Sperm chromatin tests in relation to other semen analyses results for diagnosing male infertility

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Why is it so important to develop a better test?

- Infertility is now a public health issue across Europe
- Conventional semen parameters have low prognostic value
- Success rates of ART have remained low over the past 30 years
- Sperm DNA damage can have impact on the health of future generations

Fertility rates and future population trends: will Europe's birth rate recover or continue to decline?

Wolfgang Lutz

- After a sustained decline, EU birth rate is now 1.4 children/couple
- By 2050, EUs working population will drop by 18% while senior citizens increase by 60%
- Why? – choice? most couples would like more children

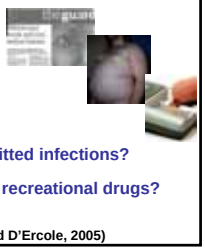
reduced fertility?

caused by environmental pollution

lifestyle factors-

obesity, diabetes, sexually transmitted infections?

alcohol, tobacco, recreational drugs?



(D'Addidio and D'Ercole, 2005)

The role of ART is finally recognised

Assisted reproductive technologies are an integrated part of national strategies addressing demographic and reproductive challenges

In 2008, European Parliament acknowledged for the first time that falling fertility rates were a major cause of its demographic decline. Over mortality and migration, infertility is the major determinant of the future size and population composition in Europe

Europe performs 60% of world ART
1-5% of births in Europe are by ART

The European Parliament (resolution adopted by parliament on 21 February 2008) 'calls on the member states to ensure the right of couples to universal access to infertility treatment.

Improving diagnosis and success rates is essential

The role of semen analysis

- Tells mainly about the functions of the male reproductive organs
  - Sperm production (numbers, morphology)
  - Sperm transport in the male reproductive tract
  - Secretory functions and ejaculation
- Functional aspects very rudimentary
  - Sperm motility
    - Sperm structures
    - Ejaculation (i.e. admixture of prostatic fluid)
    - Negative influence due to infections, presence of antibodies etc
  - Sperm survival

The role of semen analysis

- A semen sample is
  - Heterogenous
    - Sperm and prostatic fluid expelled first
    - Complete mixing only occurs in the laboratory
  - Without efficient homeostatic control
    - pH
    - Osmolality

The role of semen analysis

- Causes for non pathological variability
  - Between laboratories
    - Lack of technical standardization in methods and training
  - Between different men
    - Testis volume
    - Mitosis rate
    - Frequence of ejaculation
  - Between different samples from the same man
    - Frequency of ejaculation
    - Sexual arousal – quality and duration of stimulation

The role of semen analysis

- WHO manual on semen analysis
  - Technical guidelines for laboratories
  - Reference values
    - Distribution of results from recent fathers
    - No indication of overlap of results from men in infertile couples
    - Very limited help for diagnosis and prognosis
- Combined assessment of three variables
  - Total, motile, morphologically normal count
- Three levels instead of normal/abnormal
  - Normal fertility
  - Intermediate fertility
  - Subfertility

Guzick et al, 2001; Alvarez et al, 2003

So what **sperm function** and **fertilization competence** tests would be more useful to select the sperm that will create healthy offspring?

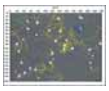
Consensus workshop on advanced diagnostic andrology techniques

- CASA
- Acrosome reaction tests
- Zona free hamster egg penetration test
- Sperm zona pellucida binding tests

Guidelines on the application of CASA technology in the analysis of spermatozoa

ESHRE

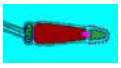
Sperm function tests



- Quantitative motion (CASA)
  Donnelly, Lewis et al, 1998; Hirano et al, 2001

- Hyperactivation (CASA)
  Sukchareon et al, 1995
- Cervical mucus penetration
  Eggert-Kruse et al; 1989 Shara et al, 1995

- Sperm-zona recognition and penetration
  Liu and Baker, 2004; Cabellero- Capo et al, 2006
- Acrosome reactions - basal and induced – ARIC
  Cummins et al, 1991



Oxidative stress tests

- XS production of ROS, H_2O_2 and O_2^-
  Jones et al, 1979; Aitken and Clarkson, 1987; Aitken et al, 2006
- Inadequate antioxidant protection
  Lewis et al, 1995;Agarwal et al, 2003; Aitken, 2005
- Chemiluminescence tests - Lucigenin and Luminol
  Donnelly, Lewis et al, 1994; Said et al, 2004
- Leucocyte contamination - use of anti CD beads
  Aitken 1996
- OS measured by lipid peroxidation and nDNA and mtDNA damage
  Lewis et al, 2005; Aitken, 2006

Advances in ART success rates?

- *1997: clinical pregnancy rate in IVF/transfer = 26.1%
- *1997: clinical pregnancy rate in ICSI/transfer = 26.4%
- *2002: clinical pregnancy rate in IVF/transfer = 29.5%
- *2002:clinical pregnancy rate in ISCI/transfer = 29.4%
- 2007: CPR IVF= 28.8%, ICSI = 35.3%, (n=611;Bungum et al, 2007)
- 2009: LBB/ET for donors= 28.8% (n=577, Patrizio and Sakkas, 2009)

*****Results generated from European registers by ESHRE*

The success of ISCI has led to a cessation of research into sperm dysfunction
- yet allowed the indiscriminate use of immature sperm

Sperm DNA damage Male Infertility

Occupation
Plastics and resins, solvents,
wood processing, metal industry,
Automobile, truck and aircraft mechanics
Sedentary or stressful job

Environment
Endocrine disruptors
xenoestrogens
Anti-androgens
Toxic compounds

Genetic Inheritance
CABVD
Robertsonian
translocations
Y-chromosome deletions
Paternal Age

Lifestyle
diet
smoking
alcohol
recreational drugs
STIs
injury
infection

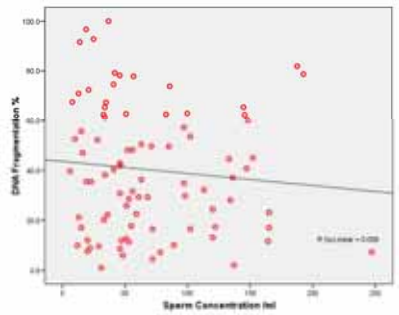
Semen analysis

IVF Patients (n=114)

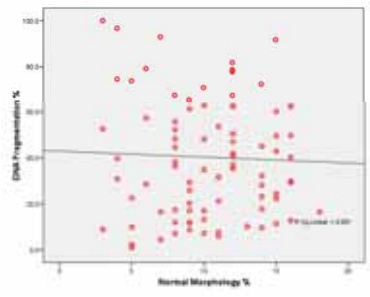
	Pregnant	Non-Pregnant
Semen volume (ml)	2.9 ± 0.2	3.3 ± 0.1
Sperm concentration (10^6/ml)	57.0 ± 7.2	70.6 ± 4.2
Sperm motility (%)	46.4 ± 4.4	52.3 ± 2.0
Abnormal morphology (%)	69.1 ± 3.6	70.7 ± 0.9

Mean ± SE

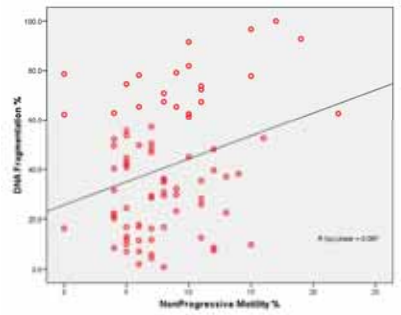
DNA Fragmentation vs Sperm concentration



DNA Fragmentation vs Morphology of sperm

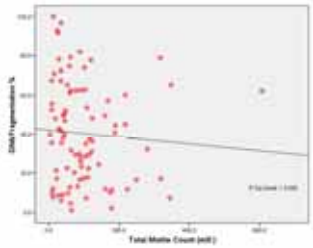


DNA Fragmentation vs NonProgressive Motility



DNA Damage ↑ with ↑ non-progressive motility

DNA Fragmentation vs Total Motile Count



Kodama et al, 1997; Shen et al, 1999; Irvine et al, 2000, Chan et al, 2001
Tomlinson et al, 2001; Saleh et al, 2003, McVicar et al, 2004
Spano et al, 1998; Evenson et al, 1999; Larson et al, 2000

Seminal plasma and SCSA

- **Defragmentation Index (DFI) positively correlated with**
  - seminal vesicular fluid in to sperm-rich fractions
    - creates a risk of depleting chromatin zinc and thereby impairing zinc-dependent chromatin stability
  - duration of sexual abstinence
- **Negative correlation to sperm concentration**

Richthoff et al, Human Reproduction Vol.17, No.12 pp. 3162–3169, 2002

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DNA test reproducibility compared to conventional parameters

- **Sperm DNA has lower CV (20%)**

Zini et al, 2001; Loft et al, 2003

- **DNA tests has 'high monthly repeatability' within donors**
  - CV 10% cf 44% for conc, 78% for motility and 69% for morphology

Evenson et al, 1991; Smit et al, 2007

- **CV of DNA Fragmentation Index (DFI) for repeated SCSA measurements was 29%.**
  - 37% of patients with DFI >30% in the first test had DFI <30% in the second test.
  - 27% of patients with DFI 21–30% in the first test had DFI >30% in the second test.

Erenpreiss et al, 2006

Are we expecting too much from one test?

Other factors with important roles-

- Sperm function
- Oocyte quality
- Embryo quality
- Uterine competence

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