with erectile dysfunction: the first at-home clinical trial. Int J Impot Res 2001;13:192

Erasmus MC
University Medical Center Rotterdam

MALE ACCESSORY GLAND INFECTION DIAGNOSIS AND TREATMENT

GERT DOHLE, MD, Ph.D
ERASMUS MC, ROTTERDAM
THE NETHERLANDS

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*

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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

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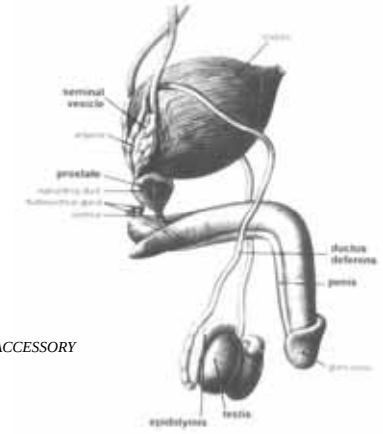

THE MALE ACCESSORY GLANDS

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

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DOES MAGI INFLUENCE MALE FERTILITY? What is the evidence?

- MOST ISOLATED BACTERIA SHOW NO IMPACT ON SPERM PARAMETERS IN VITRO
- LOW BACTERIA COUNTS IS 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

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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.
- - Chlamydia 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.
- - Ureoplasma urealyticum is a commensal in the male reproductive tract.
- - One of the manifestations of MAGI is sperm antibodies.

- ***K. Purvis, E Christiansen. Int J. Androl, 16,1-13, 1993.***

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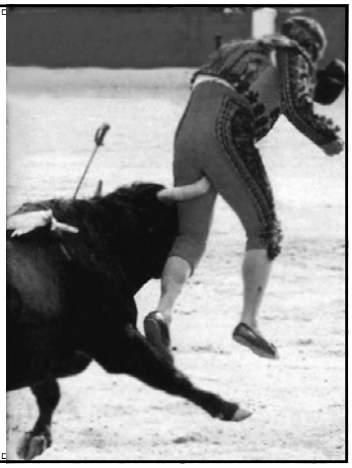
THE PROSTATITIS SYNDROME



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)	

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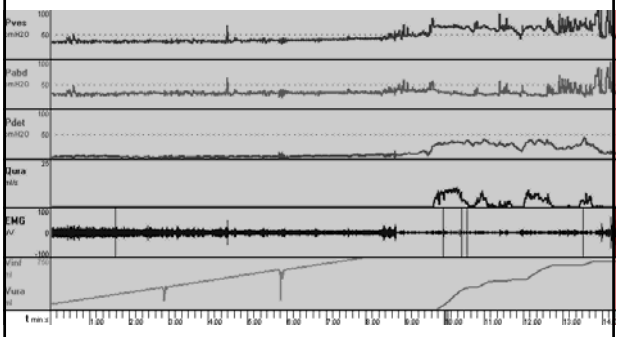
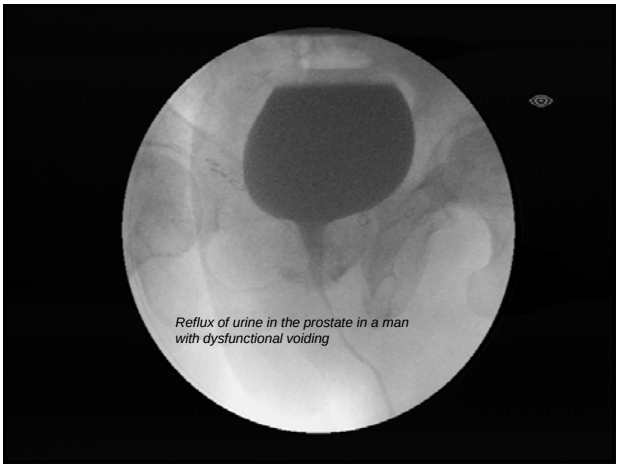
EPS = expressed prostatic secretion; WBC = white blood cells.

Urodynamics of a man with chronic pelvic pain symdrome (CPPS)





Reflux of urine in the prostate in a man with dysfunctional voiding

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 MAGI, CHEMICAL PROSTATITIS (CPPS) AND AUTO-IMMUUN 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

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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

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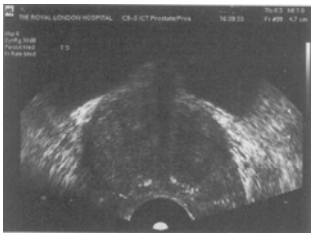

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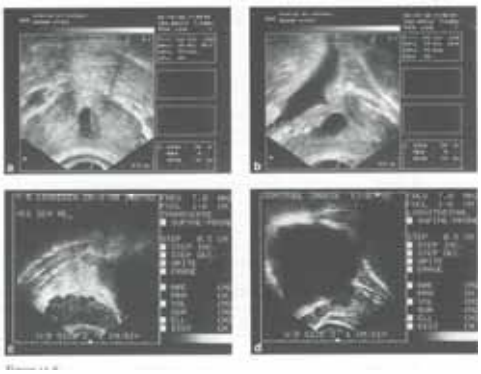
TRANSRECTAL ULTRASOUND OF THE PROSTATE

- 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)

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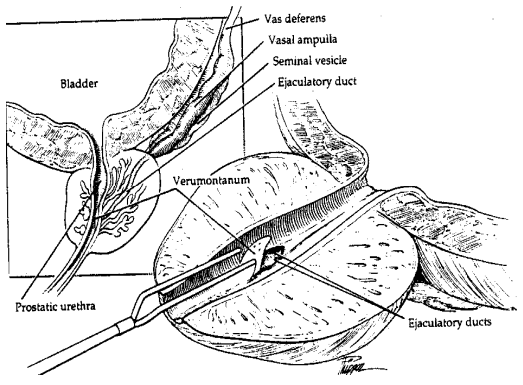

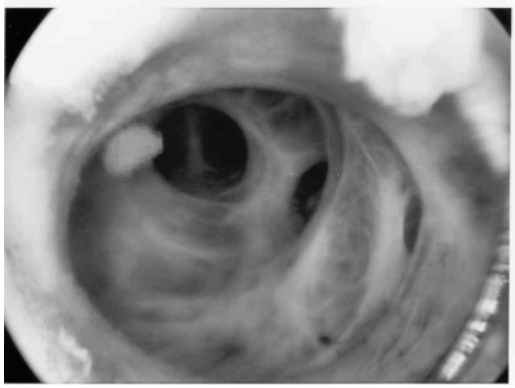

EPIDIDYMITIS

- Etiology:
- - Usually idiopathic
- - Due to obstruction
- - Ascending infection with urethritis/prostatitis
- In young men usually caused by STD`s (Chlamydia, Gonorrhoa)
- 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

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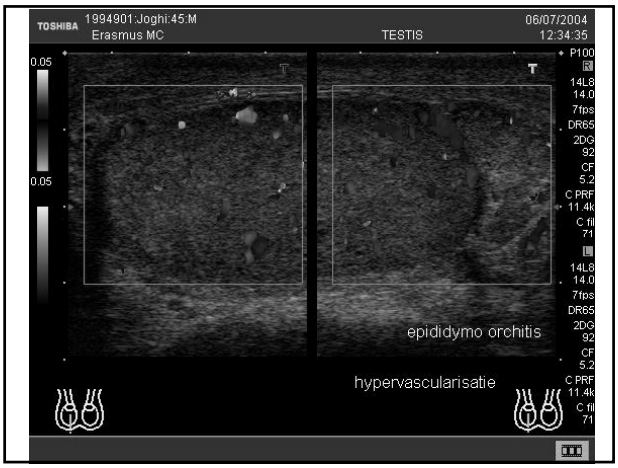

REACTIVE OXYGEN SPECIES (ROS)

- THE DELETERIOUS EFFECTS OF ROS ON SEMEN QULITY 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: ANTI-OXYDANTS ARE ABSENT IN SPERMATOZOA
- - MITOCHONDRIA ARE PARTICULARY 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

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(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/ORCHIDOPEXIA 10(12.8%)
  - MALE ACCESSORY GLAND INFECTION 10(12.8%)

- *Dohle G. R., Andrologia 2003; 35,321-324*

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Signs of a (Partial) obstruction of the seminal path

- Decline in sperm quality in a 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)

Dohle G. R., Urol Res 2003; 31,22-24

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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

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TREATMENT 2

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

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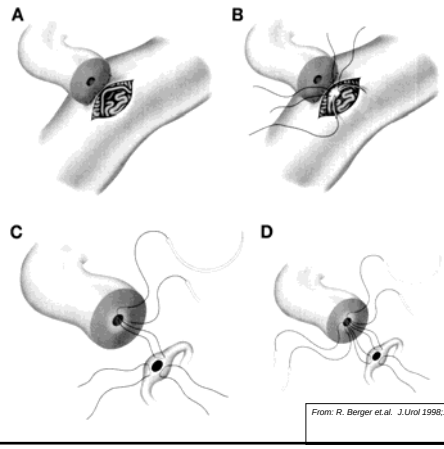
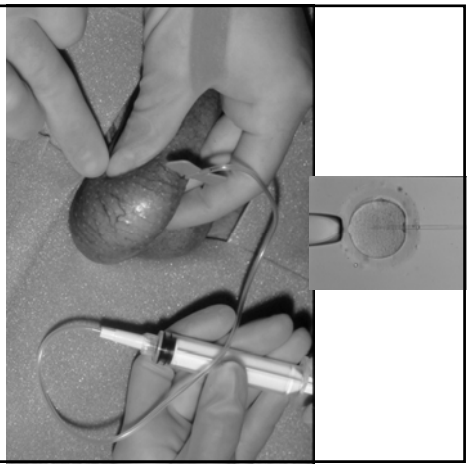


From: R. Berger et.al. J.Urol 1998;159:1951-1953

Percutaneous sperm aspiration for ICSI



KEY REFERENCES

- 1. Purvis K, Christiansen E (1993) Infection of the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. Int J Androl 16: 1-13.
- 2. G.R. Dohle, A. Jungwirth, Z Kopa, A. Giwercman, T. Diemer, T.B. Hargreave. EAU guidelines on male infertility 2009. In: European Association of Urology Guidelines 2009 edition, pp 41-49. ISBN/EAN 978-90-79754-09-0.
- 2. Weidner W, Krause W, Ludwig M. (1999) Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis. Hum Reprod Update 5: 421-432.
- 3. Tremellen K. (2008) Oxidative stress and male infertility-a clinical perspective. Hum Reprod Update 14;3:243-258.
- 4. Everaert K, Mahmoud A, Depuydt C, Maeyaert M, Comhaire F. (2003) Chronic prostatitis and male accessory gland infection--is there an impact on male infertility (diagnosis and therapy)? Andrologia. 35(5):325-30.
- 5. Dohle GR. (2003) Inflammatory-associated obstructions of the male reproductive tract. Andrologia. 3;35(5):321-4.
- 6. Vicari E. (2000) Effectiveness and limits of antimicrobal treatments on seminal leucocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. Hum Reprod 15;12:2536-44.

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Disclosures

- Member of advisory boards of
  - Lilly Netherlands
- Clinical research sponsored by
  - GSK
  - Bayer Schering
  - Prostrakan

ESHRE 280609

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

Prof Dr Eric JH Meuleman
Urologist, Free University Medical Centre

ESHRE 280609

Learning Objectives

- Male hypogonadism several different clinical entities
  - Reproductive medicine ←→ Men's health
- Pathophysiology of male hypogonadism
- Benefits and risks of (testosterone) treatment
- Alternatives

ESHRE 280609

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

Guidelines on male infertility. European Association Urology, march 2009. ISBN:978-79754-09-0

ESHRE 280609

Endocrinological investigation in subfertile men

When ?

•In extreme oligo- and azoospermia

Why ?

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

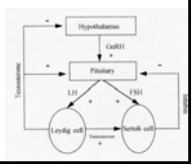
How ?

•LH – FSH - Testosterone

•Prolactine on indication

- •Anosmia
- •Visual disturbances
- •Low Testosterone

•MRI scan of sella tursica



ESHRE 280609

Two diagnostic groups

Hypogonadotroop Hypogonadism

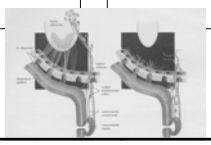
(LH, FSH ↓)

- •Kallmann
- •Idiopathic (IHH)
- •Pituitary Tumor
- •Anabolic steroids
- •Morbid obesity
- •Granulomatous diseases
- •Haemochromatosis

Hypergonadotroop Hypogonadism

(LH, FSH ↑)

- •Testicular dysgenesis
- •Klinefelter
- •Anorchia
- •Castration
- •Cytotoxic medication
- •Irradiation



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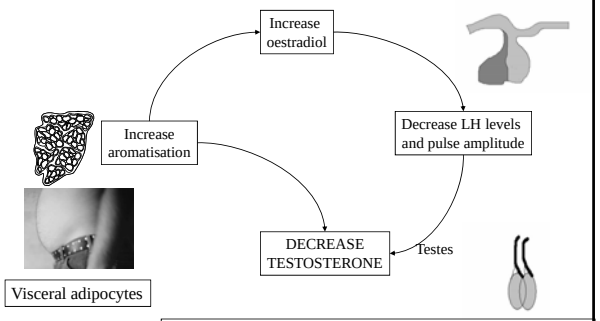
Morbid obesity and fertility



1. Kapoor D et al. *Eur J Endocrinol* 2006; 154: 899–906. 2. Dhindsa S et al. *J Clin Endocrinol Metab* 2004; 89: 5462–5468. 3. Kupelian V et al. *J Clin Endocrinol Metab* 2006; 91(3): 843–850. 4. Kapoor D et al. *Clin Endocrinol* 2005; 63: 239–250. 5. Svartberg J et al. *J Intern Med* 2006; 259(6): 576–582. 6. Hak AE et al. *J Clin Endocrinol Metab* 2002; 87(8): 3632–3639. 7. Shores MM et al. *Arch Int Med* 2006; 166: 1660–1665.

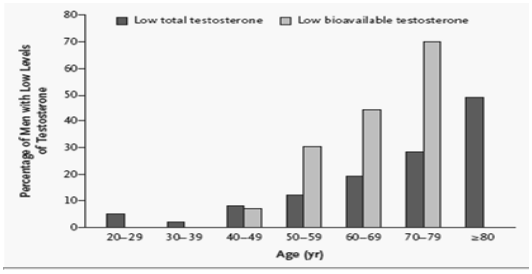
Treatment of hypogonadal men in reproductive medicine

- 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

Guidelines on male infertility. European Association Urology, march 2009.
ISBN:978-79754-09-0

Late Onset Hypogonadism

Late onset hypogonadism



ESHRE 280609

Testosterone Deficiency Syndrome

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

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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

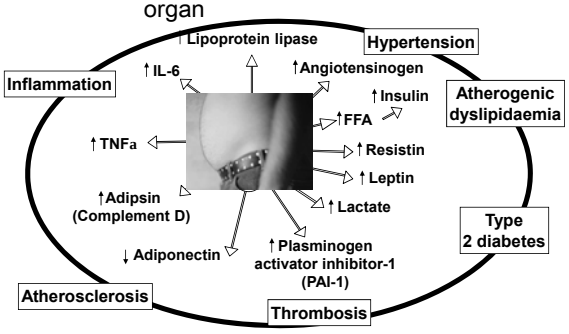
ESHRE 280609

Metabolic syndrome
- Insuline resistance
- Type 2 diabetes
- Accelerated atherosclerosis
- Obesity

Hypogonadism is associated with the metabolic syndrome and accelerated atherosclerosis

Kapoor D et al. *Eur J Endocrinol* 2006; 154: 899–906. Dhindsa S et al. *J Clin Endocrinol Metab* 2004; 89: 5462–5468. 3. Kupelian V et al. *J Clin Endocrinol Metab* 2006; 91(3): 843–850. 4. Kapoor D et al. *Clin Endocrinol* 2005; 63: 239–250. 5. Svartberg J et al. *J Intern Med* 2006; 259(6): 576–582. 6. Hak AE et al. *J Clin Endocrinol Metab* 2002; 87(8): 3632–3639. 7. Shores MM et al. *Arch Int Med* 2006; 166: 1660–1665.

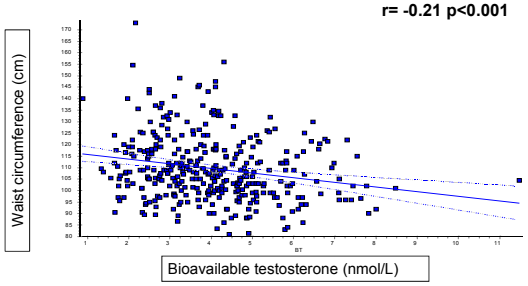
Visceral fat is an active endocrine organ



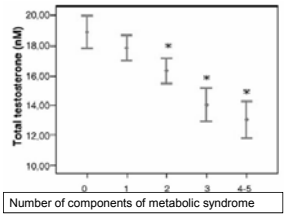
Lyon CJ *et al. Endocrinology* 2003; 144: 2195–2200. Trayhurn P *et al. Br J Nutr* 2004; 92: 347–355.Eckel RH *et al. Lancet* 2005; 365: 1415–1428.

Correlation between bioavailable testosterone and waist circumference

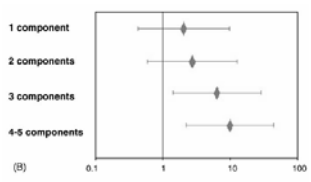
r= -0.21 p<0.001



Total testosterone is correlated with number of risk factors for metabolic syndrome



Number of components of metabolic syndrome



Relative risk of hypogonadism (TT<8.0nmol/L)

In a cohort of 803 male outpatients

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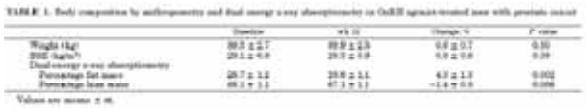
Corona G et al. *Eur Urol* 2006; 50: 595–604.

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



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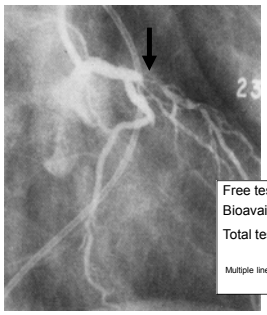
Matthew R et al. *JCEM* 2006; 91(4): 1305–1308

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 BMIAdjusted difference controls vs cases (mean +/- 95% CI)

English et al. Eur Heart J. 2000 Jun;21(11):890-4.

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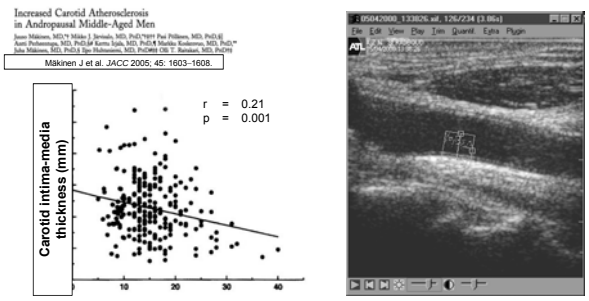
Correlation between serum testosterone and maximum intima-media thickness of the carotid bulb in 236 middle-aged men



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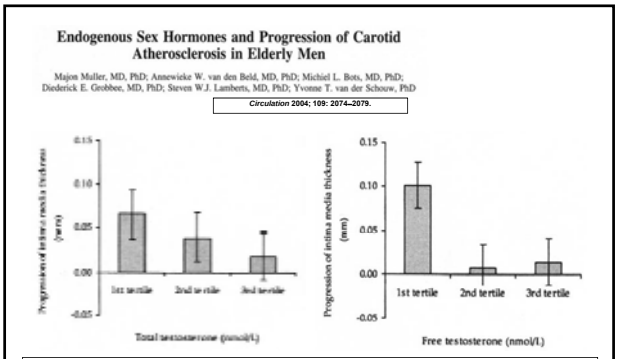

Progression of mean IMT of common carotid artery in tertiles of serum total and free testosterone concentrations (nmol/L)

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Hypogonadism – male sexual functions

The literature

Hypogonadism

Decrease of sexual desire

Decrease of sexual activity

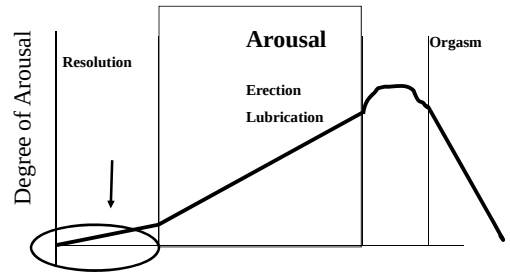
Decrease of fantasy / sleep induced erection

?

ED

ESHRE 280609

Indirect effect of testosterone on arousal



- Testosterone

Human Sexual Response
Kaplan (1979)

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Initiation of Arousal:
"Dual Pathway"

Testosterone

- Internal stimuli
  Erotic Imagery
- External stimuli

AudioVisual
Olfactorial
Tactile

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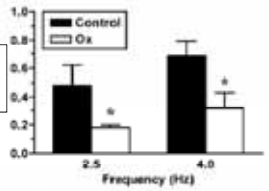
Direct hemodynamic effects of testosterone on erectile function

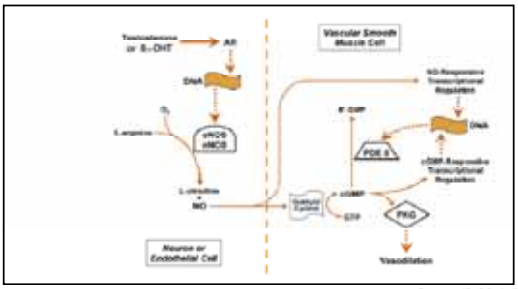
Intracavernous pressure / Systemic Arterial Pressure



Traish AM et al. J Androl. 2005 Mar-Apr;26(2):242-8.

ESHRE 280609

Changes in NO and PDE-5 transcriptional regulation following castration



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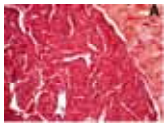
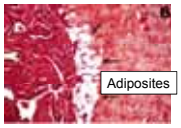
Histological changes in CC following castration

Sham Surgery

Bilateral orchidectomy

Hematoxylin + eosin

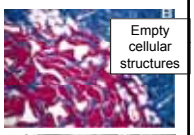




Adiposites

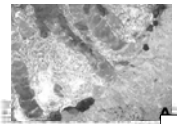
Rabbit CC 2 wks after surgery

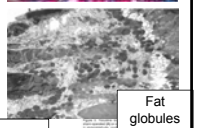
Trichrome Masson



Empty cellular structures

Toludine blue staining





Fat globules

Traish AM et al. J Androl. 2005 Mar-Apr;26(2):242-8.

Histological changes

Proposed mechanism of regulation of cellular differentiation by androgens in penile corpus cavernosum

ESHRE 280609

Medications associated with T-deficiency

Mechanism	Example
• Decreased T-production	Alcohol Ketoconazole Opoids LHRH Agonists
• T-Antagonisten	Cimetidine Spironolactone
• Increased prolactin	Metoclopramide Domperidone Methyldopa
• Increased SHGB-levels	Barbiturates
• Decreased DHT Levels	Dutasteride

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Questions

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

↓

Intervention studies

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Potential risks of Testosterone Therapy

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

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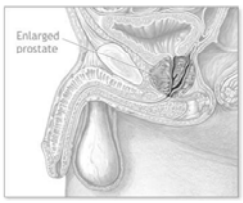
BPH and LUTS

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



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Prostate cancer

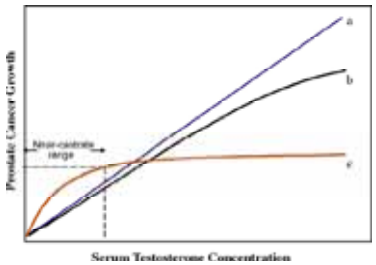
Table 3. **Prostate Cancer in Trials of Testosterone-Replacement Therapy.***

Study	Duration	Increase in PSA		Prostate Cancer		Method of Administration
		Placebo	Testosterone	Placebo	Testosterone	
	mo	*number/total number*				
Hajjar et al. (1997)[32]	24	–	–	0/27	0/45	Intramuscular
Sih et al. (1997)[9]	12	0/15	0/17	0/15	0/17	Intramuscular
Dobs et al. (1999)[11]	24	–	1/33	–	2/33	Intramuscular
		–	0/33	–	1/33	Nonscrotal patch
Snyder et al. (1999)[8]	36	7/54	13/54	0/54	1/54	Nonscrotal patch
Snyder et al. (2000)[6]	36	–	–	–	0/18	Scrotal patch
Wang et al. (2000)[20]	6	–	0/76	–	0/76	Nonscrotal patch
		–	1/73	–	0/73	Transdermal (50 mg)
		–	4/78	–	1/78	Transdermal (100 mg)
Kenny et al. (2001)[7]	12	3/33	8/34	0/33	0/34	Nonscrotal patch

Rhoden EL, Morgentaler A. N Engl J Med. 2004 Jan 29;350(5):482-92.

ESHRE 280609

The saturation model

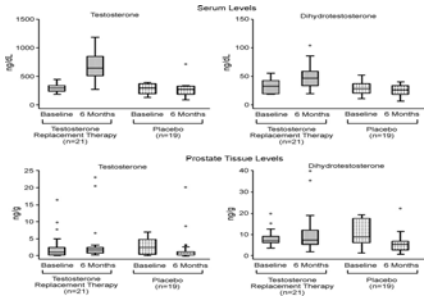


The traditional model of testosterone (T)–dependent prostate cancer (PCa) growth suggests that higher serum T concentrations lead to some degree of greater PCa growth (curves a and b). The saturation model (curve c) describes a steep T-dependent curve at T concentrations at or below the near-castrate range, with a plateau representing little or no further growth above this concentration.

ESHRE 280609

Morgentaler A, Traish AM. Eur Urol 2009;55:310–21.

The effect of exogenous testosterone (T) administration on the prostate tissue levels of T and dihydrotestosterone (DHT) in hypogonadal men.



Marks LS, Mazer NA, Mostaghel E, et al. Effect of testosterone replacement therapy on prostate tissue in men with late-onset hypogonadism: a randomized controlled trial. JAMA 2006;296:2351–61.

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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

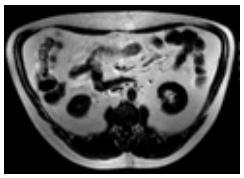
ESHRE 280609

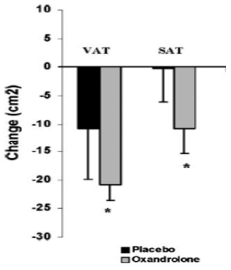
Potential benefits of testosterone Therapy

- **Metabolic syndrome**
- **Sexual function**
- **Quality of (sexual) live**

ESHRE 280609

Testosterone replacement therapy may be beneficial for some components of the metabolic syndrome in overweight men with low T-levels





Effects of Androgen Therapy on Adipose Tissue and Metabolism in Older Men

E. TODD SCHROEDER, LING ZHENG, MICHELLE D. ONG, CARMEN MARTINEZ, CARLA FLORES, YOLANDA STEWART, COLLEEN AZEN, and FRED B. SAFFILER

The Journal of Clinical Endocrinology & Metabolism 89(10):4863–4872

ESHRE 280609

Cardiovascular benefits

Improvement in distance achieved in the shuttle walk test

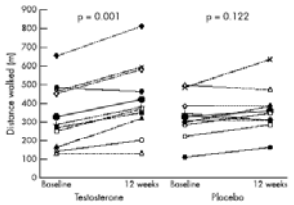


Figure 1 Changes in distance achieved in the shuttle walk test. Lines in bold with large solid circles indicate mean values in each group. Mean (SD) distance walked at baseline was similar in testosterone and placebo treated groups (328 (174) m v 314 (92) m, p = 0.825). In the testosterone treated group, exercise duration increased significantly to 419 (200) m, p = 0.001, but there was no significant change with placebo, to 340 (101) m, p = 0.122.

Testosterone treatment for men with chronic heart failure

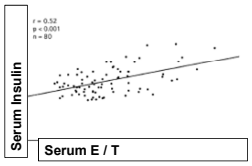
P J Pugh, R D Jones, J N West, T H Jones, K S Channer

Heart 2004;90:445–447. doi: 10.1136/hrt.2003.014639

ESHRE 280609

T-supplementation may improve insulin resistance

Reverse relationship between fasting insuline and testosterone levels



Laaksonen DE et al Diabetes Care. 2004 May;27(5):, 1036-41

ESHRE 280609

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®)
- Baselinescore IIEF5: 12

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

ESHRE 280609

The addition of T replacement after failure of sildenafil alone

N = 75, T < 400 ng / ml
Sildenafil 100 mg non-responders

T gel 1% + sildenafil 12 weeks | **PBO + sildenafil 12 weeks**

IIEF Domain	Delta baseline	Delta baseline	p – value
	T Gel + S	PBO + S	
Erectile function	5.65	2.97	0.037
Orgasmic function	1.53	0.36	0.019
Sexual desire	0.44	0.0	0.211
Intercourse satisf	1.21	0.7	0.250
Satisfaction	1.62	0.61	0.046
Total score	10.44	4.64	0.022

ESHRE 280609

Oral testosterone replacement in symptomatic late-onset hypogonadism: effects on rating scales and general safety in a randomized, placebo-controlled study

Jean-Jacques Legros, Eric J H Meuleman[1], Jolanda M H Elbers[2], T B Paul Geurts[3], Marion J G H Kaspers[4], Pierre M G Bouloux[5] for the Study 43203 Investigators

Legros JJ, Meuleman EJ, Elbers JM, Geurts TB, Kaspers MJ, Bouloux PM; Study 43203 Investigators. Oral testosterone replacement in symptomatic late-onset hypogonadism: effects on rating scales and general safety in a randomized, placebo-controlled study. Eur J Endocrinol. 2009 May;160(5):821-31.

ESHRE 280609

Table 2 Effects of oral testosterone undecanoate (TU) and placebo on the Aging Males' Symptoms (AMS) rating scale (plus sub-domains) after 6 and 12 months of treatment (mean ± s.d.; intent-to-treat group).

	Placebo n=75	Oral testosterone undecanoate 80 mg/day n=76	160 mg/day n=82	240 mg/day n=77
AMS rating scale – total score				
Baseline	29.8 ± 10.1	36.0 ± 11.4	37.3 ± 10.1	37.6 ± 11.6
Month 6	25.6 ± 9.5	30.2 ± 10.2	31.7 ± 8.9	32.2 ± 9.5
Δ from baseline	−4.2 ± 8.5	−4.8 ± 8.8	−5.6 ± 9.9	−5.4 ± 10.3
Month 12	25.5 ± 9.7	30.2 ± 10.2	31.7 ± 8.9	32.6 ± 10.3
Δ from baseline	−4.2 ± 9.1	−4.7 ± 8.1	−5.6 ± 8.8	−5.0 ± 10.4
AMS rating scale – psychological symptoms sub-score				
Baseline	10.8 ± 3.8	10.1 ± 4.2	9.6 ± 3.9	9.7 ± 4.3
Month 6	9.6 ± 3.5	8.8 ± 3.8	8.4 ± 3.6	8.3 ± 3.5
Δ from baseline	−1.2 ± 3.3	−1.2 ± 3.6	−1.2 ± 3.1	−1.3 ± 3.2
Month 12	9.3 ± 3.7	8.5 ± 3.5	8.3 ± 2.2	8.3 ± 3.6
Δ from baseline	−1.4 ± 3.6	−1.5 ± 2.9	−1.3 ± 3.1	−1.4 ± 3.4
AMS rating scale – somatic symptoms sub-score				
Baseline	15.6 ± 4.6	15.2 ± 5.1	14.3 ± 4.4	15.0 ± 5.4
Month 6	13.6 ± 4.8	13.1 ± 4.3	12.6 ± 4.4	12.8 ± 4.2
Δ from baseline	−2.0 ± 4.0	−2.1 ± 3.7	−1.7 ± 4.4	−2.3 ± 4.8
Month 12	13.8 ± 4.7	13.2 ± 4.7	12.4 ± 3.8	13.0 ± 4.5
Δ from baseline	−1.8 ± 4.1	−2.0 ± 4.0	−1.9 ± 4.0	−2.0 ± 4.7
AMS rating scale – sexual symptoms sub-score				
Baseline	13.5 ± 3.7	12.7 ± 3.7	13.3 ± 3.8	12.9 ± 3.9
Month 6	12.4 ± 3.9	11.3 ± 4.1	10.7 ± 4.0	11.0 ± 4.2
Δ from baseline	−1.1 ± 3.7	−1.4 ± 3.8	−2.6 ± 4.1*	−1.9 ± 3.8
Month 12	12.5 ± 3.9	11.5 ± 3.9	11.0 ± 3.9	11.1 ± 4.2
Δ from baseline	−0.9 ± 3.4	−1.2 ± 3.8	−2.3 ± 3.9*	−1.8 ± 3.7

*P<0.05; †P<0.01 versus change in placebo.

Legros JJ, Meuleman EJ, Elbers JM, Geurts TB, Kaspers MJ, Bouloux PM; Study 43203 Investigators. Oral testosterone replacement in symptomatic late-onset hypogonadism: effects on rating scales and general safety in a randomized, placebo-controlled study. Eur J Endocrinol. 2009 May;160(5):821-31.

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

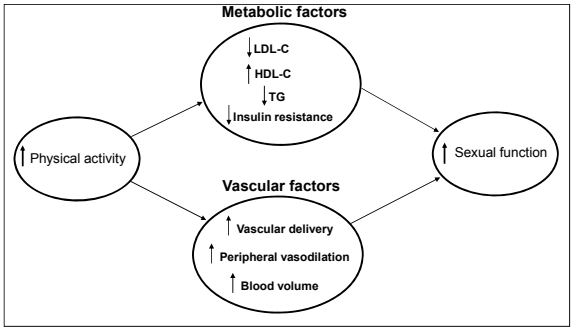
Young brain

Old brain

Taking hormones to replace those lost during menopause helps many women with their symptoms yet it may also cause cognitive decline. Could the age at which hormones are taken determine whether they will be beneficial or harmful? **Tom Siegfried** reports.

ESHRE 280609

Life-style: Physical activity: An alternative!



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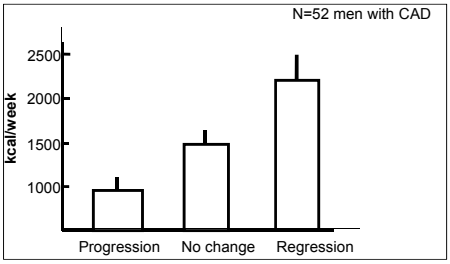
Physical activity and progression/regression of coronary atherosclerosis



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Hambrecht R et al. JACC 1993; 22: 468–477.

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

49 consecutive men > 40 yrs with LUTS and/ or ED

Criterion for sedentary lifestyle

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

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References

- Guidelines on male infertility. European Association Urology, march 2009. ISBN:978-79754-09-0
- Kapoor D et al. *Eur J Endocrinol* 2006; 154: 899–906. 2.
- Dhindsa S et al. *J Clin Endocrinol Metab* 2004; 89: 5462–5468.
- Kupelian V et al. *J Clin Endocrinol Metab* 2006; 91(3): 843–850.
- Kapoor D et al. *Clin Endocrinol* 2005; 63: 239–250.
- Svartberg J et al. *J Intern Med* 2006; 259(6): 576–582.
- Hak AE et al. *J Clin Endocrinol Metab* 2002; 87(8): 3632–3639. 7. Shores MM et al. *Arch Int Med* 2006; 166: 1660–1665
- Lyon CJ *et al. Endocrinology* 2003; 144: 2195–2200. Trayhurn P *et al. Br J Nutr* 2004; 92: 347–355.Eckel RH *et al. Lancet* 2005; 365: 1415–1428.
- Corona G et al. *Eur Urol* 2006; 50: 595–604.
- Matthew R et al. *JCEM* 2006; 91(4): 1305 1308.
- English et al. Eur Heart. 2000;1(11):890.
- Mäkinen J et al. *JACC* 2005; 45: 1603–1608.
- *Muller M et al.Circulation* 2004; 109: 2074–2079.
- Meuleman EJ, van Lankveld JJ.BJU Int. 2005 Feb;95(3):291-6.
- Traish AM et al. J Androl. 2005 Mar-Apr;26(2):242-8
- Rhoden EL, Morgenthaler A. N Engl J Med. 2004 Jan 29;350(5):482-92.
- Morgentaler A, Traish AM. Eur Urol 2009;55:310–21
- Marks LS, et al. JAMA 2006;296:2351–61.
- Laaksonen DE et al Diabetes Care. 2004 May;27(5):. 1036-41
- Legros JJ et al. Eur J Endocrinol. 2009 May;160(5):821-31.

ESHRE 280609

What does poor sperm DNA quality mean?

A critical review of methods, interpretation and clinical value

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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.

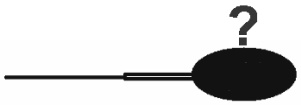
Learning objectives

- 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?

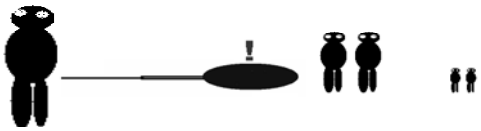
Sperm DNA quality
Sperm quality
Sperm integrity are ” political terms” an increase

Methods give RESULTS



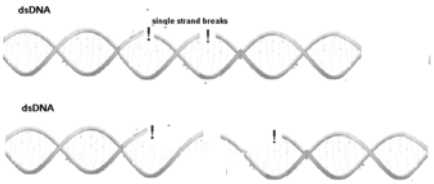
Investigators should tell about the method used and focus on RESULTS

The spermatozoon is a messenger cell carrying messages for healthy grand-children.



Messages are

- **The intact DNA – the genome**
- Structural defects
- Numerical defects
- DNA- strand – breaks
- **The ”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

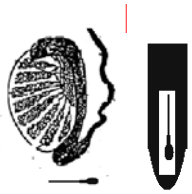


Unrepaired chromsomal deletion

Repaired by the oocyte
Normal,
Inversion or
Translocation (balanced, unbalanced

DNA strand breaks

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

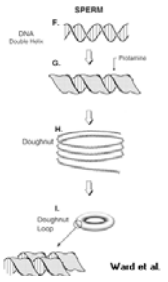


May result in a  grand-child with an unbalanced translocation with

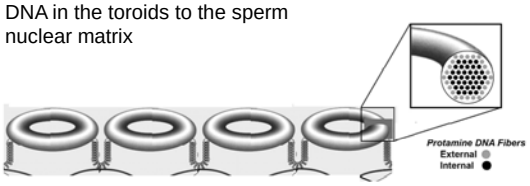
impaired psychomotor development and malformations

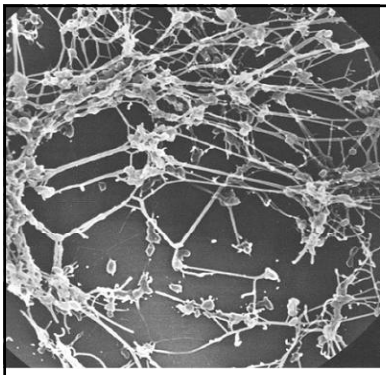
The protamin covered sperm DNA.

- 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

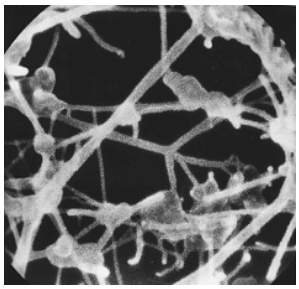


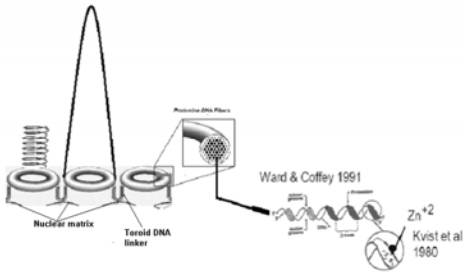


1 zinc/1 protamine/ 10 bp DNA

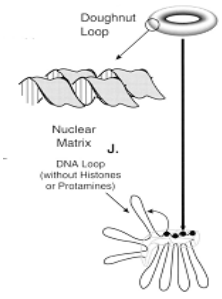
	Zinc/Sulfur x 1000
Fertile men	150 (97-182)
Childless men without prostatic affection	134 (110-201)
Childless men with prostatic affection	62 (48-77)

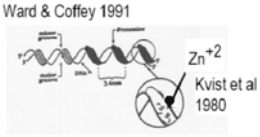
SDS exposed after EDTA pre-exposed at ejaculation





Organized in loops forming doughnuts

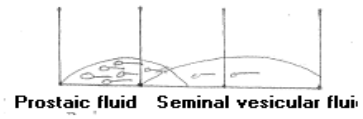




The Sequence of Ejaculation

- Man offers the woman spermatozoa in prostatic fluid

In vitro Split ejaculate

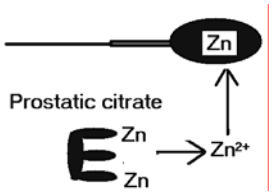


Prostaic fluid Seminal vesicular flui

No gel	Gel
Vitality +	Motility-
Motility +	Vitality-
Chromatin stability +	Chromatin zinc -
Chromatin zinc +	Chromatin stability -- ++ (SS)

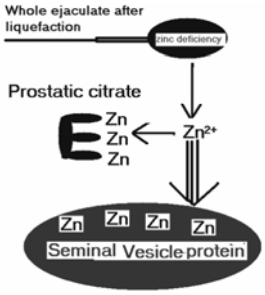
Chromatin zinc is retained by prostatic fluid

- The physiological ejaculate is spermatozoa suspended (emitted) in prostatic fluid and expulsed 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

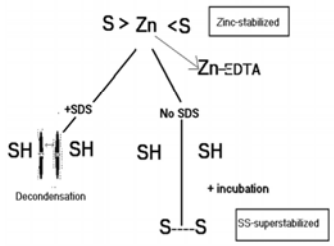
- Spermatozoa expelled in vesicular fluid at ejaculation reveal lower zinc content in the chromatin (Björndahl, 1990).
- Spermatozoa incubated in seminal vesicular fluid loose zinc

Chromatin zinc

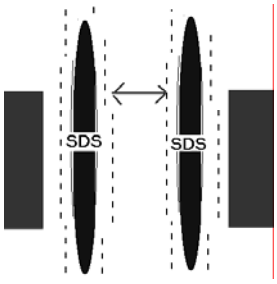
- Fertile donors have higher zinc content in chromatin than infertile men.
- Men with signs of prostatic inflammation had the lowest chromatin zinc content (Kvist, 1988).

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

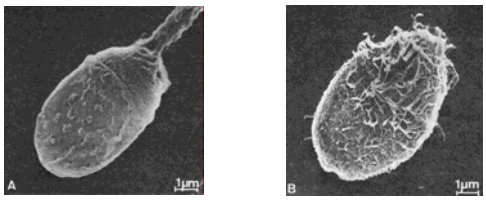
- Removal of zinc gives two possibilities!
- 1) immediate decondensation
- 2) otherwise develops superstabilization in air atmosphere.



Sodium Dodecyl Sulphate introduces negative repulsive forces



+ SDS



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Totally Resistance vs Fast delivery of DNA

- Sulfonuklein
- 90% decondensed < 5min after ejaculation
  If exposed also to zinc-chelating EDTA

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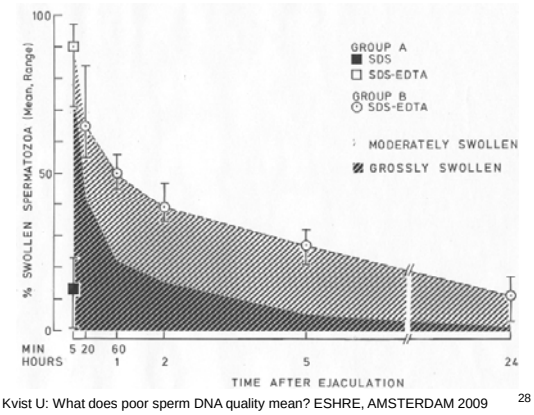
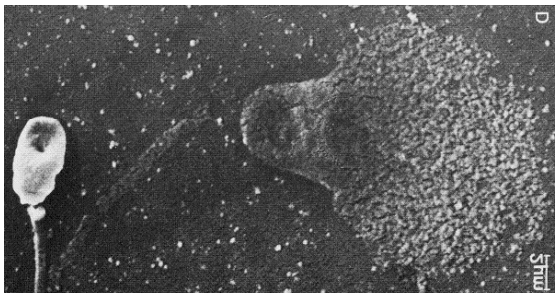
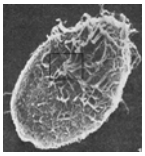


Exposed to SDS-EDTA one resistant, one decondensed



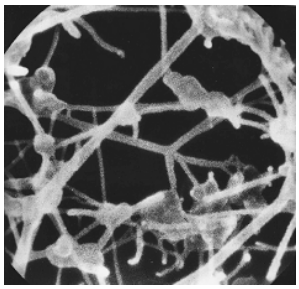


Scanning Electron Microscopy

Kvist & Nilsson1980

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

SDS exposed after EDTA pre-exposed at ejaculation

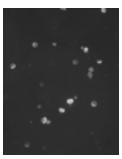


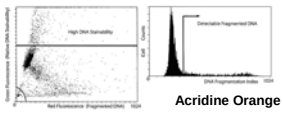
Methods

- 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

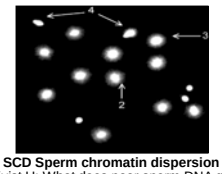
TUNEL

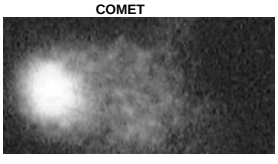




Acridine Orange

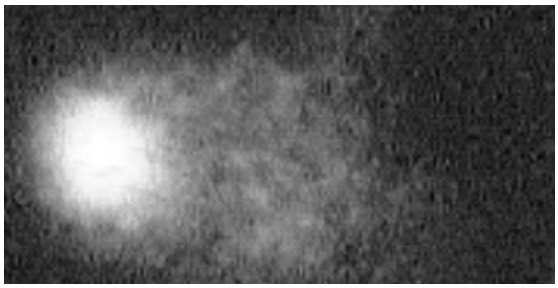
COMET



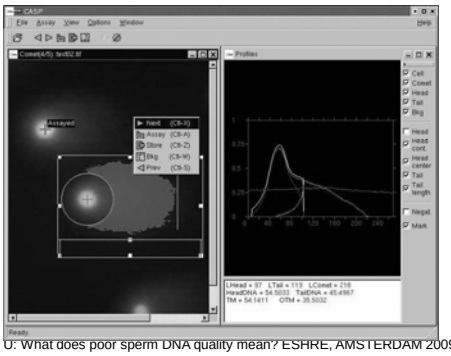


SCD Sperm chromatin dispersion

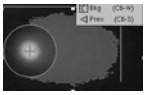
A sperm comet
Comet head and comet tail



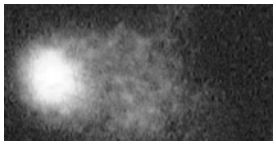
Calculation of results CASP – Comet Assay Software Project www.casplab.com



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



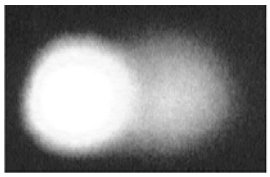
- **HeadArea** Area of the comet head
- **TailArea** Area of the comet tail
- **HeadDNA** Sum of intensities of pixels in the head
- **TailDNA** Sum of intensities of pixels in the tail
- **HeadDNA%** Percent of intensity of pixels in the comet head
- **TailDNA%** Percent of intensiyt of pixels in the 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
- **OliveTailMoment** TailDNA% x (TailMeanX-HeadMeanX)

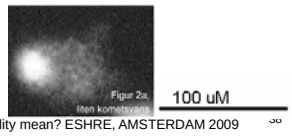


- **Principle**
- **Lysis:** Take away all proteins binding DNA leaving the naked and free DNA
- **Electroforesis:** Put current on it and small pieces will move.

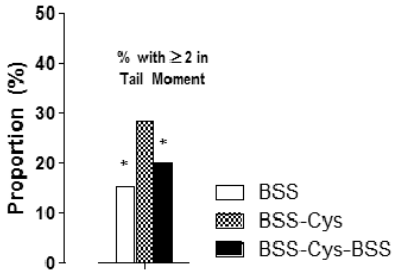
The lysis does not reveal all

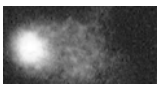
- Cysteine (zinc-chelating and S-S cleaving) **increased** the amount of DNA avaliable to the assay.
- The effect was significantly related to the to the conc of zinc in the ejaculate



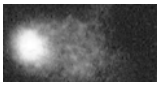


After cysteine treatment the structure re-stabilizes (S-S)





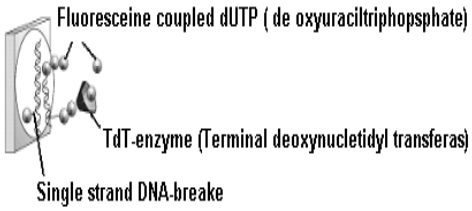
- Conclusion:
- In toxicology studies clear differences between controls end exposed
- In human standard lysis protocol does not reveal all DNA.
- The lysis respons related to zinc concentration in seminalplasma.
- Zinc removal stabilizes the chromatin towards the lysis protocol



- 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.

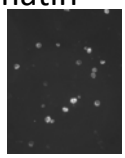
- **Thus there is the risk of false negatives!**

TUNEL= TdT-mediated dUTP Nick End Labeling



DNA- breakes

TUNEL assay and chromatin stability



- **Using In Situ Cell Detection Kit (Fluorescein)**
  - **Positive controls only stained 15-53% 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**

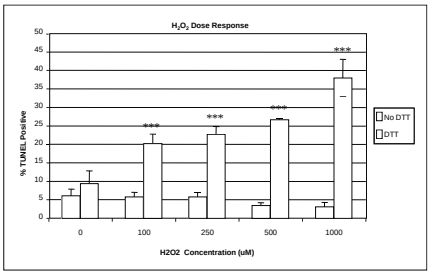
From Björk et al, 2009, Poster presentation at the American Society of Andrology, Philadelphia, PA, USA, April 4-7.

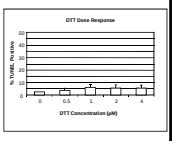
% TUNEL (N=9), coefficient of correlation (r_c)

	r_c	r_c^2	P=
Zinc conc. (mM)	-0.73	0.53	0.026
Abstinence time (days)	0.80	0.63	0.01
Analysis delay (min)	0.95	0.90	0.0001
% TUNEL (Pos. control)	0.31	0.09	ns (0.42)
Sperm conc. (10^6/mL)	-0.17	0.03	ns (0.67)

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*

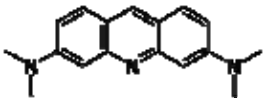




Conclusion TUNEL. Intelligent! Identifies breaks but only if given access!!.

- Stained dots means strand-breaks – bad sign.
- 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

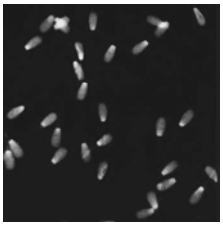


Acridine orange is prepared from coal tar and creosote oil.

The rethorical 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

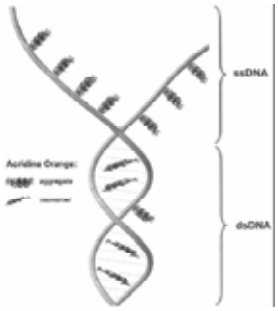


Acridine Orange and somatic cells

- Nucleic acid selective fluorescent cationic*(+)* dye useful for cell cycle determination *(i.e the shift between 2n an 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 *(eg 488 nm*) and an emission maximum at 525 nm (green). *(530+-30nm)*
- 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.

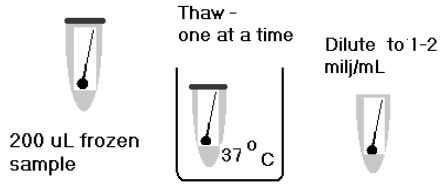
AO bound to ssDNA by electrostatic forces

+ forming aggregates with other AO molecules



•AO binds to dsDNA by interchelation

Procedure flow cytometry with AO



Freeze

When ?

-20C, -196 -80

Why?

Why?

Standardizations

- 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 (etylene diamine tetraacetic acid) (Sigma), pH 7.4)

Add " acid"

400 uL acid detergent solution to 200 uL TNE diluted sample

Why?

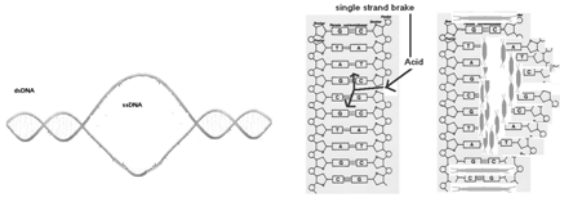
To produce single stranded DNA

Denaturation (= dsDNA into ssDNA) can be induced by
elevated temperature
alkali
acid
solvents
some drugs

and is used in lab protocols e.g FISH and SCSA

In somatic cells:
Acid denatures DNA.

Plus the idea that this will be enhanced at strand break points.



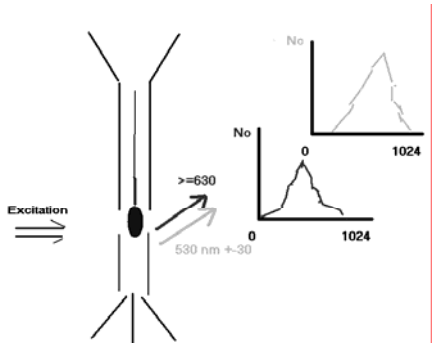
NB the denaturation increases with time

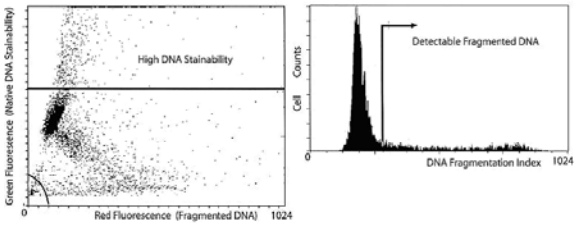
Production of single stranded DNA is stopped and AO given!

At 30 s exactly add solution pH 6,0 with AO

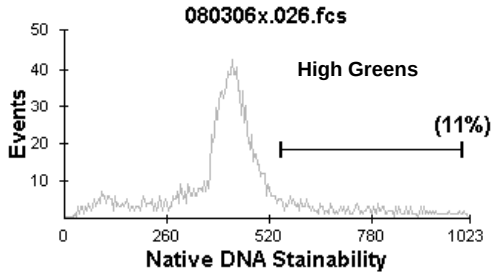
AO 6 mg/L in pH 6,0:

0.1 M citric acid (Sigma),
0.2 M Na2PO4 (Sigma),
1 mM EDTA(Sigma),
0.15 M NaCl, pH 6,0

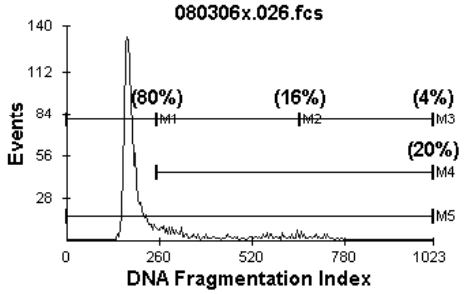




Collection of green fluorescens

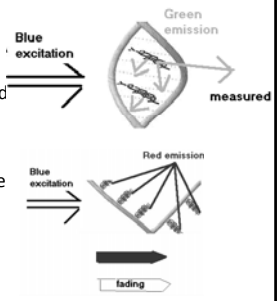


The proportion of red light of all light emitted. (red/ red+green)



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



Tytus Bernas et al 2005; Photochemistry and Photobiology 81(4): 960-969

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

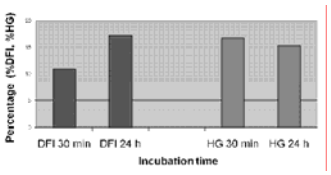
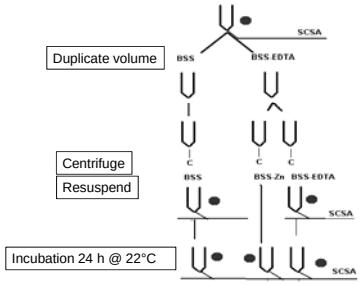
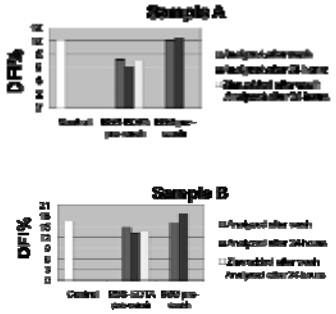


Figure 1: Defragmentation index (DFI%) increased signficantly during 24 hour storage in original seminal plasma (N=13, *P*<0.01). No significant difference was observed in the proportion High Green (HG%).

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



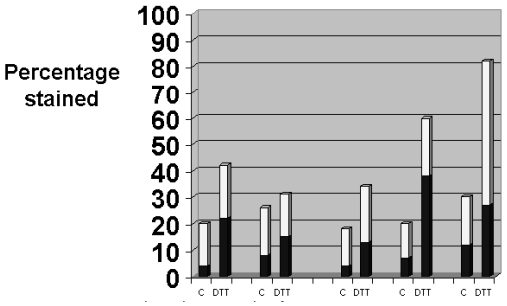
DFI changes (decreases) after sperm zinc depletion



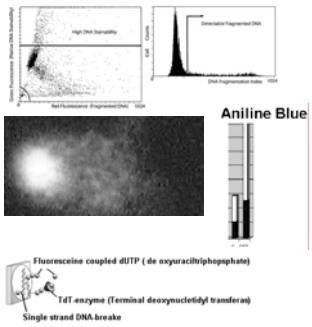
Conclusion AO-staining

- 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-superstabilizationon.
- **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 accessability

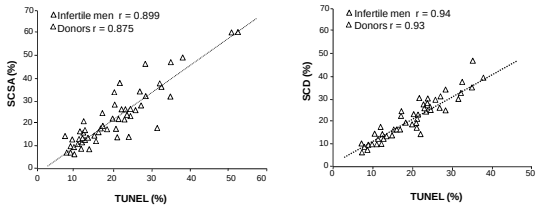


False negatives

Calls for more methodological work and standardisation

Relationships between DNA damage assays

Chohun et al. J Androl 27, 53-59, 2006



Consequences of Reported DNA damage in the male germ line

- **Reduced pregnancy rates following natural or assisted conception**
  - (Loft et al., 2003, Duran et al., 2002; Bungum et al., 2004).
- **Impaired fertilization**
  - (Benchaib et al., 2003; Virro et al., 2004; Aitken 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 **(3% 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 spermatozoa (Bungum et al 2007).
- One study- no rush!!!
- The gradient selected spermatozoa used, had mean DFI of some 4% (Bungum et al 2008).
- Thus, the bad IUI results were obtained with spermatozoa 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 AO FACS if not on whole semen?

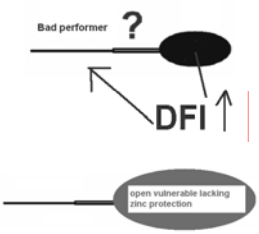
Therefore, as clearly stated in the SCSA guidelines (Evenson *et al*., 2002), SCSA analysis should be performed on raw semen aliquots.

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?



Or selection bias during IVF ICSI

Final remarks 1

- Many methods has been designed to characterize the ” integrity of the sperm DNA”.
- The original protocols developed and validated on somatic cells and not on spermatozoa.

Final remarks 2

- None of the protocols take in consideration Neither that the availability to the sperm chromatin undergoes severe changes with the 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

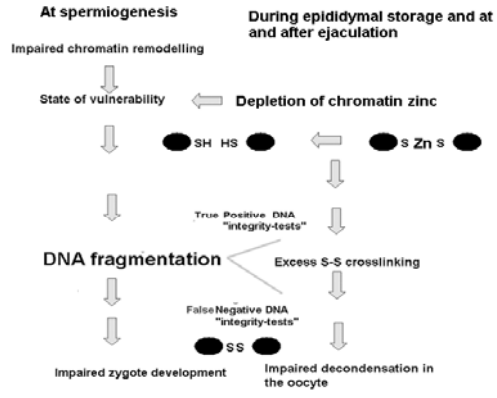
- A positive signal (pos TUNEL, AO-red, Increased DFI%, Toludine+,Sperm swelling in SDS, positive COMET) tells that the sperm chromatin is available and susceptible to damage and probably damaged.
- A negative signal can be true or false due to superstabilization. A zinc-deficient chromatin is both vulnerable and likely to undergo excess superstabilisation by S-S,which, which in turn decrease its availability and give 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



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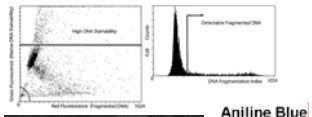
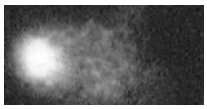
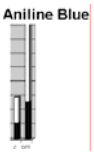






False negatives

Calls for more methodological work and standardisation

Or choose physiology! The first split ejaculate fraction at ejaculation

Kvist U: What does poor sperm DNA quality mean? ESHRE, AMSTERDAM 2009 82

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 → endocrine Rx ± IUI**
- **2 blocked tubes → surgery *or* IVF**
- **1 cervical factor → IUI**

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

5 idiopathic infertility → IUI → IVF → ICSI

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

Why Not Just Use ICSI On Everybody ?

<u>Pros</u>:
- **Will minimize the risk of fertilization failure.**
- **No need to investigate the male or his sperm.**

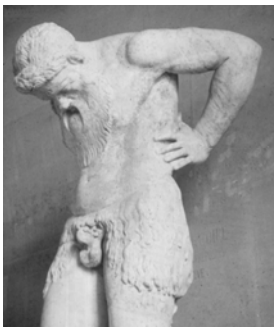
<u>Cons</u>:
- **Usually more expensive to the patients.**
- **More time consuming for the lab (workload / workflow).**
- **More invasive, with inherent risk of oocyte damage.**
- **Fertilization rate is lower than can be achieved by IVF in the absence of sperm dysfunction.**
- **Bypasses the natural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

The Argument Against "ICSI For All"

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

Parameter	Criteria	IVF Rate	IVF #	ICSI Rate	ICSI #	Criteria
COCs retrieved			11			
Maturity	% MIIs	(85%)		85%	9.35	
ICSI damage rate				-3%	9.07	
Fertilization rate	as % of COCs inseminated	80%	8.80	70%	6.35	of MIIs injected
Cleavage rate	% of zygotes	98%	8.62	98%	6.22	
Utilization rate	Day 3 embryos for ET or cryo	65%	5.61	65%	4.04	
Embryos transferred		2		2		
	Implantation rate (fresh)	30%	60%	30%	60%	Equal chance
Embryos cryopreserved			3.61		2.04	
Cryosurvival rate		75%	2.70	75%	1.53	
	Implantation rate (frozen)	20%	54%	20%	31%	
Cumulative implantation potential			114%		91%	
Loss of outcome for patient				23%		

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.

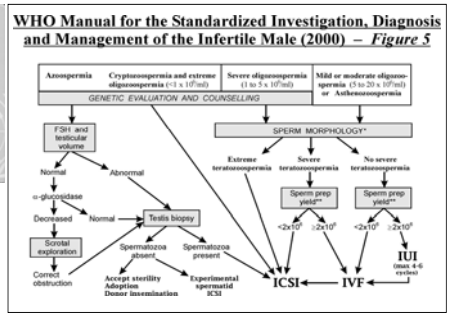
Speakers: David Mortimer, Chris de Jonge
Denny Sakkas, Gabor Huszar, Chris **Barratt**

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.

The WHO and Structured Management

(2000)



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 (HAmax) / 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 A_3**

SEMEN ANALYSIS

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

<u>Purpose</u>:

- **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 >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**)**

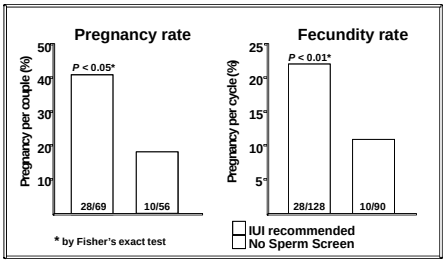
- **SpermScreen assessment was applied to 485 new referrals.**
- **Of 266 patients with "normal" WHO semen analysis, 103 (39%) had abnormal results in the other SpermScreen tests, so ICSI recommended.**
- **But 12/67 men with poor semen analysis results had good post-wash motility and hyperactivation ("OK for IVF").**
- **Incidence of low/failed fertilization in IVF fell from 6% to just 1% of cycles.**

ST Mortimer *et al.* (2002) ESHRE abstract P-317.

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.



ST Mortimer *et al.* (2002) ESHRE abstract P-317.

SFA and IUI

"Simplified SFA" (no CASA)

Victoria Fertility Centre, BC, Canada

Pregnancy Rate	IUI Recommended	IUI *Not* recommended	SFA Not done
Per IUI cycle	38% (8/21)	20% (9/45)	21% (8/38)
Per couple	57% (8/14)	31% (9/29)	31% (8/26)

C. Lawrence *et al.* (2004) CFAS abstract F02.

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:

Period	stims/yr	IUI/yr	ICSI	IVF	R/ICSI
2000	550–600	minimal	60%	40%	active programme
2001–2002	650–700	~600	40%	60%	almost none

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) . . .**

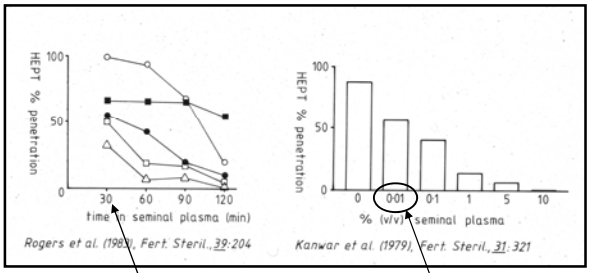
Culture Conditions for Capacitation

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: <u>minimum</u> of 10 mg/ml (**mid-cycle oviduct fluid contains about 30 mg/ml, serum ~45 mg/ml**)**

Seminal Plasma and Fertilizing Ability

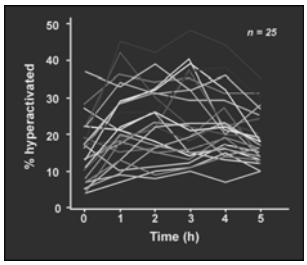
The need for prompt and efficient sperm preparation:



max 30 min

i.e. 1-in-10,000

Sperm Hyperactivation



Spontaneous hyperactivation is very variable both between men and over time.

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

Karande *et al.* (1990) J. Androl., 11(sup): P-28 (Abstract 31).

Hyperactivation agonists include progesterone and pentoxifylline.

<u>"HAmax" assay</u>:

Agonist* gives within 10% of the maximum spontaneous hyperactivation for >90% of men after 1-hr incubation.

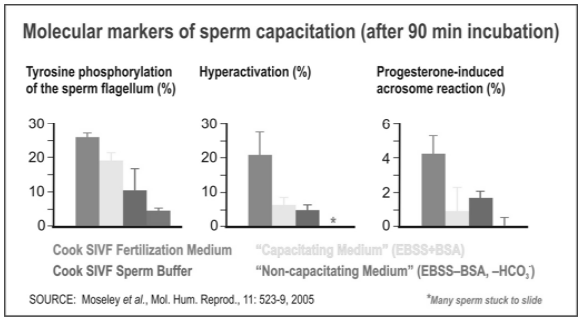
*1 µg/ml P4 + 3.6 mM POF in IVF Medium

CASA "sort" criteria (60 Hz IVOS):

VCL <u>></u>150 µm/s AND LIN <u><</u>50% AND ALH <u>></u> 7.0 µm

Capacitation Dynamics

Not all IVF media are created equal:



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 <u>after</u> 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

Björndahl L *et al. A Practical Guide to Basic Laboratory Andrology.* Cambridge University Press, Cambridge (UK), 2009.

Comhaire F, Mahmood A. Male factor infertility: State of the ART. In: Risk B *et al*. (eds), *Infertility and Assisted Reproduction.* Cambridge University Press, New York (NY, USA), 2008.

Gagnon C (ed). *The Male Gamete: From basic sciences to clinical applications.* Cache River Press, Vienna (IL, USA), 1999; chapters 32–36.

Mortimer D, Mortimer ST. Laboratory investigation of the infertile male. In Brinsden PR (ed), *Textbook of In Vitro Fertilization and Assisted Reproduction.* Taylor & Francis, Abingdon (UK), 2005, pp.61-91.

Rowe PJ *et al. WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male.* Cambridge University Press, Cambridge (UK), 2000.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 4th Edition.* Cambridge University Press, Cambridge (UK), 1999.

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