Freeze Your Future: Cryopreservation of Semen and Surgically Retrieved Sperm

Sheena E. M. Lewis

Reproductive Medicine
Queen's University Belfast
s.e.lewis@qub.ac.uk

EAA ESHRE Brussels Nov 2007

The History of Semen Cryopreservation

- 1776: Spallanzani → ↓ motility on snow
- 1866: Montegazza proposed a soldier could beget a legal heir with stored semen before battle
- 1930-1940: *some* sperm survived after freezing
- 1949: first cryoprotectant- glycerol (Polge *et al* , 1949)
- 1950: glycerol- egg yolk- citrate → ↑ animal semen use
- 1963: Freezing in liquid Nitrogen at –196^{0}C
- Last 50 years - ↑ types of sperm frozen
- Little improvement in success or our knowledge of cryo-physiology

The Milestones of Cryopreservation

1953- first birth from frozen ejaculated sperm

Bunge and Sherman, 1953

1995- first birth from frozen epididymal sperm

Devroey et al, 1995

1996- first birth from frozen testicular sperm

Gil- Salmon et al, 1996

Uses of Semen Cryopreservation

Freezing provides people with future fertility potential

- Cancer and Multiple Sclerosis
- Oncologists' ambivalence
  - low incidence of later use
- ART - patient
- - donor
- Pre- operative insurance
- Vasectomy insurance
- Post mortem sperm retrieval

Procedures for Cryopreservation

- Consent forms
- Costs of storage
- Fate of gametes in event of man's death
- Screening for Hepatitis, Syphilis, Chlamydia, CMV and HIV
- Separation in tanks for 6 mths quarantine
- Straws or vials- what material?
- Labelling systems
- Monitoring of stored samples
- Security

British Fertility Society Lab Practice, Lewis et al, 2005

The effects of cryopreservation on sperm quality

Cryo-injury is expressed as

Structural Damage-

- loss of membrane integrity
- ↓ intact acrosomes
- cytoplasmic vacuolation
- mitochondrial distortion
  (reviewed by Nijs and Ombelet, 2001)
- ↓ normal morphology
  (~40%, ↑ amorphous heads and midpiece abnormalities)
  (Verheyen et al,1997; Hammadeh et al, 1999; O'Connell et al, 2002)
- Alterations in permeability and conformation of phospholipid bilayers
  (Mazur et al, 1984; Parks and Graham, 1992)

Cryo-injury is expressed as

Loss of viability

(Nijs and Ombelet, 2001)

Functional Damage to the surviving population-

- Loss of Motility (25-75%), VSL, VCL, VAP

(Hammadeh et al, 1999; Esteves et al, 2000; Nijs et al, 2000; Donnelly et al, 2001)

- Infertile mens' sperm more susceptible to cryoinjury

(Holden et al, 1997; Nijs et al, 2000, Donnelly et al, 2001; de Paula et al, 2006)

- Better indicator of fertility than SA?

Alterations in plasma and mitochondrial membrane potentials

- ↓ $[Ca2^{+}]_i$ and ↓ response to Progesterone
- → $[Ca^{2+}]_e$ entry → Capacitative motility
- ↓ intact acrosomal caps → ↓ acrosin activity
- ↓ Progressive motility -41% (greater in infertile sperm)
- ↓ R123 uptake -36%
- ↓ R123 activity -47%

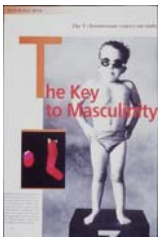
(Mack and Zanevald, 1987;Alvarez and Storey, 1993; Rossato et al, 2000; O' Connell et al, 2002)

Cryo-injury is expressed as

- No chromosomal damage
  (Li, Overstreet et al, 2007)
- ↑ Abnormal DNA condensation→ ↓ fertilization
  (Hammadeh et al, 2000, 2001)
- ↑Chromatin structure alterations
  (DNA – Protamine relationships) → ↓ fertilization
  (Royere et al, 1991)
- ↑ DNA fragmentation
  (Donnelly et al, 2001; Dalzell et al, 2001; de Paula et al, 2006)
-

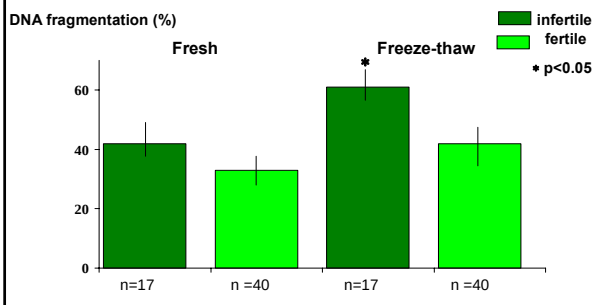
Can DNA integrity predict ART success?

Nuclear DNA anomalies lead to:-



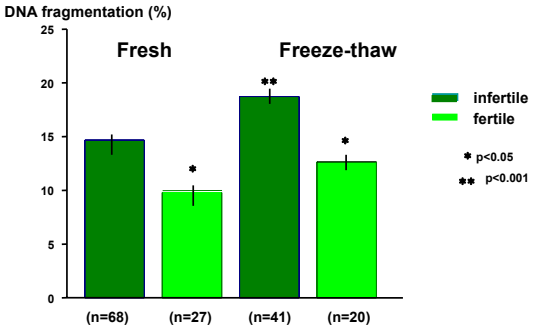
- Failure of fertilization in IVF
  Bianchi et al, 1993; Sun et al, 1997
- Failure to implant in ICSI
  Sakkas et al, 1996; Lopes et al, 1998
- Increased time to conception
- Increased miscarriage rate
  Evenson et al, 1999; Carrell et al, 2003
- Poor embryo development
  Morris et al, 2002; Tomsu et al, 2002
- Post-implantation loss and malformations
  Robaire et al, 1985
- Childhood cancers
  Knight and Marrett, 1997

Effects of freezing on ejaculated sperm DNA from fertile and infertile men

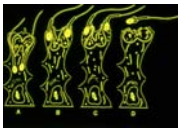


Donnelly et al, Human Reproduction 2001

Effects of freezing on testicular sperm DNA from fertile and infertile men



Dalzell et al, Human Reproduction 2001



A testicular sperm

Compaction of DNA by crosslinkage in epididymis

An ejaculated sperm

When does Cryo-injury Occur?

During freezing

- intracellular ice formation
- Osmotic stresses

(Muldrew and McGann, 1988; Watson, 1995; Devireddy et al, 2000, Morris et al, 2007)

During thawing

- Rapid warming prevents recrystallization

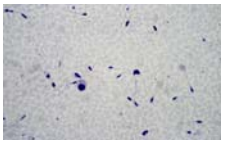
(Watson, 1995)

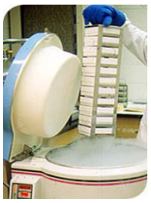
Not during storage

- Cryoprotectants are crucial for protection

(Mortimer, 1994; Yildiz et al, 2007)

The effects of cryopreservation on ART outcomes with ejaculated and epididymal sperm

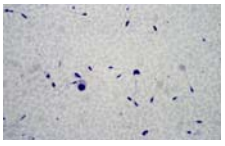


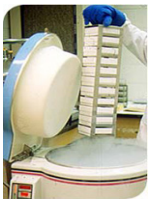


Cryo-injury leads to reduced success in ART

- IUI by husband- ↓ CPR
  (Sherman, 1973)
- IUI by donor - ↓ CPR
  (Richter et al, 1984)
- IVF and ICSI - ↓ FR, IR, CPR
  (Critser et al, 1987; Crabbe et al, 1999; Hammadeh et al, 1999)
- ICSI/MESA- ↑ CPR with F-T sperm
  (Devroey et al, 1995, Wood, Lewis-Jones et al, 2002)

The effects of cryopreservation on ART outcomes with testicular sperm





Current success rates of fresh v frozen testicular (OA) cycles

- FR, IR and CPR significantly reduced

  (Verheyen et al, 1997; Wood et al, 2002; Nicopoullos et al, 2003)

- IR significantly impaired

(RR1.75,95% CI 1.10-2.80, p=0.02: meta-analysis-of 1476 cycles Nicopoullos et al, 2004)

- No impairment in outcome- FR, IR, CPR or LBR

  (Friedler et al, 1998; De Croo et al, 1998; Ben- Yosef et al, 1999; Tournaye et al, 1999; Habermann et al, 2002 ; Thompson- Cree et al, 2003)

Reasons for Conflict in Literature

- Lack of Randomization
- Patient Choice
- Previous failure with frozen sperm or
- Their frozen sperm may be fitter!
- Should include only first time cycles
- But this can ↓ cases by 50%

Success rates depend on aetiology of azoospermia

- FR are higher and miscarriage rates are lower for men with acquired azoospermia but CPR and LBR are similar

  (*meta- analysis Nicopoullos et al, 2004)*

- ↓ CPR with time post vasectomy

  (Abdelmassih et al. 2002; Borges et al., 2003; McVicar et al, 2004)

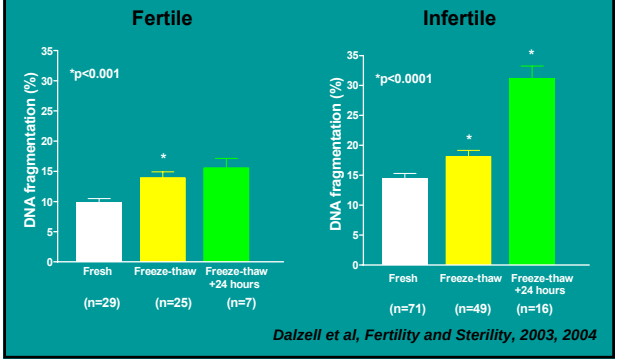
- ↓ CPR with NOA (usually fresh)

A danger of incubating post-thaw testicular sperm to acquire motility

Overnight incubation of post-thaw testicular sperm

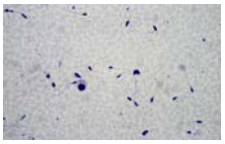
- routine clinical practice
- reasons: convenient
  non invasive
  quick viability test

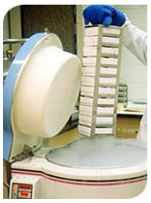
Effects of post-thaw incubation on testicular sperm DNA



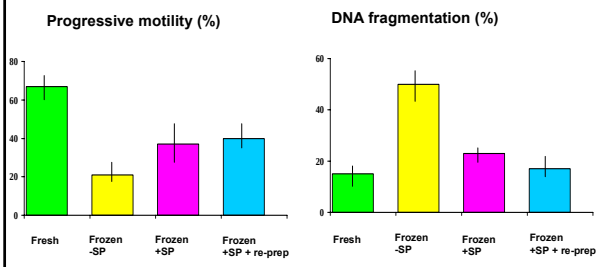
Dalzell et al, Fertility and Sterility, 2003, 2004

A better way to freeze sperm





The benefits of poor sperm removal and seminal plasma addition



Donnelly et al, Fertility and Sterility 2001

Challenges of Cryopreservation

Reduction of cryoinjury from-

- **Ice crystals- reduction or prevention**
  (Mudrew and McGann, 1988; Devireddy et al, 2000)

Combat with

- **programmable freezing**
- **cryoprotectants**

(Mortimer, 1994; Watson, 1995)

Challenges of Cryopreservation

Reduction of cryoinjury from-

- **Reactive oxygen species generation**

(Alvarez and Storey, 1992; ell et al, 1993, Kumar and Das, 2005; Peris et al, 2007)

Depletion of antioxidants

- **GSH ↓78%, SOD ↓50%**

(Bilodeau, Gagnon et al, 2000; Peris et al, 2007)

Benefits of antioxidant therapy

- **Catalase maintains motility**
  (Foote, 1967)
- **∝- tocopherol and ascorbate → ↑ viability**
- **SOD and Catalase → ↑ embryo numbers**
  (Roca et al, 2005)
- **Ascorbate → ↑ hamster egg penetration**
  → ↑ implantation in cows

(Beconi et al, 1993, Kumar et al, 2003)

Latest Advances and Challenges

- **Cryobanking testicular tissue for prepubertal boys**
- **Testicular stem cell transplantation**
- **Clinical application and safety**

(Schlatt et al, 2000-7; reviewed by Tournaye et al, 2004)

Acknowledgements

Ciara Hughes
Kristine Steele
Michael O'Connell
Lauren Dalzell

Eilish Donnelly
Ishola Agbaje
Carmel McVicar
Margaret Kennedy

The Wellcome Trust

NI

r&d office